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Research article

Antioxidant capacity and pigment synthesis of marigold (*Calendula officinalis* L.) as influenced by benzyladenine and epibrassinolide

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Abstract: The impact of various rates of benzyladenine (BA) and epibrassinolide (EPL) was explored on antioxidant capacity and pigment biosynthesis of marigold in a factorial experiment on the basis of a Randomized Complete Block Design with two factors including benzyladenine (BA) and epibrassinolide (EPL), both at four rates of 0, 1, 5 and 10 mg/L with three replications. Means comparison revealed that the highest antioxidant capacity was devoted to the treatment of "0 mg/L BA × 5 mg/L EPL", the highest flavonoid concentration at three wavelengths of 270, 300 and 330 nm was observed in the treatment of "10 mg/L BA × 10 mg/L EPL", and the highest anthocyanin was obtained from the treatment of "1 mg/L BA × 0 mg/L EPL". According to results, it can be said that the application of BA and EPL significantly influenced catalase, peroxidase, phenol, chlorophyll *a*, anthocyanin, and carotenoid in marigold plants.

Keywords: antioxidant capacity; epibrassinolide; flavonoid; marigold; pigment

1. Introduction

Marigold (*Calendula officinalis* L.) is one of the widely used medicinal herbs and a flower commonly employed in the urban landscapes and as a pot flower [1]. At present, medications with plant origins compose one-third of all medications consumed by people and this amount is undoubtedly increasing [2]. It has been shown that cytokinin can improve the production and growth of suckers [3]. Foliar application of cytokinin on *Hemerocallis itrina* showed that this group of

regulators increase the size of suckers by postponing cell division and improve their number by stimulating the growth of lateral buds [4]. Exogenous application of cytokinin entailed the strongest impact on carnation longevity. Zeatin, trans-zeatin and isopentenyladenine all retarded the aging of roses [5]. The treatment of marigold with benzyladenine was associated with the increase in plant height, the number of branches, plant fresh weight, and leaf fresh and dry weight as compared to control [6].

Brassinosteroids are a class of polyhydroxylated steroidal plant hormones [7]. They are the derivates of 5-alpha-cholestane and are synthesized in plants through mevalonate pathway. These compounds occur almost in all parts of the plants, but they are mostly observed in reproductive parts [8,9]. These compounds exert diverse physiological influences on the growth and development of plants. They induce growth and cell division, impact seed germination, and influence electrical characteristics, permeability, structure, stability, and activity of membrane enzymes [7,9]. In addition, brassinosteroids change gene expression and the metabolism and biosynthesis of nucleic acids and proteins at the molecular level [10]. They improve drought tolerance in plants by enhancing the amount of proline and protein in the affected plants [8]. As steroid plant hormones, brassinosteroids play a crucial role in plant growth and development, e.g. pollen tube growth, stem elongation, leaf epinasty, ethylene biosynthesis, and fruit development and maturity [11,12]. They increase photosynthesis and photosynthesizing pigments production in plants [13]. Swamy and Rao [14] showed that the improved growth of geranium (*Pelargonium graveolens*) due to the application of 24-epibrassinolide resulted in higher leaf photosynthesis rate and consequently, higher biomass accumulation in the shoot. Zhang et al. [15] reported that 24-epibrassinolide treatment influenced some antioxidant enzymes resulting in the mitigation of oxidative effects. Given the medicinal and ornamental importance of marigold, the present study aimed to explore the impact of benzyladenine and epibrassinolide on the biosynthesis of pigments in marigolds and the increase in the compounds of marigolds.

2. Materials and methods

The present study was a factorial experiment with two factors arranged in an RCBD with 16 treatments, three replications, and two samples per plot. Both benzyladenine (BA) and epibrassinolide (EPL) were applied at four levels (0, 1, 5 and 10 mg/L). The experimental pots amounted to 96 pots. The plants were planted in a substrate composed of 50% cocopeat, 25% garden soil, and 25% perlite. Hormones were applied at three stages once two weeks. The recorded traits included chlorophyll *a* and *b*, total chlorophyll, anthocyanin, phenol, flavonoid, catalase and peroxidase activity, antioxidant property, and carotenoid content.

To measure antioxidant capacity, 1 g of the plant was folded in a piece of foil and was kept in liquefied nitrogen for 2–3 minutes. Then, it was ground with 10 mL 85% methanol. The samples were placed at room temperature for one hour. They were infiltrated and centrifuged for five minutes. Then, 150 mL was taken and was added with 850 μL DPPH. The resulting solution was shaken and was kept in darkness for 20 minutes. After blank placement and instrument reset, first only DPPH was poured into coquette and it was read. Then, the sample was read by a spectrophotometer at 517 nm. The antioxidant capacity of the samples was calculated as DPPH inhibition percentage according to Eq 1 [16,17]:

$$\%\ DPPH_{sc} = \frac{A_{cont} - A_{samp}}{A_{cont}} \times 100 \quad (1)$$

Where, % $DPPH_{sc}$ = inhibition percentage, A_{samp} = absorption rate (sample + DPPH), and A_{cont} = DPPH absorption rate.

To measure chlorophyll content, 0.5 g of the sample was weighed and was pounded in a Chinese mortar with 50 mL 80% acetone. The extract was infiltrated and adjusted to 50 mL and was poured into a coquette. Chlorophyll content was read at 643 and 660 nm by a spectrophotometer. Then, they (A) were put in the following formula to derive the amount of chlorophyll *a*, chlorophyll *b*, and total chlorophyll according to Eqs 2, 3 and 4, respectively [18].

$$\text{Total chlorophyll (mg/ml)} = 7.12\,(A_{660}) + 16.8\,(A_{643}) \quad (2)$$

$$\text{Chlorophyll a (mg/ml)} = 9.93\,(A_{660}) - 0.777\,(A_{643}) \quad (3)$$

$$\text{Chlorophyll b (mg/ml)} = 71.6\,(A_{643}) - 2.81\,(A_{660}) \quad (4)$$

To measure carotenoid content, 0.5 g of samples was weighed and was pounded in a Chinese mortar with 50 mL 80% acetone. The extract was infiltrated and poured into a coquette after being adjusted to 50 mL. They were read at 645, 663, and 660 nm. The results (A) were put in the Eq 5 to yield carotenoid contents [1].

$$\text{Carotenoid content} = 4.69\,(A_{660}) - 0.268\,(A_{645}) + 8.02\,(A_{663}) \quad (5)$$

Anthocyanin content was estimated in 0.5 g of each sample pounded in a Chinese mortar with 50 mL ethanol-hydrochloric acid (85% ethanol 95% + 15% hydrochloric acid). It was infiltrated, adjusted to 50 cc, and poured into coquettes. They were placed in a refrigerator at 4 ℃ for 24 hours followed by keeping in darkness for two hours. To determine anthocyanin content, the extracts were read at 535 nm by a spectrophotometer and the Eqs 6 and 7 were applied [18].

$$\text{Total sample absorption} = \frac{e \times b \times c}{d \times a} \times 100 \quad (6)$$

Where, *a* = sample size (0.5 g), *b* = the volume taken for measurement (5 mL), *c* = total volume (50 mL), *d* = fraction taken for 0.1 sample, and *e* = the figure read at 535 nm.

$$\text{Total anthocyanin content of sample (\%)} = \frac{\text{Sample total absorption}}{98.2} \quad (7)$$

One g of fresh leaves was rasped in 10 mL methanol for two minutes. The solution was infiltrated. Then, 0.5 mL of diluted extract (1:10 g/L) was added with 5 mL diluted folin-ciocalteu and then with 4 mL sodium carbonate solution (7.5% v/v). The samples were kept at laboratory temperature for 15 minutes and its absorption was read at 765 nm with a spectrophotometer.

The standard curve was prepared on the basis of gallic acid concentrations of 0, 10, 20, 30, 40 and 50 mg/L. Then, the line equation was obtained to be $y = bx + a$. The read absorptions were put for *y* to yield the concentration *x* [19,20].

To measure peroxidase (POD) activity, the relevant extract was prepared and then, OD variation was read at 430 nm with a spectrophotometer once every 30 seconds for two minutes [21]. The procedure to measure catalase (CAT) activity was as below [22]:

0.01 mol phosphate buffer (pH = 7), 0.5 mL 0.2 mol H_2O_2, and 2 mL acid reagent (dichromate/citric acid mixture) were added to 1 g of plant tissue that had been ground in 4 mL ethanol. The absorption was read at 610 nm with a spectrophotometer.

Data were statistically analyzed in MSTATC Software Package; the means were compared by LSD test.

3. Results and discussion

According to the results of ANOVA (Table 1), the simple effects of BA and EPL and their interaction was not significant for chlorophyll *b* and total chlorophyll. Also, they implied the insignificant impact of BA on chlorophyll *a*. However, EPL and its interaction with BA changed chlorophyll *a* significantly ($p < 0.05$). We obtained the highest chlorophyll *a* from the plants treated with 5 mg/L EPL and the lowest one from those not treated with EPL (Table 2). A study on *Eriobotrya japonica* confirmed the desirable effect of EPL on chlorophyll content. The application of 24-EPL improved stem dry weight and leaf area versus control. The application of EPL improved plant growth by alleviating the harmful impacts of salinity [23].

The highest chlorophyll *a* content was observed in plants treated with 0 mg/L BA × 5 mg/L EPL and the lowest one was observed in those treated with 0 mg/L BA × 0 mg/L EPL (Table 3). The interaction between BA and EPL was not significant for chlorophyll *b* and total chlorophyll. A study on *Zantedeschia aethiopica* "Childsiana" revealed that the increase in BA rate improved chlorophyll production [24].

ANOVA (Table 1) indicated that BA and EPL did not influence carotenoid content significantly but their interaction was significant ($p < 0.05$). The highest carotenoid content was obtained when the plants were treated with 1 mg/l BA × 5 mg/l EPL and the lowest one was related to those treated with 0 mg/l BA × 0 mg/l EPL and 10 mg/l BA × 5 mg/l EPL, showing an insignificant difference with 0 mg/l BA × 10 mg/l EPL (Table 3). Sardoei Kara et al. [25] reported that marigold seed priming and foliar application of 24-EPL improved growth parameters and photosynthesis pigments, which is consistent with our finding that 24-EPL influenced some growth parameters significantly.

ANOVA (Table 1) revealed that the simple effects of BA and EPL and their interaction were significant for anthocyanin. Plants treated with 1 mg/l BA showed the highest anthocyanin content and the lowest one was related to those treated with 0 mg/l BA or 5 mg/l BA (Table 4). This implies that the application of BA had a favorable impact on anthocyanin content of marigold plants, but this effect was not linear. Petridou et al. [26] reported that cytokinin positively impacted petal anthocyanin in chrysanthemum. In a study on cut Lisianthus, Karimi and Hassanpour Asil [27] found that short-term treatment with BA was effective on the maintenance of petal anthocyanin. They stated that BA postponed ethylene production and extended vase life by four days.

Table 1. Analysis of variance of the impact of experimental factors on the recorded traits.

S.O.V.	df	Means of squares									
		Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Phenol	Flavonoid (330 nm)	Flavonoid (300 nm)	Flavonoid (270 nm)	Antioxidant property	Catalase	Peroxidase
BA	3	0.33 ns	0.24 ns	0.81 ns	4.24**	2.83*	4.06 ns	9.19*	141.27*	0.01*	0.26**
EPL	3	1.38*	0.23 ns	1.81 ns	15.8**	10.67**	15.41**	10.25*	289.48**	0.01*	0.011 ns
BA × EPL	9	0.65*	0.12 ns	1.19 ns	4.05**	7.20**	15.33**	12.03**	412.43**	0.01*	0.24*
Error	30	0.3	0.12	0.68	0.43	0.87	2.22	2.76	46.58	0.005	0.05
C.V. (%)		11.58	15.34	11.73	15.7	13.56	20.46	16.03	8.97	15.31	27.43

**: Significant at $p < 0.01$; *: Significant at $p < 0.05$ level; ns: Non-significant.

Table 2. Means comparison for the simple effect of epibrassinolide (EPL) on the measured traits.

Treatments	Chlorophyll *a* (mg/L)	Anthocyanin (mg/100 g)	Phenol (mg/g)	Flavonoid at 270 nm (μmol/g FW)	Flavonoid at 300 nm (μmol/g FW)	Flavonoid at 330 nm (μmol/g FW)	Antioxidant property (% inhibition)	Catalase enzyme $(UNIT)^2$
0 mg/l	4.38 c	21.38 a	3.05 c	10.64 a	7.97 a	6.84 b	72.96 b	0.41 b
1 mg/l	4.63 bc	15.90 b	5.77 a	9.02 b	5.68 b	5.58 c	80.67 a	0.44 ab
5 mg/l	5.09 a	12.27 c	3.79 b	10.74 a	7.30 a	7.64 a	79.85 a	0.41 b
10 mg/l	4.87 ab	18.87 a	4.17 b	11.10 a	8.19 a	7.51 ab	70.85 b	0.48 a

Table 3. Means comparison for interactions of experimental hormones for the measured traits.

Treatments	Chlorophyll *a* (mg/L)	Carotenoid (mg/L)	Anthocyanin (mg/100g)	Phenol (mg GAE/ g DW)	Flavonoid (µmol/g FW)			Antioxidant property (% inhibition)	Catalase	Peroxidase
					270 nm	300 nm	330 nm			
0 mg/L BA ×0 mg/L EPL	4.03 c	1.05 d	8.33 f	3.12 eh	8.53 cde	5.85 de	5.64 eh	54.73 g	0.33 d	0.98 abc
0 mg/L BA ×1 mg/L EPL	4.64 bc	2.50 bcd	19.58 bc	5.64 b	6.81 e	4.35 e	4.66 h	71.17 def	0.37 cd	0.51 f
0 mg/L BA ×5 mg/L EPL	6.17 a	3.09 ad	12.50 ef	2.81 gh	1.48 ab	9.50 a	7.99 abc	88.03 a	0.50 abc	0.94 ae
0 mg/L BA ×10 mg/L EPL	5.10 b	1.37 d	21.97 b	3.97 def	10.90 abc	8.75 abc	7.12 cde	82.57 abc	0.44 ad	0.95 ad
1 mg/L BA ×0 mg/L EPL	4.67 bc	1.65 cd	34.14 a	2.31 h	10.55 bc	6.37 cde	6.30 def	80.95 ad	0.50 ab	1.10 abc
1 mg/L BA ×1 mg/L EPL	4.45 bc	2.77 bcd	10.32 ef	3.81 dg	8.54 cde	6.10 de	4.93 fgh	83.13 abc	0.56 a	1.22 ab
1 mg/L BA ×5 mg/L EPL	4.88 bc	5.25 a	13.17 ef	4.23 cde	8.60 cde	6.32 cde	8.84 ab	75.75 be	0.43 bcd	0.83 bf
1 mg/L BA ×10 mg/L EPL	4.36 bc	2.34 bcd	19.70 bc	3.73 dg	12.40 ab	9.09 ab	9.00 a	62.37 fg	0.47 abc	0.54 f
5 mg/L BA ×0 mg/L EPL	4.54 bc	1.67 cd	23.89	3.01 fgh	12.07 ab	10.22 a	8.83 ab	82.75 abc	0.35 cd	0.47 f
5 mg/L BA ×1 mg/L EPL	4.38 bc	3.84 ab	14.31 cde	8.54 a	9.78 bcd	6.93 bcd	6.50 cde	85.42 ab	0.39 bcd	1.05 abc
5 mg/L BA ×5 mg/L EPL	5.00 b	2.05 bcd	11.80 ef	3.54 dg	11.23 abc	6.48 cde	6.42 def	69.47 ef	0.39 bcd	0.58 def
5 mg/L BA ×10 mg/L EPL	5.02 b	2.19 bcd	13.24 def	3.81 dg	7.50 de	4.04 e	4.72 gh	56.67 g	0.56 a	0.55 ef
10 mg/L BA ×0 mg/L EPL	4.27 bc	2.38 bcd	19.13 bcd	3.78dg	11.41 ab	9.45 a	66.00 cde	73.38 cf	0.46 abc	0.96 ad
10 mg/L BA ×mg/L EPL	5.11 b	1.52 cd	19.41 bc	5.09 bc	10.95 abc	5.36 de	6.24 dg	82.95 abc	0.45 abc	0.80 cf
10 mg/L BA ×5 mg/L EPL	4.68 bc	1.05 d	11.60 ef	4.61 bcd	10.66 bc	6.92 bcd	7.33 bcd	76.17 ab	0.36 cd	1.00 abc
10 mg/L BA ×10 mg/L EPL	4.96 b	3.64 abc	20.58 b	5.19 bc	13.60 a	10.88 a	9.21 a	81.81 ad	0.45 abc	1.29 a

Similar letter(s) in each column shows insignificant differences. Phenol is expressed in terms of mg gallic acid per g DM, flavonoid in terms of µmol/g FW, and catalase and peroxidase in terms of µmol consumed H_2O_2 per min per mg protein.

Table 4. Means comparison for simple effects of benzyladenine (BA) on the measured traits.

Treatments	Anthocyanin (mg/100g)	Phenol (mg/g)	Flavonoid (330 nm)	Flavonoid (270 nm)	Antioxidant	Peroxidase	Catalase (Unit)[1]
0 mg/L	15.59 b	3.88 b	6.35 b	9.68 b	74.12 b	0.84 ab	0.40 b
1 mg/L	19.33 a	3.52 b	7.27 a	10.02 b	75.55 ab	0.92 a	0.49 a
5 mg/L	15.81 b	4.73 a	6.62 ab	10.15 b	73.58 b	0.66 b	0.42 b
10 mg/L	17.68 ab	4.66 a	7.34 a	11.66 a	81.08 a	1.01 a	0.43 b

Similar letter(s) in each column show insignificant difference. Catalase and peroxidase enzymes are expressed in terms of μmol consumed H_2O_2 per min per mg protein.

The highest anthocyanin contents of 21.38 and 18.87 mg/100 g were obtained from the plants treated with 0 and 10 mg/L EPL, respectively. The lowest one was extracted under 5 mg/L EPL (Table 2). A study on excised red cabbage showed that the application of 0.1 and 10 mmol EPL enhanced anthocyanin content at 21 and 52%, respectively [28]. The interaction "1 mg/L BA × 0 mg/L EPL" was associated with the highest anthocyanin content and the interaction "0 mg/L BA × 0 mg/L EPL" with the lowest one. According to Petridou et al. [26], the treatment of chrysanthemum with cytokinin influenced petal anthocyanin positively.

Analysis of variance (Table 1) indicated that the simple effects of BA and EPL and their interactions were significant for phenol index ($p < 0.01$). Plants treated with 5 and 10 mg/L BA exhibited the highest phenol contents and those treated with 1 mg/L BA had the lowest one, showing no significant difference with those not treated with BA (Table 4). Karimi Qale'ehTaki [29] observed the highest phenol content at BA rate of 1 mg/L. The highest phenol content was obtained from the plants treated with 1 mg/L EPL and the lowest one from those that did not receive EPL (Table 2). The higher EPL rate that was used to treat maize plants resulted in the decrease in phenolic compounds [30]. Similar results have been reported for cucumber [31]. In our experiment, the application of 1 mg/L EPL increased phenol content as compared to control, but as EPL rate was increased, phenol content showed a descending trend.

The highest phenol content was obtained from the interaction "5 mg/L BA × 1 mg/L EPL" and the lowest one was obtained under "1 mg/L BA × 0 mg/L EPL" (Table 3). It has been reported that 3 and 6 μmol brassinosteroid increased total phenol content of the tomato fruits significantly, but they did not differ significantly with one another ($p < 0.05$) [32].

The simple effect of BA, EPL and their interactions were significant for flavonoid at 270 nm. According to Table 1, the simple effect of BA was insignificant on flavonoid at 300 nm, whereas the simple effect of EPL and its interaction with BA was significant for it ($p < 0.01$). Table 1 depicts that flavonoid at 330 nm was significantly influenced by BA ($p < 0.05$) and by EPL and BA × EPL ($p < 0.01$). The highest flavonoid at 270 nm was obtained from plants treated with 10 mg/L BA. No BA application exhibited the lowest flavonoid (Table 4). BA did not change flavonoid at 300 nm significantly. The highest flavonoid at 330 nm was observed in plants treated with 10 mg/L BA and the lowest one was related to no BA treatment (Table 4). The results revealed that the highest flavonoid at 270 and 300 nm was related to plants treated with 10 mg/L EPL. But, they did not differ significantly with that obtained from plants treated with 5 or 0 mg/L EPL. The lowest one was observed in plants treated with 1 mg/L EPL. Flavonoid at 330 nm was the maximum in plants treated with 5 mg/L EPL and was the minimum in those treated with 1 mg/L EPL (Table 2). This implies that EPL changes flavonoid favorably at all three wavelengths. Accordingly, EPL at higher rates was found to be effective on flavonoid content of marigolds.

The highest flavonoid at 270 nm was obtained from "10 mg/L BA × 10 mg/L EPL" and the lowest one from "0 mg/L BA × 1 mg/L EPL". The highest flavonoid at 300 nm was observed in plants treated with "10 mg/L BA × 10 mg/L EPL". It did not differ significantly from "5 mg/L BA × 0 mg/L EPL", "0 mg/L BA × 5 mg/L EPL" and "10 mg/L BA × 0 mg/L EPL". The lowest one was related to "0 mg/L BA × 1 mg/L EPL", showing insignificant difference with "5 mg/L BA × 10 mg/L EPL". The highest flavonoid contents at 330 nm (9.21 and 9.00 μmol/g FW, respectively) were found in plants treated with "10 mg/L BA × 10 mg/L EPL" and "1 mg/L BA × 10 mg/L EPL". The lowest one was related to the treatment of "0 mg/L BA × 1 mg/L EPL" (Table 3). The highest flavonoid concentrations at 270, 300 and 330 nm were produced at BA rate of 1 mg/L in *Lilium ledebourii* "Bioss" [29].

Results (Table 1) indicated that the simple effects of BA and EPL and their interactions were significant for antioxidant property ($p < 0.05$). The highest antioxidant property with 81.08% inhibition was observed in plants treated with 10 mg/L BA and the lowest one was obtained when no BA was applied (Table 4). This shows that as BA rate is increased, antioxidant property is linearly strengthened. The highest antioxidant property was associated with plants treated with 1 mg/L and 5 mg/L EPL. The lowest one was related to plants treated with 10 mg/L EPL, showing insignificant differences with no EPL application (Table 2).

Among interactions, "0 mg/L BA × 5 mg/L EPL" yielded the highest antioxidant property and "0 mg/L BA × 0 mg/L EPL" resulted in the lowest one with no significant difference with that of "5 mg/L BA × 10 mg/L EPL" (Table 3). It implies that the use of EPL had favorable impact on antioxidant property, but its excessive use lessened its favorable impact.

As analysis of variance showed (Table 1), the simple effect of BA and EPL and their interactions were significant for catalase ($p < 0.05$). Also, BA effects and BA × EPL interactions were significant for peroxidase ($p < 0.01$). But, EPL did not account for peroxidase significantly. Plants treated with 1 mg/L BA showed the highest catalase content of 0.49 μmol consumed H_2O_2 min/mg/protein and those that were not treated with BA showed the lowest one (Table 4). It seems that the use of BA had a positive impact on catalase of marigold, but this impact was not linear so that as BA rate exceeded the optimum level, catalase activity was suppressed. Catalase is an effective antioxidant in the defense system of most plants against abiotic stresses. It can directly transform hydrogen peroxide into water and oxygen and remove the toxicity of this free oxygen radical [33].

The treatment of 10 mg/L BA resulted in the highest peroxidase (1.01 μmol consumed H_2O_2 /min/mg protein) with no significant difference with BA rates of 1 and 5 mg/L. The lowest one was obtained when no BA was applied (Table 2). The results of a research on *Alstroemeria* showed that as plants were treated with higher concentrations of BA, peroxidase was linearly decreased [34]. Our results were inconsistent with this study. In a study on tomato, significant reductions were observed in relative water content, stomatal conductance, intercellular CO_2 concentration, and photosynthesis activity under water stress. But, EPL treatment alleviated the adverse effects of water stress to a great extent and improved relative water content and photosynthesis activity. Also, it was revealed that the application of 24-epibrassinolide enhanced the activities of antioxidant enzymes significantly [35].

The highest peroxidase content (1.29 μmol H_2O_2 min/mg/protein) was obtained from the treatment of "10 mg/L BA × 10 mg/L EPL" and the lowest one from "5 mg/L BA × 0 mg/L EPL" (Table 3). The highest amount of catalase was observed in plants treated with "1 mg/L BA × 1 mg/L EPL" and "5 mg/L BA × 10 mg/L EPL" and the lowest in plants that were not treated with BA and EPL (Table 3). This shows that the simultaneous use of BA and EPL favorably impacted catalase activity so that its content was much lower when none of them was applied. Behnamnia et al. [36] reported that brassinolide application improved root volume, antioxidant content, free proline content, and APX, POD, CAT and SOD activity in tomato seedlings. On the contrary, MDA and H_2O_2 were significantly decreased in plants treated with brassinosteroid.

In conclusion, we found that BA and EPL positively influenced the recorded traits of marigold. They enhanced catalase activity, anthocyanin, antioxidant property, phenol, and flavonoid. Both BA and EPL increased marigold petal flavonoid content and similarly, improved antioxidant capacity. According to the results, BA and EPL were influential on the quality of marigold flowers and enhanced their pigments, essential oils, and yield.

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Conflict of interest

All authors declare that they have no conflict of interests.

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