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EXOGENOUS APPLICATION OF 24-EPIBRASSINOLIDE ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL ATTRIBUTES AND ESSENTIAL OIL CONTENTS OF *Jasminum sambac* L.

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The current investigation was carried out to evaluate the effect of 24-Epibrassinolide (EBL) on growth, physiological and biochemical attributes along with oil contents of *Jasminum sambac*. Present experiment was laid out according to Randomized Complete Block Design and foliar application of 24-Epibrassinolide were applied in four different concentrations viz. 1, 2, 3 and 4 µM. Present study results showed that EBL application enhanced the plant growth, chlorophyll contents and rate of photosynthesis of the *J. sambac*. The results indicated that EBL concentration 2 µM proved to be the best treatment for morpho-physiological and bio-chemical traits like number of leaves (25.55), chlorophyll contents (6.84 mg g^{-1}), leaf area (25.95 cm^2), fresh and dry weight ratio (4.97), number of flower per plant (1324), photosynthetic rates (4.80 µmol m^{-2} S^{-1}), transpiration rate (0.776 mmol m^{-2} S^{-1}), stomatal conductance (0.158 mmol m^{-2} S^{-2}) and sub-stomatal cavity (286.05 µmolmol^{-1}) compared with other treatments. However, plants treated with 3 µM showed better results for growth indices like plant height, number of branches per plant, fresh weight of flower, and dry weight of flower as compared to rest of the treatments. In case of flower diameter (2.16 cm) and leaf water potential (22.28 MPa), control treatment gave the maximum values compared to other treatments. However, application of EBL @1 µM showed good results for bud diameter only. The quantitative analysis of *J. sambac* oil was also carried out and results exhibited that the plants treated with 3 µM EBL, revealed an increase in essential oil contents. The present study revealed encouraging results related to EBL application on the growth performance and essential oil contents in *J. sambac*.
Keywords: 24-Epibrassinolide, plant growth regulator, essential oil extraction, oil contents, jasmine

INTRODUCTION

Jasminum sambac (Jasmine) belongs to family Oleaceae, is one of the ancient fragrant flowers cultivated globally (Irulappan, 1994). In spite of ornamental purposes, it can also be cultivated for extraction of essential oil, which is accredited as king of fragrance (Younis *et al*., 2011a). Brassinosteroids (BR) distinguish a sixth class of plant hormones with widespread occurrence in plants in addition to abscisic acid, auxins, and ethylene. BR was identified in different plant parts like seeds, anthers, and flowers. Research studies on BRs were exploited by different researchers (Clouse and Sasse, 1998; Khripach *et al*., 1999; Sakurai *et al*., 1999). Many investigators emphasized on particular features of its site of action and structures (Li and Chory, 1999; Schumacher and Chory, 2000; Khripach *et al.*, 2000; Bishop and Yokota, 2001; Friedrichsen and Chory, 2001; Mussig and Altmann, 2001; Clouse, 2001, Clouse, 2002ab; Zullo and Adam, 2002; Bajguz and Tretyn, 2003; Fujioka and Yokota, 2003; Zullo *et al*., 2003). Brassinosteroids have distinguishing biological properties on plants and have the capability to protect plants from different environmental stresses, like drought and salinity (Sasse, 1997; Sasse, 1999). The 24-epibrassinolide (EBL) application had increased the chlorophyll contents, photosynthesis rate and essential oil contents in geranium (Swamy and Rao, 2009). EBL promising effect on plant growth is connected with photosynthetic ability.

Foliar application of plant growth regulators is helpful to improve the quality parameters of cut flowers (Younis *et al.*, 2011b; Younis *et al*., 2013; Ramzan *et al*., 2014). Foliar spray with alpha-tocopherol on faba bean plant induced increments in yield components reported El-Bassiouny *et al*. (2005). Foliar application of ascorbic acid and alpha-tocopherol in *Hibiscus* plants significantly increased number of flowers and its oil contents (El-Quesni *et al*., 2009). EBL foliar application at 3-µM concentration had increased the essential oil contents in rose scented geranium (*Pelargonium graveolens* L.) and it was also revealed an increase in geraniol content and decrease in citronellol content (Swamy and Rao, 2009).

Keeping in view the potential of *J. samabac* as non-traditional high value crop, the present study was planned to evaluate the effect of 24- Epibrassinolide on morphological, physiological and biochemical parameters of jasmine plants along with its essential oil contents. In the present study, exogenous application of 24-epibrassinolide at early plant growth stage was applied.

MATERIALS AND METHODS

The present research was conducted at Rosa project, IHS, University of Agriculture, Faisalabad. An optimization trial was carried out to find the best possible concentration of 24-Epibrassinolide. This optimization trail was done prior to conduct this experiment. The row-row and plant-plant distance among plants were maintained at three feet. Pruning was done to attain equal height. Exogenous applications of 24-Epibrassinolide using concentrations (1, 2, 3 and 4 μM) were applied after 30, 60 and 90 days after pruning. Tween-20 @ 0.1% was added to ensure the maximum absorption of EBL in leaf tissues. After 120 days of application, data were recorded. Randomized Complete Block Design (RCBD) was used to lay out the present experiment.

The healthy jasmine plants were selected for flowers from Rosa project, IHS, University of Agriculture, Faisalabad. This plain region with mild climate considered suitable for cultivation of jasmine plants. Thus, the climatic conditions provide a good opportunity for perfect plant growth. Jasmine flowers were harvested early morning in order to ensure the least loss of volatile compounds with maximum essential oil recovery. The flowers were prepared for essential oil extraction as done by Younis (2006).

***Growth parameters*:** The plant growth was recorded in terms of plant height, number of branches per plant, number of leaves per plant, leaf area, number of flowers per plant, flower fresh weight, dry weight, fresh and dry weight ratio and flower diameter.

***Physiological parameters*:** Measurements of the photosynthesis rate (A), CO_2 difference, stomatal conductance (gs), transpiration rate (E), sub-stomatal cavity (Ci) and water use efficiency (Pn/E) were made on the 2nd leaf from the top of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical development company, Hoddesdon, England). All measurements were computed at 10 a.m. to 2:00 p.m. with the following specifications: leaf surface area 11.35 cm^2 ambient CO_2 concentration (C ref.) 342.12 (μmol m^{-1}), temperature of leaf chamber (Tch) varied from 36.2 to 42.9 ^{0}C, leaf chamber volume gas flow rate (v) 396 mL min^{-1}, leaf chamber molar gas flow rate (U) 251 μmol s^{-1}, ambient pressure (P) 99.95 kPa, molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}, PAR (Q leaf) at leaf surface was maximum up to 918 μmol m^{-2} s^{-1}.

Biochemical parameters:

***Chlorophyll contents (mgg^{-1})*:** The chlorophyll contents of fresh leaf samples were computed by using Minolta (SPAD) chlorophyll meter SPAD-502; Konica, Minolta sensing Inc., Japan.

***Essential oil content*:** The essential oil from flowers of *J. sambac* was extracted through hydro-distillation. 1000 g jasmine petals were filled in 4L flask and 1L of distilled water had been added into it and then distillation was carried out. Distillation process was completed in 3hrs. The jasmine oils obtained at the end of distillation were collected and oil contents were measured by adopting the following formula.

Oil content (%) in herb = Vol. of oil (ml) x specific gravity/Weight of fresh herb x 100

***Statistical analysis*:** Experiment was laid out according to Randomized Complete Block Design with five treatments, four different levels of (24-EBL) along with control. All treatments were replicated four times with five plants in each replication. The data were subjected to statistical analysis using analysis of variance technique at 5% probability (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Exogenous application of EBL substantially improved growth of *J. sambac* as reflected in increase of all growth parameters studied (Fig. 1 & 2). EBL at concentration of 2 μM, exhibited the maximum number of leaves/plant (25.55), number of flower/plant (1324), fresh/dry weight ratio (4.97) and leaf area (25.95 cm^2) while at 3 μM concentration, increased plant height (66.72 cm), number of branches per plant (62.15), fresh weight (0.408 g) and dry weight of flower (0.118) were observed compared to rest treatments (Fig. 1 & 2). Results regarding plant indices viz. plant height, all treatments gave non-significant results, in case number of leave/plant and leaf area EBL concentrations of 1, 3 and 4 μM gave non-significant results as well.

Exogenous application of EBL resulted in a significant increase in carbon dioxide fixation (Fig. 3). EBL treatment having concentration 2 μM exhibited maximum photosynthetic rate (4.80 μmol m^{-2} S^{-1}), chlorophyll contents (6.84 mg g^{-1}), transpiration rate (0.776mmol m^{-2} S^{-1}), stomatal conductance (0.158mmol m^{-2} S^{-2}) and sub-stomatal cavity (286.05 μmolmol^{-1}) compared to rest of the treatments (Fig. 3). Exogenous use of 24-Epibrassinolide improved the rate of photosynthesis and increased chlorophyll content in *Pelargonium* (Swamy and Rao, 2009). High levels of chlorophyll connected with a rise in photosynthesis may be contributed to increase carbohydrate levels. There are several reports of the higher chlorophyll levels in plants after brassinosteroid application. In *Chlorella vulgaris* brassinosteroid enhanced the chlorophyll levels (Bajguz and Czerpak, 1998). There is an increase in the net photosynthetic rate and seed yield per plant in mustard crop (Hayat *et al*., 2000). Comparative efficacy of optimum levels (10 μM) with abscisic acid and kinetin exhibited that brassinosteroids showed most promising among the other regulators and enhanced the chlorophyll contents (Hayat *et al*., 2001).

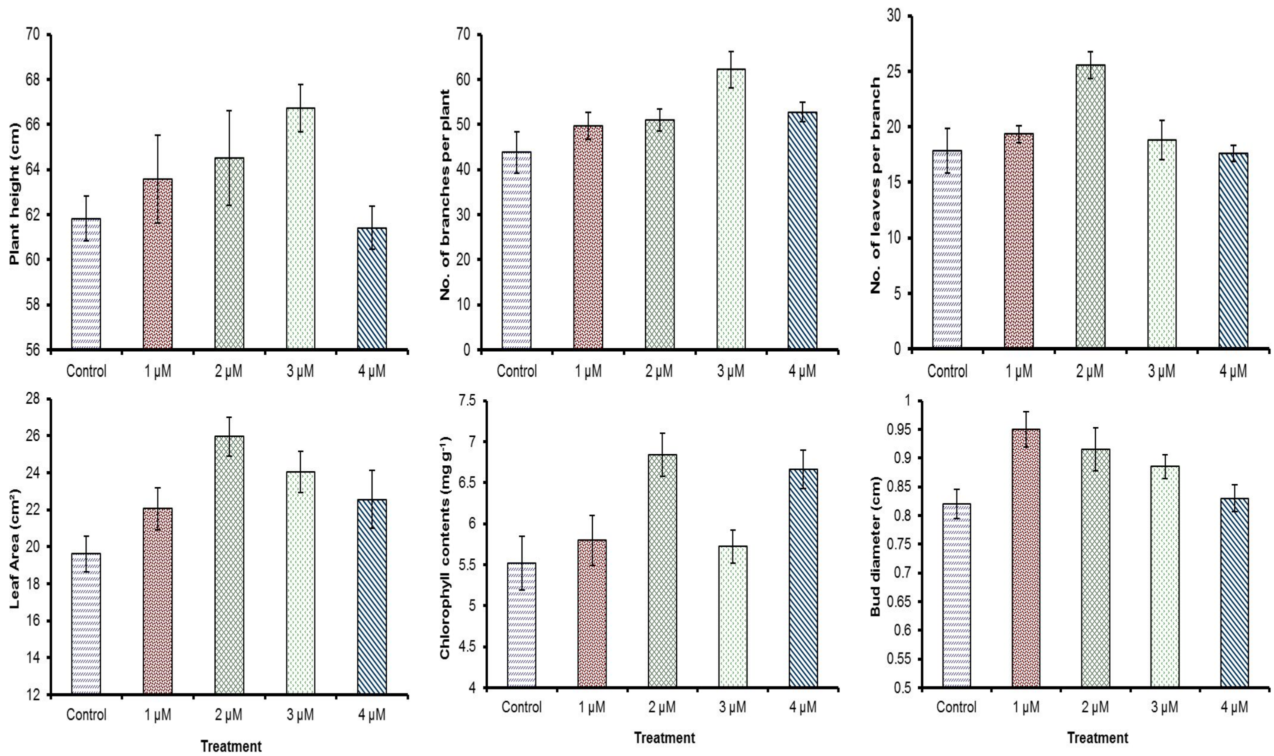

Figure 1. Effect of EBL on morphological attributes and chlorophyll contents in *Jasminum sambac*

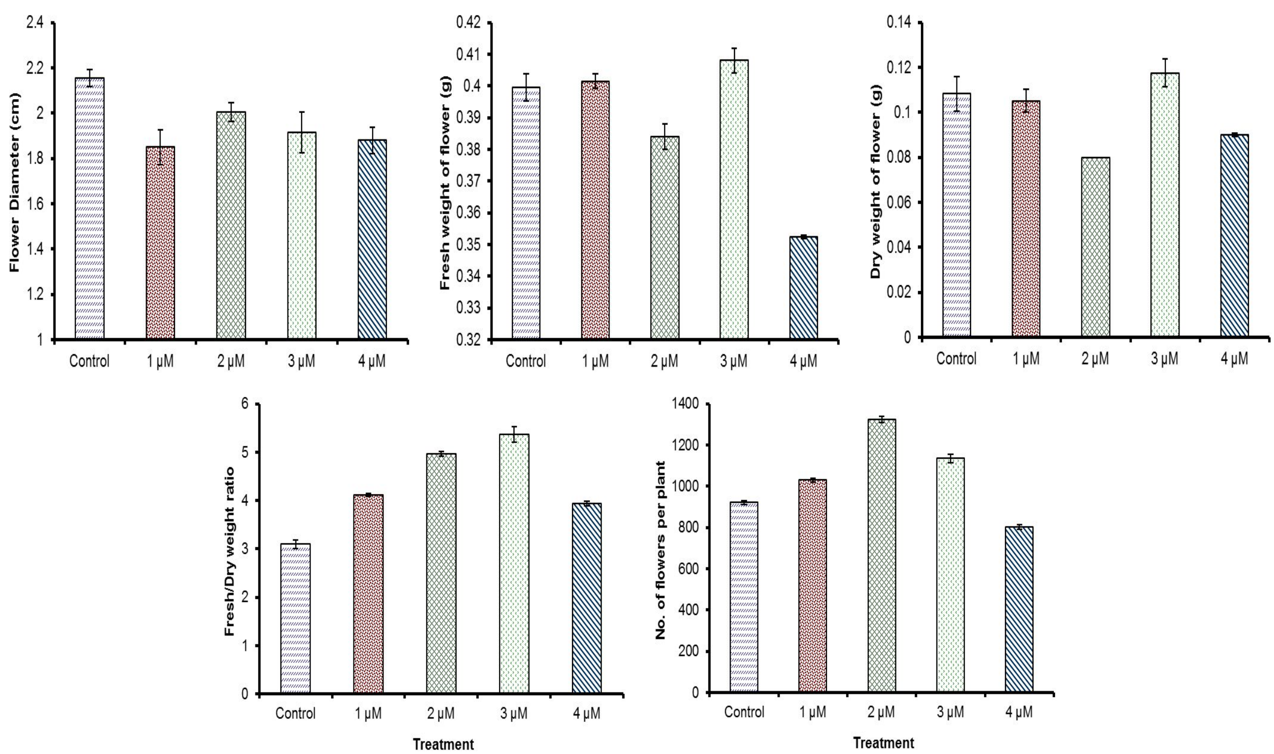

Figure 2. Effect of EBL on floral attributes of *Jasminum sambac*

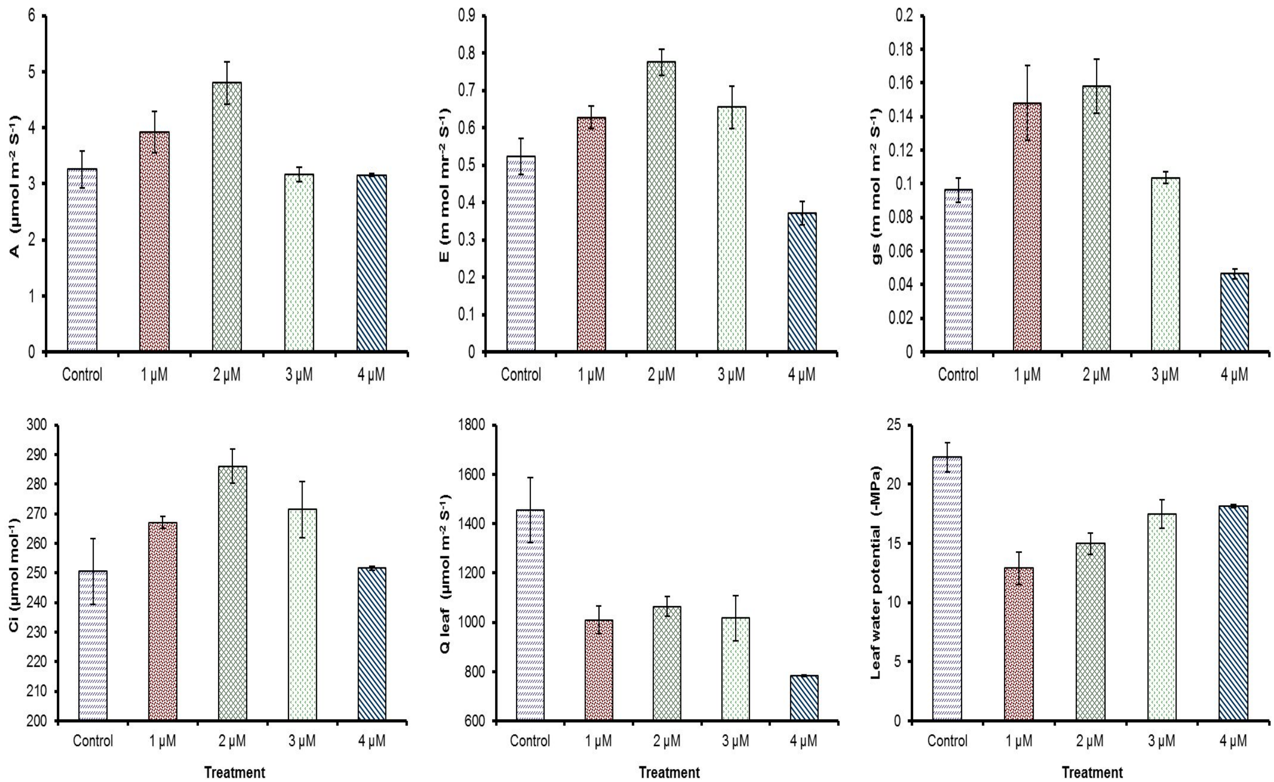

Figure 3. Effect of EBL on physiological attributes of *Jasminum sambac*

EBL employed in the study caused a significant increase in the essential oil contents of *J. sambac* when applied at 2 µM concentration (Table 1). EBL effect on oil yield might be determined through the influence of metabolism and growth. EBL might activate the inherent genetic potential of the plant to yield more oil. More essential oil contents in lavender were obtained by judicious EBL applications (Youssef and Talaat, 1998). A substantial increase in oil accumulation in fragrant Japanese mint (*Mentha arvensis*) by application of chlormequat chloride was reported (Farooqi and Sharma, 1988).

Table 1. Effect of EBL on essential oil contents of *Jasminum sambac*

Treatments	Essential oil contents (g)
Control	0.0091±0.00051 B
1 µM	0.0085±0.00027 B
2 µM	0.0112±0.00091 A
3 µM	0.0094±0.00066 AB
4 µM	0.0086±0.00022 B

Means sharing similar letters are statistically non-significant ($P>0.05$).

24-Epibrassinolide application on plant growth, chlorophyll contents, photosynthesis rate, and essential oil yield in geranium was exploited by Swamy and Rao (2009). Present study results are in accordance with findings of Swamy and Rao (2009). A promising role of brassinosteroids in modern agriculture is being foreseen (Khripach *et al.*, 2000) and the current findings add another dimension to the practical utility of EBL.

***Conclusion*:** It was concluded that the 24-Epibrassinolide's application (2 µM) proved an effective method to enhance the morphological and physiological attributes in *Jasminum sambac*. EBL promising effect on plant growth is connected with photosynthetic ability. EBL application @ 3 µM had increased the essential oil contents and its quality in *J. sambac*. Therefore, vegetative and reproductive growth and essential oil contents can be improved by applying optimal concentration of 24-epibrassinolide and its application can further be exploited in modern agriculture for quality crop production.

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