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RESEARCH ARTICLE

Effect of chitosan, brassinosteroids and myo-inositol on pot marigold plants

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Abstract

The annual herbaceous pot marigold (*Calendula officinalis* L.), valued for its ornamental and medicinal properties. Different plant biostimulants could use for minimizing the negative effects of agricultural chemicals on ecosystems and human health *Calendula officinalis* was subjected to a split-plot experiment. Main plot treatments consisted of chitosan (CH) at 0.00, 0.20, 0.40, and 0.60 g/L, while subplots received either brassinolide (BR) at 0.10 or 0.20 g/L, or myo-inositol (MYO) at 0.25 or 0.50 g/L. All treatments improved plant and flower fresh and dry weights, and there were clear interactions between CH and BR/MYO. The highest total biomass (64.39 g/plant) came from 0.60 g/L CH + 0.10 g/L BR, while 0.60 g/L CH + 0.50 g/L MYO produced the most flowers (50) and the heaviest dry flowers (144.5 g/plant). Plants with no treatment had the lowest yield and chlorophyll content (SPAD = 40.17), whereas 0.40 g/L CH + 0.50 g/L MYO achieved the highest chlorophyll level (SPAD = 46.87). Both CH and BR/MYO significantly boosted N, P, and K levels in the plants. For optimal fresh and dry flower yield, application of 0.60 g/L CH combined with either 0.20 g/L BR or 0.50 g/L MYO is recommended.

Keywords: *Calendula officinalis*, Flower yield, Growth promotion, Myo-inositol, Plant Biostimulants

1. Introduction

Calendula officinalis L., commonly called pot marigold, is an annual herbaceous plant from the Asteraceae family. Originally from the Mediterranean area, pot marigold has spread worldwide and is frequently cultivated due to its vibrant bright orange or yellow flowers and extended blooming season (Zolfaghari *et al*., 2013). It is extensively grown for its decorative appeal, medicinal benefits, and industrial applications. The flowers are rich in bioactive compounds such as flavonoids, and carotenoids, making them valuable in cosmetic, food industries as well as pharmaceutical uses for its anti-inflammatory, antimicrobial, and wound-healing properties Preethi *et al*., 2006 and Barbour *et al*., 2004). Minimizing the negative effects of agricultural chemicals on ecosystems and human health is a crucial issue. In this context, plant biostimulants have emerged as promising innovations that promote more economical, sustainable, and environmentally friendly farming practices (Soliman *et al*., 2024; Gahory *et al*., 2025). These biostimulants may stimulate plant growth, yield, and stress tolerance and serve as safe alternatives to conventional fertilizers (Soliman *et al*., 2024; Gahory *et al*., 2025). Chitosan (CH) is natural, biodegradable biopolymer commonly used in agriculture as a potential biocatalyst agent. It is applied in farming to boost plant defenses against various stresses and significantly improve several physiological traits of crops. Chitosan activates the plant immune system (Katiyar *et al*., 2015). As a result, it promotes the production of several plant enzymes that help protect plants from microbial infections as well as oxidative damage (Faqir *et al*., 2021). Its effectiveness is also attributed to its low cost (Azmana *et al*., 2024). Brassinolides are a class of plant growth regulators which play a vital role in controlling plant growth and development by influencing numerous physiological processes including cell elongation, reproduction, vascular

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differentiation, leaf development, male fertility, and the timing of senescence (Bajguz, 2011; Fariduddin *et al*., 2014; Vardhini and Anjum, 2015). Myo-inositol (MYO) is a biological molecule with nutritional and medicinal benefits (Bonnet *et al*., 2006)., It plays a crucial role in plant growth and development, stress response, cell wall formation, osmotic regulation, and membrane transport (Mechri *et al*., 2015). These functions are likely linked to their involvement in signal transduction pathways related to auxin, phosphatidylinositol, and cell wall processes. Numerous studies indicated that MYO can enhance plant stress tolerance by modifying gene expression (Yildizli *et al*., 2018). Additionally, it may serve as a chelator for certain metal cations, aiding their absorption and transport (Perera *et al*., 2008; Munnik and Vermeer, 2010). Therefore, the aim of this study was to evaluate the effect of chitosan, brassinosteroids and myo-inositol on growth and flowering attributes as well as chemical content of pot marigold plants.bacter-positive.

2. Materials and Methods

A randomized complete block design with a split-plot arrangement in 3 replicates as described by Clewer and Scarisbrick (2001) was implemented over two seasons, 2020/21 and 2021/22. The physical and chemical characteristics of the experimental soil are detailed in Table 1 as described by Black *et al*. (1981).

Table 1 . Physical and chemical analysis of experimental soils

Coarse sand, %	Fine sand, %	Silt, %	Clay, %	PH	EC (dSm)	organic matter %
73.7	16.8	5.8	3.7	8.17	0.44	0.89
Na Ppm	K ppm	Ca ppm	Mgppm	Cl ppm	SO4 ppm	$CaCO_3$ ppm
20.03	10.38	13.33	22.11	64.17	4.81	3.4

Seeds were planted nursery on Oct. 1st, then 45-day-old seedlings were transplanted into rows spaced 60 cm apart, with 40 cm between plants within the row. All plants were fertilized with 300 kg/fed ammonium sulfate (20.6% N), 200 kg/fed calcium superphosphate (15.5% P_2O_5), and 50 kg/fed potassium sulfate (48% K_2O). The N fertilizer was split into 3 equal doses, at 15, 30, and 45 days after transplanting. Potassium fertilizer was applied alongside the 1st N dose, while P fertilizer was incorporated into the soil during land preparation for both seasons. All other agricultural practices were performed according to local farming customs. The main plot consisted of CH at 0.00, 0.20, 0.40 and 0.60 g/l, while the subplots included BR at 0.10 and 0.20 and MYO at 0.25 and 0.50 g/l in addition to the control treatment. Each subplot treatment was assigned to one row containing 6 plants. In total, the experiment involved 20 different treatments.

2.1. Vegetative growth attributes

At the end of the growth season (15th of May), plants were cut just above the soil surface and their fresh weight was estimated, then the plants were drying at 70℃ for two days to assess the dry weight.

2.2. Flowering attributes

During the flowering stage, full bloom flowers were collected every 5 days and total number of flowers was counted. For each cut, the flowers fresh weights per plant was measured directly after cutting, then flowers were air drying under shade (almost one week) to measure total flowers dry weights.

2.3. Chlorophyll content

During flowering and after 2 weeks of the last treatment, chlorophyll content was determined by a portable Minolta chlorophyll meter SPAD-502 (Spectrum Technologies, Inc., Plainfield, IL, U.S.); which has a 0.71 cm^2 measurement area and based on absorbance measurements at 660 and 940 nm (Richardson *et al*., 2002). Thirty separate measurements were made using the fifth-fully developed leaves from the top of 18 plants in each treatment.

2.4. Total sugars content

A simple weight of 1 g oven-dried leaves (dried for 24 hours at 70ºC) of each season were collected together. Anthrone method as described by Yem & Willis (1954) method was used to assess total sugars content

2.5. NPK Herb Percentage

One gram of oven-dried leaves (dried for 24 hours at 70ºC) was ground into a fine powder to determine the N, P, and K%. The sample underwent wet mineralization using 96% sulfuric acid with phosphorus-free hydrogen peroxide (30% W/V) at 300 ºC to measure N (Tel and Hagarty, 1984). Nitrogen was determined using the micro-Kjeldahl digestion technique. Ground samples (one gram) were digested with a mixture of nitric, perchloric, and sulfuric acids (AOAC, 1990) procedures. Phosphorus content was measured calorimetrically using the vanadomolybdate method. Potassium levels were assessed with a flame photometer (AOAC, 1990).

2.5. Statistical Analysis

Analysis of variance (ANOVA) and the LSD at 5% significance were calculated for all recorded data (Clewer and Scarisbrick, 2001) using the MSTAT program (version 4.0), developed in 1986 by the MSTAT development team at Michigan University and the Agricultural University of Norway.

3. Results and Discussion

3.1. Plant fresh weight

The plant fresh weights increased due to CH treatment in both seasons. Untreated plants showed the lowest value (253.34 and 288.39 g/plant in both seasons, respectively), while the highest one (269.12 and 295.06 g/plant in both seasons respectively) was for those treated with the highest CH concentration (Table 2).

Table 2: Effect of chitosan, brassinolide, and myo-inositol on plant fresh weight (g) of *Calendula officinalis* plants during two seasons (2020/2021 and 2021/2022).

BR/MYO* (g/l) (B)		Chitosan (g/l) (A)				
		0.00	0.20	0.40	0.60	Mean (B)
First season						
Control		246.53	255.90	262.69	266.40	257.88
BR	0.10	248.78	258.79	264.52	268.88	260.24
BR	0.20	260.20	255.21	266.69	270.79	263.22
MYO	0.25	255.08	260.29	258.06	269.44	260.72
MYO	0.50	256.11	261.23	260.78	270.09	262.05
Mean (A)		253.34	258.28	262.54	269.12	
LSD 5 %		A: 0.95		B: 0.62		AB: 1.25
Second season						
Control		286.50	288.00	291.21	293.47	289.79
BR	0.10	287.98	290.01	293.53	295.44	291.74
BR	0.20	288.00	290.09	294.08	296.31	292.12
MYO	0.25	289.46	292.86	292.24	294.70	292.31
MYO	0.50	290.03	293.39	292.08	295.42	292.73
Mean (A)		288.39	290.87	292.62	295.06	
LSD 5 %		A: 0.82		B: 0.49		AB: 0.97

BR: Brassinolide and MYO: Myo-inositol

Application of BR/MYO also had a significant impact as the control plants had the lowest fresh weights in both seasons, whereas those treated with 0.20 g/l of BR and 0.50 g/l of MYO exhibited the highest values (263.22 and 292.73 g/plant) in the 1st and 2nd season respectively. Overall, the interaction between the two tested factors showed that the lowest fresh weights (246.53 and 286.50 g/plant in the first and second seasons, respectively) were for untreated plants. Meanwhile those treated with 0.6 g/l of CH + 0.20 g/l of BR had 270.79 and 296.31 g/plant, in both seasons respectively.

3.2. Plant dry weight

The results indicated that the plant dry weights were influenced by all studied factors, showing a similar pattern to that of the fresh weights in both seasons (Table 3).

Table 3. Effect of chitosan, brassinolide, and myo-inositol on plant dry weights (g) of *Calendula officinalis* plants during two seasons (2020/2021 and 2021/2022)

BR/MYO* (g/l) (B)		Chitosan(g/l) (A)				
		0.00	0.20	0.40	0.60	Mean (B)
First season						
Control		48.38	51.43	55.32	59.24	53.74
BR	0.10	52.27	52.61	62.23	64.39	57.88
BR	0.20	53.21	53.82	62.96	65.30	58.82
MYO	0.25	50.16	50.77	59.96	61.53	55.36
MYO	0.50	52.49	51.41	62.50	63.66	57.51
Mean (A)		51.30	52.01	60.59	62.82	
LSD 5 %		A: 1.05		B: 0.74		AB: 1.48
Second season						
Control		47.31	52.32	50.78	51.28	50.99
BR	0.10	47.77	50.70	51.26	52.71	50.61
BR	0.20	48.29	51.38	51.71	53.48	51.21
MYO	0.25	49.61	49.81	50.67	51.87	50.17
MYO	0.50	48.46	50.36	51.27	52.59	50.67
Mean (A)		48.29	50.91	51.14	52.38	
LSD 5 %		A: 0.55		B: 0.37		AB: 0.74

BR: Brassinolide and MYO: Myo-inositol

In general, increasing the concentration of CH led to higher plant dry weights at any level of BR/MYO. The lowest significant dry weight (48.38 and 47.31g/plant in both seasons, respectively) was recorded in untreated plants, while the highest value in the 1st season, with no significant difference between them (65.30 and 64.39 g/plant), were observed in plants treated with 0.60 g/l of CH combined with 0.10 or 0.20 g/l of BR, respectively. Meanwhile the 2nd season these treatments yielded less plant dry weight (53.48 and 52.59 g/plant) nevertheless they still the highest values. These findings suggest that CH, acts as an effective biostimulant for pot marigold plants. Its application has been associated with improved vegetative growth, including notable increases in fresh weight, and dry biomass, compared to untreated plants. These enhancements are attributed to its ability to stimulate nutrient uptake, boost photosynthetic activity, and promote cell division and elongation (Sharif *et al*., 2018). Moreover, CH can affect internal hormone levels, particularly by increasing auxin and gibberellin-like activities, which support shoot elongation and biomass growth (Gornik *et al*., 2008). Similar achievements were found on pot marigold by Akhtar *et al*. (2022). A recent study has demonstrated that MYO can improve vegetative characteristics in pot marigold, including a about 9% increase in biomass. It acts as a precursor for several key molecules involved in cell wall formation, and signal transduction mechanisms (Gillaspy *et al*., 1993). Recent findings revealed that applying 0.2 g/l of BR increased fresh and dry plant weights by 9%. These hormones are crucial for promoting cell elongation, division, and differentiation, which contribute to greater biomass, and root growth (Clouse and Sasse, 1998). In pot marigold, BR treatment may boost vegetative growth by improving photosynthetic efficiency through increased chlorophyll content and enhancing nutrient uptake, as reported by Vardhini and Rao (2003). Previous studies suggested similar responses (Swamy and Rao, 2008, 2009 ; Bakr *et al*., 2024). Exogenous application of MYO has also been found to promote root development and nutrient absorption, which supports overall plant growth (Loewus and Murthy, 2000). Studies on other crops (Wang *et al*., 2025) have highlighted its beneficial effects on growth and productivity, by regulating certain enzymes.

3.3 Number of flowers/plant

The number of flowers/plant was significantly increased following CH application in both seasons as the control plant shad the lowest values (31 and 35 respectively) to reach 44 and 46 for these seasons once plants treated with the highest concentration of CH. In both seasons, plants treated with BR at 0.10 g/l had significantly a smaller number of flowers 33 and 37 compared with the control plants 38 and 41 in both seasons respectively. However, plants treated with the highest concentration of MYO showed a significantly the greatest number (47 in both seasons) (Table 4). The minimum flower number in both seasons respectively (32 and 30 flower/plant) was for plants treated solely with 0.1 g/l BR, although this value did not differ significantly from those treated with 0.2 g/l BR, 0.25 g/l of MYO, or untreated plants. The highest flowers/plant in both seasons (50) was recorded in plants treated with 0.60 g/l of CH + 0.50 g/l of MYO, with no significant difference compared to plants treated with either 0.60 g/l of CH + 0.25 g/l of MYO or 0.40 g/l CH+0.25 g/l of MYO (Table 4).

3.4. Total flower fresh weights/plant

Results for both seasons indicated that CH significantly increased the total fresh weight of flowers. For example, in the 1st season the highest value of 447.5 g/ once plants were treated with 0.60 g/l of CH, while untreated one had the lowest yield (291.5 g). The control plants showed the lowest yield weight (344.5 g/plant), while the highest one was for 0.20 g/l of BR or 0.50 g/l of MYO treatments (478.6 and 459.1 g/plant, respectively). The interaction between the two factors was significant; the lowest and highest weights (180.7 and 512.7g/plant, respectively) were for untreated plants and 0.6 g/l of CH + 0.20 g/l of BR (Table 5).

Table 4. Effect of chitosan, brassinolide, and myo-inositol on number of flowers of *Calendula officinalis* plants during two seasons (2020/2021 and 2021/2022)

BR/MYO* (g/l) (B)		Chitosan (g/l) (A)				
		0.00	0.20	0.40	0.60	Mean (B)
First season						
Control		32	38	40	45	38
BR	0.10	28	32	37	38	33
BR	0.20	32	35	40	43	37
MYO	0.25	30	37	44	47	39
MYO	0.50	36	39	47	50	43
Mean (A)		31	36	41	44	
LSD 5 %		A: 3		B: 1		AB:4
Second season						
Control		30	41	45	48	41
BR	0.10	34	36	39	41	37
BR	0.20	39	42	42	44	41
MYO	0.25	34	42	39	43	39
MYO	0.50	40	47	43	56	47
Mean (A)		35	41	41	46	
LSD 5 %		A: 2		B: 1		AB: 3

BR: Brassinolide and MYO: Myo-inositol

Similar results were observed in the 2nd seasons. The total dry weight of flowers followed a similar pattern across both seasons, mirroring the trends seen in fresh flower weights (Table 6). Overall, the results demonstrated that increasing CH levels enhanced total flower dry weight regardless of the BR/MYO concentration. Similarly, raising BR/MYO concentrations boosted dry flower yield at any CH concentration. Table 6 also shows that untreated plants had the lowest yield (47.36 and 43.50 g/plant, in both seasons respectively), which increased to 144.50 and 135.70 g/plant respectively for plants treated with 0.6 g/l of CH + 0.50 g/l of MYO. These findings suggest that CH may be an effective natural growth enhancer, contributing not only to vegetative growth but also aesthetic value based on pot marigold based on flower number, and their higher fresh and dry weights. Compared with the control plants flower number, total flower fresh and dry weights were increased by 42, 53 and

Table 5. Effect of chitosan, brassinolide, and myo-inositol on flower fresh weight (g) of *Calendula officinalis* plants during two seasons (2020/2021 and 2021/2022)

BR/MYO* (g/l) (B)		Chitosan (g/l) (A)				
		0.00	0.20	0.40	0.60	Mean (B)
First season						
Control		180.7	316.6	439.5	441.2	344.5
BR	0.10	276.7	420.3	337.4	339.6	343.5
BR	0.20	395.7	495.3	510.7	512.7	478.6
MYO	0.25	261.9	413.5	437.2	439.6	388.1
MYO	0.50	342.5	486.4	502.5	504.8	459.1
Mean (A)		291.5	426.4	445.4	447.5	
LSD 5 %		A: 19.1		B: 16.4		AB: 32.5
Second season						
Control		204.6	377.5	336.7	338.5	314.3
BR	0.10	241.6	430.8	525.6	527.8	431.5
BR	0.20	312.4	408.6	528.5	529.4	444.7
MYO	0.25	297.5	478.8	538.7	540.6	463.9
MYO	0.50	346.4	556.3	487.6	489.6	469.9
Mean (A)		280.5	450.4	483.4	486.2	
LSD 5 %		A: 20.4		B: 18.5		AB: 39.3

BR: Brassinolide and MYO: Myo-inositol

150% respectively once plants were treated with 0.6 g/l of CH. Chitosan has gained attention as a natural biostimulant capable of enhancing floral traits of ornamental plants (Salachna and Zawadzińska, 2014; Akhtar *et al*., 2022). This effect is due to its role in stimulating physiological processes such as nutrient assimilation, photosynthesis, and hormonal regulation (Gornik et al., 2008; Sharif *et al*., 2018). Vardhini and Rao (2003) and Bajguz and Hayat (2009) thought that the promotion effect of BR on flower traits may be due to stimulating the biosynthesis of floral hormones and improving carbohydrate transport to reproductive organs. That has been confirmed in many crops (Sagar *et al*., 2018; Sadiq, 2023). Data showed that MYO played a vital role in regulating pot marigold plant reproductive development, including flowering number and their fresh and dry weights which increased for plants treated with 0.50 g/l of MYO by 13, 33 and 56% respectively over the control plants. This effect may be due to its involvement in cell signaling pathways and its function as a precursor for molecules like phosphoinositide, which influence hormonal balance and floral induction (Loewus and Murthy, 2000).

It also may support carbohydrate metabolism and nutrient mobilization (Gillaspy *et al*., 1993).

Table 6. Effect of chitosan, brassinolide, and myo-inositol on total flower dry weight (g/plant) of *Calendula officinalis* plants during two seasons (2020/2021 and 2021/2022)

BR/MYO* (g/l) (B)		Chitosan (g/l) (A) 0.00	0.20	0.40	0.60	Mean (A)
First season						
Control		47.36	59.66	68.8	79.20	63.75
BR	0.10	58.52	72.96	82.14	85.88	74.87
BR	0.20	84.16	94.85	112.40	124.27	103.92
MYO	0.25	47.10	77.70	93.72	106.22	81.18
MYO	0.50	88.56	105.60	133.95	144.50	118.15
Mean (A)		65.14	82.15	98.20	108.01	
LSD 5 %		A: 4.78		B: 5.76		AB: 11.52
Second season						
Control		43.5	60.27	69.75	80.16	63.42
BR	0.10	78.54	95.76	104.13	96.35	93.69
BR	0.20	104.13	119.7	118.86	128.92	117.90
MYO	0.25	46.92	89.04	99.45	101.05	84.11
MYO	0.50	100.4	130.19	124.27	135.7	122.64
Mean (A)		74.69	98.99	103.29	108.43	
LSD 5 %		A: 3.94		B: 5.85		AB: 11.07

BR: Brassinolide and MYO: Myo-inositol

3.5. Chlorophyll content

As the chlorophyll content of pot marigold was similar in both investigated seasons only data of the 1st one will be presented. Plant treated with 0.2 and 0.4 g/l of CH had significantly the highest chlorophyll content (about 44 SAPD unit). On the other hand, no significant variations were assessed between plants treated with any concentration of BR or MYO. However, all of them helped treated plants to have significantly higher chlorophyll content. A significant interaction between the two investigated factors was observed. The highest value (47.49) was for plants treated with 0.2 g/l of CH + 0.2 g/l of BR (Table 7). The same trend was observed in the 2nd season. The enhancement of plant chlorophyll content due to CH of many plants has been reported by Salachna and Zawadzińska (2014) and Akhtar *et al*. (2024). Loewus and Murthy (2000) suggest that MYO improved photosynthetic capacity by supporting chloroplast function and membrane integrity (Fatima *et al*., 2024). The crucial role of BR in enhancing chlorophyll content of pot marigold (Table 7) agreed with Bakr *et al*. (2024) and Ali *et al*. (2008).

That could improve photosynthetic capacity, stimulate chloroplast development and regulate genes involved in chlorophyll biosynthesis, leading to increased chlorophyll accumulation (Bajguz and Hayat, 2009).

Table 7. Effect of chitosan, brassinolide, and myo-inositol on chlorophyll content (SAPD unit) of *Calendula officinalis* plants during two seasons (2020/2021 and 2021/2022)

BR/MYO* (g/l) (B)		Chitosan(g/l) (A)				
		0.00	0.20	0.40	0.60	Mean (B)
First season						
Control		40.17	44.43	44.96	44.87	43.60
BR	0.10	42.28	46.79	47.42	44.19	45.17
	0.20	43.66	47.49	46.24	44.11	45.37
MYO	0.25	44.08	46.00	45.31	46.38	45.44
	0.50	45.40	45.49	46.87	45.10	45.71
Mean (A)		43.11	46.04	46.16	44.93	
LSD 5 %		A: 1.32		B: 1.20		AB: 2.40
Second season						
Control		39.41	42.82	43.93	42.30	42.11
BR	0.1	41.40	42.07	44.04	42.71	42.55
	0.2	42.41	42.27	42.01	43.63	42.58
MYO	0.25	42.30	41.36	42.80	43.04	42.37
	0.50	43.20	43.23	43.02	44.42	43.46
Mean (A)		41.74	42.35	43.16	43.22	
LSD 5 %		A: 2.77		B: 1.63		AB: 3.27

BR: Brassinolide and MYO: Myo-inositol.

3.6. Total sugar content

Significant increases on plant content of total sugar were observed by increasing CH under any concentration of BR/MYO. The highest content of total sugar (24.9 mg/g) was for the plant treated with 0.60 g/l of CH (Table 8). Similar to that increasing the concentration of the 2nd factor gradually and significantly increased the total sugar content achieving the highest value (24.9 and 23.7 of mg/g) for the highest concentration of MYO and BR ,respectively (Table 8). Overall, the significant interaction between the two factors showed that non-treated plants had the lowest content (17.6 mg/g) whereas the highest value was for 0.6 g/l of CH +0.50 g/l of MYO treatment with no significant difference between these of plants treated with the same concentration of CH + 0.25 g/l of MYO.

Table 8. Effect of chitosan, brassinolide, and myo-inositol on total sugars and N% of *Calendula officinalis* plants (2020/2021 and 2021/2022)

BR/MYO* (g/l) (B)		Chitosan (g/l) (A)				
		0.00	0.20	0.40	0.60	Mean (B)
Total sugars (%)						
Control		17.6	19.7	21.6	22.5	20.3
BR	0.10	19.6	21.3	22.4	23.7	21.7
BR	0.20	21.7	23.2	24.4	25.8	23.7
MYO	0.25	19.2	24.7	25.7	26.6	24.1
MYO	0.50	21.0	25.6	26.0	27.2	24.9
Mean (A)		19.8	22.9	24.0	25.1	
LSD 5 %		A: 0.2		B: 0.6		AB: 1.3
N%						
Control		2.12	2.66	2.57	2.62	2.49
BR	0.10	2.65	2.04	2.61	2.91	2.55
BR	0.20	2.38	2.52	2.69	2.45	2.51
MYO	0.25	2.21	2.82	2.72	2.49	2.56
MYO	0.50	2.33	2.45	2.49	2.72	2.50
Mean (A)		2.34	2.50	2.62	2.64	
LSD 5 %		A: 0.15		B: 0.11		AB: 0.23

BR: Brassinolide and MYO: Myo-inositol

Chitosan has shown promising effects in enhancing carbohydrate metabolism of pot marigold plants. Mondal *et al*. (2012) and Sharif *et al*. (2018) suggested that CH application has been associated with improved physiological processes that ultimately boost plant productivity and quality. The stimulation effect of BR on photosynthetic activity and sugar biosynthesis, leading to an increase in total sugar content which is crucial for plant energy balance. BR improves the efficiency of carbon fixation and promote enzyme activities related to sugar, metabolism, transport and storage (Bajguz and Hayat 2009; Siddiqui *et al*., 2019). Myo-inositol contributes significantly to total sugar content of *C. officinalis.* That could be due to stimulating photosynthesis, improving chlorophyll content and function, and supporting sugar transport and storage (Loewus and Murthy, 2000)

3.7. Nitrogen, P, and K percentage

Tables 8 and 9 demonstrate that both factors studied significantly affect the dry herb N, P, and K%, additionally, results revealed a significant interaction between these two factors. The percentages of N, P, and K were gradually increased with CH concentrations. Untreated plants showed the lowest 2.34%, 0.11%, and 3.27%, respectively. While plants treated with the highest CH concentration exhibited the highest contents (2.64, 0.15, and 3.46%, respectively).

Table 9. Effect of chitosan, brassinolide, and myo-inositol on the P and K % of *Calendula officinalis* plants

BR/MYO* (g/l) (B)		Chitosan (g/l) (A) 0.00	0.20	0.40	0.60	Mean (B)
Control		0.09	0.11	0.12	0.13	0.11
BR	0.10	0.12	0.13	0.14	0.15	0.13
	0.20	0.12	0.13	0.15	0.17	0.14
MYO	0.25	0.12	0.13	0.14	0.15	0.13
	0.50	0.13	0.14	0.15	0.16	0.14
Mean (A)		0.11	0.12	0.14	0.15	
LSD 5 %		A: 0.02		B: 0.04	AB: 0.08	
K%						
Control		2.72	2.73	3.35	3.39	3.05
BR	0.10	3.15	3.17	3.49	3.31	3.28
	0.20	3.05	3.55	3.44	3.33	3.34
MYO	0.25	3.11	3.39	3.85	3.72	3.52
	0.50	3.67	3.77	3.72	3.55	3.68
Mean (A)		3.14	3.32	3.57	3.46	
LSD 5 %		A: 0.15		B: 0.17		AB: 0.33

BR: Brassinolide and MYO: Myo-inositol

Plants that did not receive BR or MYO treatments had the lowest NPK percentages (2.49%, 0.11%, and 3.14%, respectively). Interestingly, plants treated with the lowest concentrations of BR or MYO had significantly higher N content, whereas P and K showed the opposite trend. Overall, the interaction indicated that the lowest NPK percentages (2.12%, 0.09%, and 2.72%) were for plants untreated with either substance. In contrast, the highest percentages (2.82%, 0.17%, and 3.85%) were observed in plants treated with 0.4 g/l of CH + 0.20 g/l of BR, 0.6 0g/l of CH + 0.20 g/l of BR, and 0.40 g/l of CH + 0.25 g/l of MYO. The notable increase in NPK% following CH application support results of Ohta *et al*. (2004) and Sami *et al*. (2022). Chitosan enhances nutrient uptake mainly by promoting root growth and membrane permeability, which facilitates better nutrient absorption (Gornik *et al*., 2008). The resulting rise in NPK levels contributes to improved vegetative growth and flower production (Kumaraswamy *et al*., 2008). Brassinosteroids (BR) play a crucial role in boosting uptake and assimilation of essential macronutrients like N, P, and K in plants. this might be achieved root development, increasing membrane permeability, and activating nutrient transporters (Bajguz and Hayat, 2009), as well as by modifying root structure and ion transport mechanisms (Ali *et al*., 2008). This leads to better nutritional status, supporting enhanced vegetative growth and flowering. Foliar application of MYO also increased NPK%, possibly by fostering root development and enhancing the plant's capacity to absorb and assimilate soil nutrients. Loewus and Murthy (2000) suggested that MYO is involved in membrane formation and signaling pathways that regulate ion transport and nutrient balance (Gillaspy *et al*., 1993). Studies in other species support the beneficial effects of MYO (Liu *et al*., 2025; Fatima *et al*., 2024). These findings suggest that CH, BR and MYO are promising eco-friendly option to boost both the aesthetic and commercial value of pot marigold flowers. Therefor the study suggested that pot marigold plants should be treated with CH at 0.60 g/l in addition to BR at 0.20 g/l to significantly achieved the highest flower fresh weight (512.7 g/plant). However, the highest flower dry weight (144.50 g/plant) resulted in plants treated with CH at 0.60 g/l in addition to 0.50 g/l of MYO.

4. Conclusion

From the obtained results of the present study it could be recommended that spraying micronutrients and amino acids for obtaining the best fruit quality with reasonable yield and healthy fruits. For decreasing the fruit cracking and increase the fruit firmness it could be recommended that to spray the fruit with calcium and potassium.

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Not applicable

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The authors disclosed no conflict of interest.

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