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A New Concept in Organic Farming: Efficacy of Brassinosteroids as Foliar Spray to Ameliorate Growth of Marigold Plants

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Abstract

Organic farming an alternative agricultural system, strives for sustainability and relies on use of natural fertilizers and soil amendments. The commonly used natural fertilizers such as compost, green manure, and bone meal have yielded positive results in a wide range of crops. In a global trend of Good Agricultural Practices (GAP), integrated methods are being adopted for raising crop plants and also flowers like lilies and carnations. Such practices have become important as chemically grown plants be it fruits, vegetables or flowers impact consumer health. These bio-safe methods help manage soil, control pests and provide post- harvest care.. It is therefore, imperative that we find more natural products that can be used as fertilizers and help raise crops organically. In one such attempt Epibrassinolide (EBL), a synthetic hormone belonging to Brassinosteroid group of plant hormones (other being homobrassinolide) was used as foliar spray. *Tagetes erecta* (L.) commonly known as marigold was sprayed with 24-Epibrassinolide at concentration of 10^{-8} M at 15-days stage and later at interval of another 15 days the second spray was given. Ameliorative effect of EBL on growth and photosynthetic parameters was studied and EBL emerged as a non-toxic, and eco-friendly foliar spray with a potential to increase both quality and yield of marigold.

Introduction

After investigating ten possible cases of pesticide poisoning among Miami florists in 1979, the American Journal of Public Health recommended that safety standards for residual pesticides on cut flowers to protect both florists and consumers should be implemented (https://www.greenamerica.org/green-living/say-it-organic-flowers), During 1980´s a wide variety of micronutrient 'chelates' and 'complexes' (e.g. synthetic chelates using EDTA, glucoheptonates, polyols, amino-acids, or lignosulphonates, among many other types) were offered as an alternative to the application of inorganic compounds (Fernandez *et al*., 2013). It is now shown that chelated micronutrients for foliar applications are often more effective than trace elements from inorganic sources. This may be largely because chelates not only guarantee the availability of micronutrients, but also facilitate absorption of the trace elements by the leaves (https://micronutrients.akzonobel.com/applications/foliar/). The proposition that stomata could contribute to the foliar penetration process was assessed by Eichert and co-workers at the end of the 1990's and subsequently validated (Eichert *et al*, 1998; Eichert and Burkhardt, 2001; Eichert and Goldbach, 2008; Fernández and Eichert, 2009).

Though foliar application is a lucrative option but it is important to understand that such applications are not practical for nutrients that plants require in large amounts, such as nitrogen, phosphorous, and potassium. However, micronutrients can be easily administered through foliar application. At present a wide spectrum of foliar sprays are available, some are commercial while others are simple and can be prepared at home (Figure 1). Foliar sprays have certain limitations such as (a) they cannot be practiced in xerophytic plants with special anatomy to protect water losses (b) spray drift is another limitation (De Schampheleire *et al*., 2008) and (c) in perennial high-value crops, foliar fertilizers must be applied during the period of highest nutrient demand since soil supply and root uptake may be inadequate to meet demands even with adequate soil-applied fertilizer (Fernandez *et al.,* 2013).

In a study, nitrogen sufficient commercial 'Washington' navel orange trees were subjected to winter application of foliar urea just prior to or during flower initiation. Increased yield (p 0.05) was observed in three successive years without a reduction in fruit size (Ali *et al*., 1993). Carvalho (1994) observed that foliar application of KNO_3 at the rate of 0.3% brought best improvement in various growth parameters of Rangpur line seedlings (*Citrus limonia* cv. Cravo). In another study, Quin *et al*. (1996) found decreased leaf drop and increased fruit weight, fruit yield, soluble solids, juice content, ascorbic acid and total acid contents in 16 year old Eureka lemon tree when sprayed with 10% KCl. In Maize (cv. Jubilee) single foliar spray of 0.1M phosphate salts solution was applied to the upper surface at the five-to six-leaf stage, 2−4 h before inoculation with *Puccinia sorghi.* This not only resulted in increased growth but also induced systemic resistance against common rust (Reuveni and Reuveni, 1998). Brassinosteroids (BRs), a class of steroidal phytohormones have pleiotropic effects on wide range of physiological responses such as growth, germination, flowering, abscission, and senescence. BRs are also known to confer resistance to plants against biotic and abiotic stresses (Ali *et al*., 2007). According to Rao *et al*. (2002) BRs comprise a specific class of phytohormones which possesses a significant growth promoting activity. BRs such as 24-

epicastasterone and 24-epibrassinolide promote root elongation up to 50% in wild-type plants of *Arabidopsis* (Müssig *et al*., 2003). Swamy and Rao (2006) studied the effect of 24-epibrassinolide and 28-homobrassinolide on an ornamental plant- *Pelargonium* sp. (geranium) bourbon type and observed better root and shoot growth. Youssef and Talaat (1998) also reported increase in the growth parameters in lavender by exogenous application of brassinosteroids.

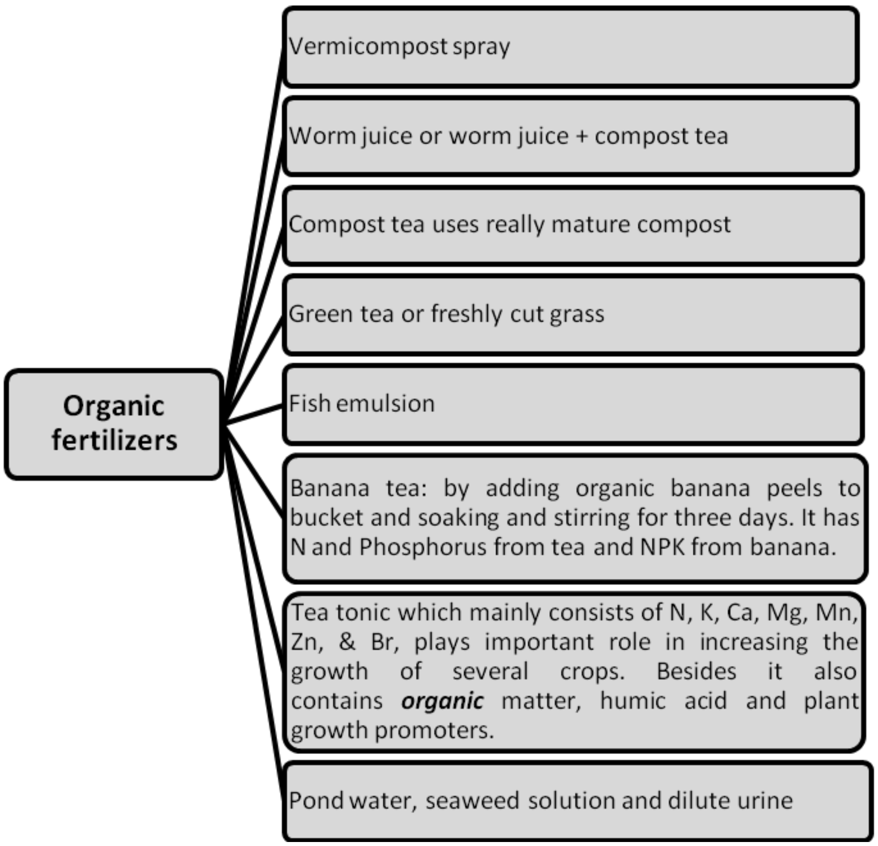

Figure 1 Types of organic fertilizers that easily prepared and administered while raising a kitchen garden.

Ameliorative effect of BRs on various plants growing under stress from salinity, drought and heavy metals are well known (Divi *et al* 2010, Cannata *et al* 2015). There remains little doubt if BRs are not able to bring about increase in growth and crop yield under stressful conditions. Similar data is also available on horticultural crops (Kang and Guo 2010). However, studies with BRs on flowers (and therefore in Floriculture) are recent. Kumar and Raju (2008) and Padamlatha *et al* (2013) have reported increase in yield of *Gladiolus* with BR application. It however, remains to be investigated if BRs can be recommended as components of commercial foliar sprays. Data is also lacking on the combinations of hormones which can give best results with BRs. Work on *Gladiolus* has indicated TIBA, GAs and CPPU combination to be effective in increasing growth and yield. It would also be worthwhile to increase anthocyanins, carotenoids and flavonoids concentration in flowers, as it would mean increase in quality of the produce. *Tagetes* L. is an annual herb that grows to about 30 cm in height. The bright orange to yellow flowers have been since ages used in religious ceremonies. They are also ground and used as chicken feed to enhance the characteristic yellow color of chicken skin and egg yolk. The pinnate leaves are a source of oil against skin problems. In foods and beverages, *Tagetes* is used as a flavor component. With a great importance attached to the plant, attempts are being made to increase quality of the produce. An experiment was therefore designed to investigate the potential of EBL in enhancing growth and yield of the plant through a sustainable method. The present investigation is only one of the few studies carried out with BR as foliar spray to increase growth and yield of a plant whose flowers are of great commercial potential.

Material and Methods

Preparation of hormone: Epibrassinolide (EBL) was purchased from Sigma Aldrich, Delhi. Stock solution (10^{-4} M) of Epibrassinolide (EBL) (Sigma Aldrich, Delhi, India) was prepared by dissolving the hormone in ethanol following protocol of Hasan *et al.,* (2011). The concentration (10^{-8} M) of EBL was prepared by dilution of stock in 0.5% Tween-20 (used as surfactant) solution of distilled water. The hormone was sprayed with atomizer all over the aerial plant surface during morning hours.

Experimental site and treatments: The experiment was carried out in a split plot completely randomized block design in Botanical Garden, SGTB Khalsa College, Delhi, India during the months of December 2017-March 2018. Seeds of *Tagetes erecta* L. (African marigold) variety Pusa narangi were purchased from National Seeds Corporation, Indian Agricultural Research Institute, New Delhi. Procured seeds were sown on a raised bed of size 110×50 cm to ensure appropriate germination. The nursery was maintained with proper irrigation and periodical weeding; seedlings that started to emerge after a fortnight were watered with a fixed amount of water daily and were covered with net to protect them from predators. After a month, the seedlings were transplanted into earthen pots of size 14x10 inches with one seedling per pot. Two series of six pots each were maintained where one series that was kept untreated, served as control and the other series sprayed with hormone was the treated one. After 15 days of transplantation (DAT) when the seedlings were about 20 cm long they were given first foliar spray of growth hormone 24-epibrassinolide (EBL). The second spray was at 30 DAT and each time a volume of 3ml 10^{-8} M EBL per plant was given. The control series was sprayed with distilled water. The treatments were named as follows:

1. Control (Pusa narangi) (PN)
2. PN+ Sprayed with EBL

There were six pots for each treatment and measurements were taken individually from each of the pots and data was pooled for statistical analysis.

Concentration of photosynthetic pigments: The chlorophyll and carotenoids concentration of leaves was determined by the non-maceration method using dimethylsulphoxide (DMSO) modified after Hiscox and Israelstem, (1979). The concentrations of chlorophyll a, chlorophyll b and carotenoids were calculated according to Arnon (1949).

$$\text{Chlorophyll a (mg g}^{-1}\text{ fresh weight)} = \frac{12.7D_{663} - 2.69D_{645} \times \text{Volume}}{1000 \times \text{weight of the sample}}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ fresh weight)} = \frac{22.9D_{645} - 4.68D_{663} \times \text{Volume}}{1000 \times \text{weight of the sample}}$$

$$\text{Carotenoids (mg g}^{-1}\text{ fresh weight)} = \frac{7.6D_{480} - 1.49D_{510} \times \text{Volume}}{1000 \times \text{weight of the sample}}$$

Where, D= optical density and Volume= final volume of aliquot

Vegetative growth parameters

Plant height: was recorded from both control and EBL sprayed 45 days old plants. Measurements were taken with help of metre scale and height of plants was expressed in centimetres (cm).

Number of leaves and leaf area: Number of leaves was counted from both control and EBL sprayed 45 days old plants. For measurement of leaf area, the leaves were harvested and immediately traced on tracing paper and transferred on graph paper. The area was then calculated and expressed in cm^2.

Number of branches and internode length: The number of branches and internode length were recorded from both control and EBL treated plants. The internode lengths (third internode from below the apex) were measured with thread and metre scale and expressed in cm.

Reproductive growth parameters

Number of buds: The number of buds were counted from both control and EBL sprayed series at two stages. The first counting was done when plants were 60 days old and second counting was done at the time of final harvesting.

Number and diameter of flowers: The observations for number of flowers and diameter were taken at three different intervals from both control and EBL treated plants. Initial measurement for number of flowers was taken from 65 days old plants when the first flush of flowers was observed. The diameter of flowers was measured with help of thread and scale. The plants were tagged so as to keep a check that next observation was made from the same plant. The second and third readings were taken from 75 and 85 days old plants respectively.

Fresh weight and dry weight: Fresh and dry weight was taken at the time of final harvest from 90 days old plants. The fresh weights of plants were recorded immediately after the harvest using a physical balance (Sartorius, Germany). For recording dry weight, the plants were dried in oven at 60ºC till there was no change in weight in consecutive readings. Both the fresh and dry weight of plants was expressed in grams.

Statistical analysis: The data for both the series are given as mean ± standard deviation of six replicates. The significance of differences between means of the two treatments was calculated using the Student's t-test in Statistical Package for Social Sciences version 16 (SPSS, USA). The significance was checked at level of P<0.05 and statistically significant differences were shown with help of asterisk (*) in histograms and tables.

Results

Concentration of photosynthetic pigments: Exogenous application of EBL has significantly affected concentration of chlorophyll a and chlorophyll b. There was 60.9% and 29.03% increase in concentration of chlorophyll a and chlorophyll b in plants sprayed with EBL over control respectively (Figure 2). While no significant change was observed in concentration of carotenoids in control and EBL sprayed plants (Figure 2)

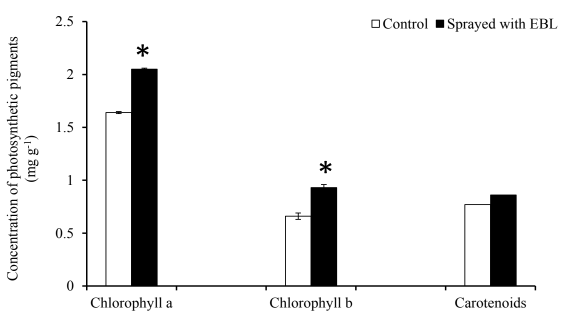

Figure 2. Effect of EBL spray on photosynthetic pigments in leaf tissue. Statistically significant differences were shown with help of asterisk (*)

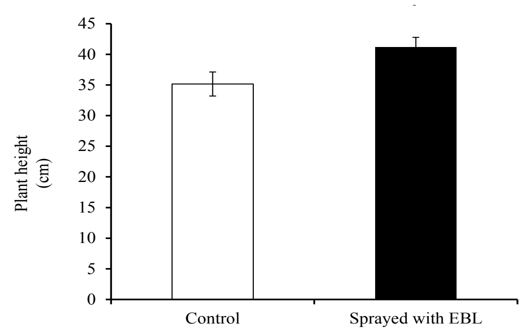

Figure 3. Effect of EBL spray on height of marigold plants

Vegetative growth parameters:

A significant difference in plant height was seen in plants sprayed with EBL as compared to control. Height of plants sprayed with EBL showed 14.57% increase over control plants (Figure 3). At the same time, numbers of leaves were also more in plants sprayed with EBL. EBL treated plants exhibited 12.33% increase in leaves as compared to control plants (Figure 4a). Further differences were seen for leaf area in plants that were sprayed with EBL. Significant increase of 39.45% was observed in leaf area for EBL sprayed plants as compared to control (Figure 4b). The marigold plants sprayed with EBL had significantly higher number of branches and internode length compared to plants without any spray. The number of branches significantly increased by 15.81%, while internode length increased by 9.73% in plants sprayed with EBL over control (Figure 5a and b).

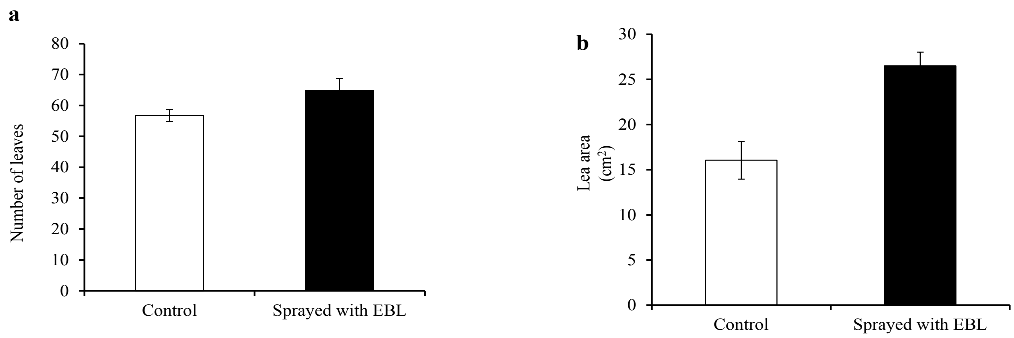

Figure 4 (a & b). Effect of EBL spray on (a) number of leaves; (b) leaf area of marigold plants.

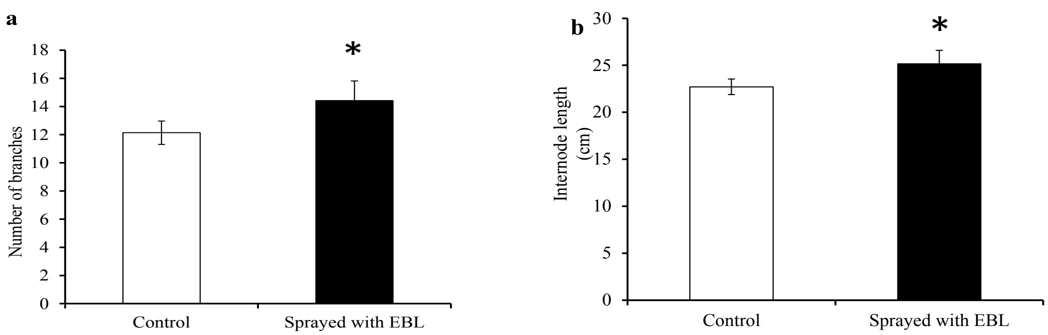

Figure 5 (a & b). Effect of EBL spray on (a) number of branches; (b) internode length of marigold plants. Statistically significant differences were shown with help of asterisk (*)

Reproductive growth: Number of buds was higher in plants sprayed with EBL as compared to control plants in both of the sampling stages (i.e. onset of reproductive phase and onset of senescence). Higher number of flowers was seen in EBL treated plants as compared to control in all the three stages of harvesting (Table 1). With each harvesting subsequently, the number flowers increased but the major difference was observed during third stage of harvesting between EBL treatment and control. There was increase in diameter of flower in EBL treated plants as compared to control in first (0.36%) and second (0.17%) stages of harvesting. However, slight decrease in diameter was observed during third stage of harvesting.

Table 1. Effect of exogenous spray of Epibrassinolide on number and diameter of flowers on three harvestings

Parameter	Number of flowers		Mean diameter of flowers	
	Control	Sprayed with EBL	Control	Sprayed with EBL
Ist harvest	6	7	4.7	6.1
IInd harvest	5	7	4.1	4.8
IIIrd harvest	13	16	3.7	3.5

Fresh weight and dry weight: Significant differences were observed in both fresh weight and dry weight of plants sprayed with EBL and those of control plants. Plants treated with EBL showed 17.03% and 27.2% higher fresh and dry weight respectively over control plants (Figures 6 and 7).

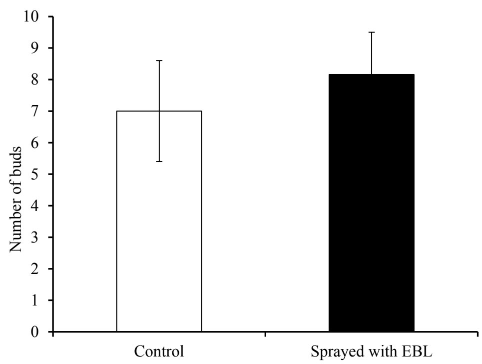

Figure 6. Effect of EBL spray on number of buds

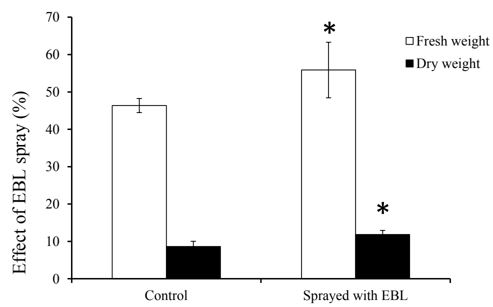

Figure 7. Effect of EBL spray on fresh and dry weight. Statistically significant differences were shown with help of asterisk (*)

Discussion

Problems associated with the use of synthetic chemicals for crop protection and weed control have been reviewed worldwide. It is known that with constant use of these chemicals - pests and weeds become resistant. The issues of human health and environmental pollution also cannot be ignored. Therefore organic fertilizers are increasingly becoming important with environmentalists, farmers and the consumers. Gradually the agro-chemicals are being replaced by fertilizers made out of many kinds of agricultural wastes such as animal dung and plant residues (Larptansuphaphal and Jitumroochokchai, 2009). In the present scenario of rising concerns of 'safe and organic' crops, foliar feeding using natural and organic fertilizer is an important tool for the sustainable and productive management of crops.

The foliage of plants exposed to EBL supplement exhibited enhanced photosynthetic pigments concentration that is in support of other studies (Ali *et al*., 2007;

Hasan *et al*., 2008). This is in compliance with earlier reports indicating the BRs-induced impact on transcription and translation involved in the expression of specific genes required for synthesis of biosynthetic enzymes involved in chlorophyll formation (Bajguz, 2000). In the present study the concentration of chlorophyll a (chl a) showed a better result as compared to chlorophyll b (chl b). The reason may be that application of BRs results in slowing down the rate of chlorophyll a degradation (Hola, 2011). There is also an increase in the chl a/b ratio in EBL supplemented plants indicating that chl b was being degraded at a higher rate than chl a. The results support the findings of Fang *et al* (1998) who demonstrated that the first step in the degradation of chl b implies its conversion to chl a. According to Khripach *et al* (1999) increase could be due to the prevention of the loss of photosynthetic pigments by BRs. The primary function of carotenoids is to absorb light energy for the use of photosynthesis, protect chlorophyll from photodamage and eliminate ROS formed in cell during various metabolic processes (Minguez-Mosquera *et al*., 2002). Carotenoid is a non-enzymatic antioxidant thus protecting chlorophyll and cell membranes against ROS (Das and Roy choudhury, 2014). In the present study, carotenoids content was not much affected thus showing no significant impact of EBL on carotenoids biosynthetic pathways.

In the present investigation all studied vegetative growth parameters were enhanced in EBL treated plants as compared to control. Improved growth could be seen in terms of increased plant height, intermodal length, number of branches and leaves, and also leaf area. Müssig (2003) reviewed that BRs could influence branch formation (also, in present work) by modulating metabolic pathway and by altering the nutrient allocation. However, the EBL induced leaf area is in viewpoint with the findings of Zhiponova *et al*. (2013) that BRs influence cell division and subsequently the leaf area and internode size. The increase in the chl a and b content along with increase in leaf area, could possibly lead to the improvement in photosynthetic efficiency of EBL treated plants.

It was observed that hormone treated plants have higher fresh and dry weight than control. The obtained results can be explained through work of Khripach *et al* (1999) that BRs prevent the loss of photosynthetic pigments thus retaining the photosynthetic efficiency and in turn affects the biomass of the tissue (Stoeva *et al*., 2005). Cutler (1991) showed that BRs increased plant growth, root size and root and stem dry weight. Çağ *et al* (2007) has also depicted similar results in red cabbage and reported fresh weight in cotyledons increased in 0.001 and 0.1μM EBL. Braun and Wild (1984) reported increased shoot fresh weight manifested by the stimulation of both elongation and radial growth in mustard. Besides enhancing vegetative parameters, effect of EBL was also observed on the reproductive parameters in present investigation. Increased bud production, flower number and size were observed in EBL treated plants as compared to control. The present work is indicative of correlation between vegetative and reproductive growth parameters. Subsequently, better vegetative growth leads to increased number of bud formation and flower size in hormone treated plants. Ethylene plays an important role in the regulation and co-ordination of senescence in climacteric flowers and a sharp increase of this hormone evolution was found during flower maturation, opening and senescence **(**De *et al.* 2014)**.** Arteca and Arteca (2008) findings support that BRs have been found to regulate ethylene levels. These above mentioned findings related to ethylene can be correlated with the present work that EBL enhanced not only the number of flowers but also the size of flower perhaps under the influence of ethylene. The result was in corroboration to the efforts of Khripach et al. (2000) where Epin supplemented varieties of roses showed enhanced production of flowers. According to Ye *et al*. (2012) in *Arabidopsis* sp. BRs regulate the timings of flowering while Yu *et al*. (2008) observed that BRs enhance carbohydrate assimilation, allocation, control of aquaporin activities and also various developmental processes and organ differentiation. These aspects however need to be investigated in *Tagetes*.

Judicious combination of sustainable practices like biofertilizer application as well as growth regulator foliar sprays creates possibilities of manipulating flowering to a rational quantity, which would prove useful in commercial species like marigold. Present study proves efficacy of BRs in bringing significant effects on plant growth and development and therefore may be one of the components of foliar sprays or nutrient supplements.

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Author's Contribution: *Inderdeep Kaur, Anjana Sagar and Piyush Mathur wrote and edited the manuscript. Inderdeep Kaur and Anjana Sagar has planned methodology and designed the work. Anirudh Mukerji, Sagar Dhama, Vibhav Singh and Jasmeen Kaur meticulously carried out the experiments and Anirudh Mukerji and Sagar Dhama also collected data. Piyush Mathur analysed the results and prepared the manuscript.*

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