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Exploiting the Epibrassinolide as a plant growth promoter for augmenting the growth, physiological activities and alkaloids production in *Catharanthus roseus* L.

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Abstract
Catharanthus roseus (L.) G. Don is a medicinal plant that bears indole alkaloids used in cancer chemotherapy. The anti-cancer alkaloids, *viz*. vinblastine and vincristine, are mainly present in the leaves of *C. roseus*. Brassinolides, or Epibrassinolide (EBL) a sub-class of brassinosteroids, have demonstrated plant growth regulator (PGR)-like activity in different crops. A pot culture experiment was carried out to explore the effect of EBL on plant growth, physiological activities and production of total alkaloids in leaves, stems and roots in *C. roseus* at 6 and 9 months after planting (MAP). Five concentrations of EBL (10^{-0}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) were applied on *C. roseus*. EBL 10^{-7} M significantly increased a number of growth, physiological and biochemical parameter at 6 and 9 MAP. The said concentration also increased leaf and root alkaloid content/ yield at both the stages. However, it was not significantly increased the stem alkaloids content/ yield.

Keywords: *Catharanthus roseus*, vinblastine, vincristine, alkaloids, EBL.

Introduction
Catharanthus roseus L. is a renowned medicinal plant, belonging to the family Apocynaceae. It is a rich source of valuable alkaloids, which are distributed in all parts of the plant. The alkaloid content of periwinkle varies considerably in various parts, the maximum being in the root bark which ranges from 0.15 to 1.34% and even up to 1.79% in some strains (Singh and Jagdev, 1996) [1]. The plant contains about 130 alkaloids of indole group, out of which 25 are dimeric in nature. Among the monomeric alkaloids, ajmalicine (raubacine) found in the roots, has been confirmed to have a broad application in the treatment of circulatory diseases, specially those that give relief in obstruction of normal cerebral blood flow. Vincristine sulphate arrests mitosis in metaphase and is very effective for treating acute leukemia in children and chronic lymphocytic leukemia in elderly adults. It is also used against Hodgkin's disease, wilkins's tumor, neuroblastoma and reticulum cell sarcoma. Vinblastine sulphate is used particularly to treat Hodgkin's disease besides lymphocarcoma, choriocarcinoma, neuroblastoma, and carcinoma of breast, lungs and other organs as well as in acute and chronic leukemia (blood cancer). In plant material, VCR and VBL are accumulated in very low concentrations viz. 20 mg t^{-1} and 1 g t^{-1}, respectively (Tyler, 1988) [2]. At present, India is the third largest manufacturer of VCR and VBL in the world and is exporting these alkaloids to European countries. High demand and low yield of these alkaloids urges to explore alternative means of their production.

Phytohormones are small molecules derived from secondary metabolism that play pivotal role in shaping plant architecture (Santner and Estelle, 2009) [3]. The presence and biological effects of some of these hormones have been recognized for more than a century. It has been well established that foliar application of plant hormones and bioactive compounds can improve the physiological efficiency and may play significant role in raising the productivity of a crop (Alam *et al*., 2012; Naeem *et al*., 2012) [4, 5]. Epibrassinolide (EBL) is an important steroidal naturally occurring brassinosteroids (BRs) with strong biological activity that induces a large range of cellular responses, including plant growth, seed germination and nitrogen fixation (Fujioka 1999; Asha *et al*., 2014) [6, 7]. BRs have pleotropic effects and can induce a broad array of cellular responses including stem elongation, leaf bending and epinasty, pollen tube growth, induction of ethylene biosynthesis, proton pump activation, xylem differentiation,

and regulation of gene expression (Clouse and Sasse, 1998; Li and Chory, 1999; Dhaubhadel *et al.*, 2002) [8, 9, 10]. Use of BRs has also been examined in agricultural production. Several studies have established that BRs influence senescence, leaf abscission and enhanced tolerance against drought and cold stress, heavy metal stress, salt stress and diseases (Nakashita *et al.*, 2003; Yu *et al.*, 2004; Allagulova *et al.*, 2015) [11, 12, 13]. As a consequence, extensive research has been undertaken to develop BRs as plant growth regulators for agricultural production (Sasse, 2003; Özdimer *et al.*, 2004) [14, 15].

Keeping in view the importance of EBL as a plant growth promoter and desired production of alkaloids of the crop, this study is aimed at investigating the effect of EBL on plant growth, enzyme activities, mineral content and content/ yield of total alkaloids of *C. roseus* plants.

2. Materials and Methods

Plant Materials and Growth Conditions

The pot experiment was conducted in naturally illuminated environmental conditions of the net house at the Botany Department, Aligarh Muslim University, Aligarh, India. Healthy periwinkle seedlings of equal size were obtained from Woodbine Nursery, Civil Lines, Aligarh. The seedlings were then transplanted to earthen pots. Prior to transplantation, each pot (25 cm diameter × 25 cm height) was filled with 5 kg of homogenous mixture of soil and farmyard manure (4: 1). Physical and chemical characteristics of the soil were texture sandy loam, pH (1 : 2) 7.5, E.C. (1 : 2) 0.46 dSm^{-1}, available N, P, and K 102.0, 7.8, and 145.6mg kg^{-1} of soil, respectively. A uniform recommended basal dose of N, P, and K (15: 25: 25 kg ha^{-1}, resp.) was applied in the form of urea, single superphosphate, and muriate of potash, respectively, at the time of transplanting the seedlings. The experiment was conducted with simple randomized block design. Each treatment was replicated five times. Each pot contained a single healthy plant. The pots were watered as and when required.

Experimental Design

Foliar spray of different concentrations of EBL was started at 10 days interval at 4 months after planting (MAP) and 7 MAP stage and harvested at 6 MAP and 9 MAP, respectively. Totally, six sprays of EBL were applied to the crop using a hand sprayer. The control plants were sprayed with distilled water only. The applied EBL treatments were comprised of 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M (Molar Concentration) of EBL. Plants were sampled for all growth, physiological and biochemical parameters carried out at 6 and 9 MAP. All yield and quality attributes were also measured on these dates.

Determination of Growth Attributes.

At sampling, five plants from each treatment were uprooted carefully and washed with running tap water to wipe off all adhering foreign particles. They were soaked thereafter using blotting sheets. Thereafter, the leaf, stem and root of each plant from each treatment were dried in a hot air oven at 80ºC for 24 hours to record the dry weight using an electronic weighing balance.

Leaf area index (LAI) was determined by using following formula suggested by Watson (1947) [16].

$$LAI = \frac{\text{Leaf area per plant}}{\text{Area occupied by plant}}$$

Total Chlorophyll and Carotenoid Contents

The total chlorophyll and carotenoid content were estimated by the method of Mackinney (1941) [17] and Maclachlan and Zalik (1963) [18], respectively. One hundred mg of fresh leaves from interveinal area was ground in 10 mL of 80% acetone using a mortar and pestle. The suspension was decanted and filtered through a Whatman filter paper No.1 into a Buchner funnel. The optical density (OD) of the solution was read at 645 and 663 nm for chlorophyll estimation and at 480 and 510 nm for carotenoid estimation using a spectrophotometer (Spectronic UV-1700, Shimadzu, Japan). The total chlorophyll and carotenoid contents were calculated and expressed as mg g−1 leaf FW.

Activity of Nitrate Reductase (NR)

Activity of nitrate reductase (E.C.1.6.6.1) was estimated in the youngest fully developed leaves by the intact tissue assay method developed by Jaworski (1971) [19]. Fresh chopped leaves, weighing 200mg, were transferred to plastic vials. Each vial contained 2.5mL phosphate buffer (pH 7.5), 0.5mL potassium nitrate solution, and 2.5mL of 5% isopropanol. The vials, containing the reaction mixture, were incubated for two hours at 30◦C. After incubation, 1% sulphanilamide and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NED-HCL) were added. The test tubes were kept for 20 minutes at room temperature for maximum color development. The OD of the content was recorded at 540 nm. Activity of NR was expressed as nM NO^{2-} g^{-1} FW h^{-1}.

Activity of Carbonic Anhydrase (CA)

The activity of carbonic anhydrase (E.C.4.2.1.1) was measured in the fresh leaves selected randomly, using the method described by Dwivedi and Randhawa (1974) [20]. Two hundred mg of the leaves (chopped leaf-pieces) was transferred to Petri plates. The leaf pieces were dipped in 10mL of 0.2M cysteine hydrochloride solution for 20 minutes at 4◦C. The solution adhering to leaf pieces was removed with the help of a blotting paper, followed by immediately transferring them to a test tube containing 4mL of phosphate buffer (pH 6.8). To it, 4mL of 0.2M sodium bicarbonate solution and 0.2mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05NHCl using methyl red as indicator. The enzyme activity was expressed as of mol (CO_2) kg^{-1} (FW) s^{-1}.

Estimation of N, P, and K content in leaves

Leaf samples from each treatment were digested for the estimation of leaf N, P, and K content. The leaves were dried in a hot-air oven at 80ºC for 24 hours. Dried leaves were powdered using a mortar and pestle and the powder was passed through a 72 mesh. The sieved leaf powder was used for N, P, and K content. One hundred mg of oven-dried leaf powder was carefully transferred to a digestion tube carrying 2 mL of AR (analytical reagent) grade concentrated sulphuric acid. Leaf-digestion was made on a temperature controlled heated assembly maintained at 80ºC temperature for about 2 h. Thereafter, the contents were cooled for about 15 min at room temperature. Afterwards, 0.5 mL of 30% hydrogen peroxide (H_2O_2) was added to the content drop by drop. The addition of H_2O_2 followed by heating was repeated until the content of the tube turned colorless. The aliquot (peroxide-digested material) thus prepared was used to estimate the N, P, and K contents in the leaves on a dry weight basis.

Estimation of nitrogen content

Leaf-nitrogen content was estimated according to the method

of Lindner (1944) [21] with a slight modification suggested by Novozamsky *et al*., (1983) [22]. The dried leaf powder was digested in H_2SO_4 in a digestion tube. A 10 mL aliquot (peroxide-digested material) was poured into a 50 mL volumetric flask. To this, 2 mL of a 2.5 N sodium hydroxide and 1 mL of 10% sodium silicate solutions were added to neutralize the excess acid and prevent turbidity. A 5 mL aliquot of this solution was poured into a 10 mL graduated test tube and a 0.5 mL Nessler's reagent was added. The OD (optical density) of the resultant solution was recorded at 525 nm using a spectrophotometer. The leaf N content was determined by standard graph prepared by using known dilutions of ammonium sulphate.

Estimation of phosphorus content

The method of Fiske and Subbarow (1925) [23] with a slight modification suggested by Rorison *et al.,* (1993) [24] was used to estimate the leaf-phosphorus content in the digested material. The same aliquot (peroxide digested material) was used to determine the leaf-phosphorus content. A 5 mL aliquot was poured into a 10 mL graduated test tube to which 1 mL of molybdic acid (2.5%) was added, followed by the addition of 0.4 mL 1-amino-2-naphthol-4-sulphonic acid. When the color turned blue, the volume was made up to 10 mL with double distilled water. The OD of the resultant solution was recorded at 620 nm using a spectrophotometer. The leaf P content was determined by standard graph prepared by using known dilutions of potassium dihydrogen orthophosphate (KH_2PO_4).

Estimation of potassium content

Potassium content in the leaves was determined flame-photometrically, using the same aliquot (peroxide-digested material). In the flame-photometer, the aliquot was discharged through an atomizer in the form of a fine mist into a chamber, where it is drawn into a flame. Combustion of the element (potassium) produces light of a particular wavelength [(λmax for K = 767 nm (violet)] that was passed through the appropriate filter to impinge upon a photoelectric cell that activates a galvanometer. The readings of leaf potassium content were recorded with the help of emission spectra using specific filter in the flame-photometer (Model, C150, AIMIL, India).

Total Alkaloid Content in Leaves, Stems and Roots

Total alkaloid content was estimated in leaves, stems and roots as described by Afaq *et al*., (1994) [25]. The leaves, stems and roots were dried in a hot air oven at 80°C for twenty-four hours. The samples were powdered and passed through a 72 mesh. Five hundred mg of the powdered sample was taken in a 100mL round bottom reflux flask. To it, a known volume of ethyl alcohol was added. Then, the mixture was refluxed for 6 hours. Thereafter, it was filtered, followed by adding 50mL of dilute HCl, and then the filtrate was transferred to a separating funnel, to which 50mL of diethyl ether was added. The mixture was shaken for 15–20 minutes. The upper diethyl ether layer was discarded and the lower water layer was decanted to a beaker. The content, collected in beaker, was made slightly basic by adding ammonia solution. The decanted content was again transferred into a separating funnel with 50mL of diethyl ether, followed by decanting the content again. To the final decant, anhydrous sodium carbonate was added. Then, the mixture was decanted in a pre-weighed dry porcelain dish, followed by evaporating the content till dryness; lastly, the dried content was weighed.

Total alkaloid content (%) was calculated using following formula:

$$\frac{W_A - W_E}{W_R} \times 100$$

W_E = Weight of empty porcelain dishing (g)
W_A = Weight of porcelain dish after evaporation (g)
W_R = Weight of the powder (g)

Statistical analysis

The experimental data were statistically analyzed using the analyses of variance techniques according to Gomez and Gomez (1984) [26]. In applying the 'F' test, the error due to replicate was also determined. When 'F' value was found to be significant at the level of probability, the least significant difference (LSD) was calculated.

3. Results

EBL applied at the concentration10^{-7} M proved best compared to the other foliar dosage of EBL. EBL, applied at 10^{-8} M to 10^{-7} M, enhanced growth, yield and quality attributes as well as physiological and biochemical parameters of the crop significantly at both the growth stages. However, EBL applied at 10^{-6} M to 10^{-5} M did not further improve all the attributes but it significantly proved better in comparison to control for above studied attributes.

Growth attributes

The foliar application of EBL significantly increased the growth attributes of *C. roseus* at both the growth stages. There was a progressive increase in values of growth parameters with the increase in EBL concentration up to 10^{-7} M. At 10^{-7} M EBL, the values declined significantly but gave higher values compared to the control (Tables 1). Application of EBL at 10^{-7} M increased the number of leaves by 33.2 and 18.5%, average leaf area by 9.2 and 10.9 %, leaf area index by 45.2 and 31.5%, leaf dry weight by 40.8 and 46.4%, stem dry weight by 61.2 and 44.3 %, and root dry weight by 60.6 and 67.4% in comparison to the control, at 6 and 9 MAP, respectively (Tables 1).

Physiological and biochemical attributes

EBL significantly increased the total chlorophyll content at both the stages i.e., at 6 MAP and 9 MAP, but the effect of EBL treatments on total carotenoid content was found not significant in the treated plants (Table 2). Of the five EBL concentrations, 10^{-7} resulted in the greatest increase in total chlorophyll content. As compared to the control, application of EBL at 10^{-7} M enhanced the total chlorophyll content by 27.1 and 35.7% at 6 and 9 MAP, respectively (Table 2). At both the growth stages, the activity of NR was significantly improved by EBL application. 10^{-7} M of EBL surpassed the control by 14.5 and 10.3% with regard to NR activity at 6 and 9 MAP, respectively (Table 2). Application of EBL also positively improved the CA activity at both the growth stages. Compared to the control, 10^{-7} M of EBL resulted in 8.1 and 6.6% increase in CA activity, at 6 and 9 MAP, respectively (Table 2). A progressive increase in leaf nitrogen, phosphorus and potassium content was noted from control to EBL 10^{-7}M that proved optimum at both the stages. However, the maximum enhancement was observed at 9 MAP. Application of EBL at 10^{-7} M increased leaf nitrogen by 28.8 and 36.9%, phosphorus by 15.7 and 21.1% and potassium content by 4.2 and 8.6% respectively at 6 MAP and 9 MAP, respectively (Table 2).

Table 3.1: Effect of five foliar concentrations of epibrassinolide (EBL) on various growth attributes of *Catharanthus roseus* L. at 6 and 9 MAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Growth attributes	EBL Concentrations (M)						
	MAP	10^{-0}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	LSD at 5%
Number of leaves per plant	6	209.3^{e}	270.0^{b}	278.8^{a}	245.0^{c}	231.8^{d}	5.7
	9	255.0^{d}	295.0^{ab}	302.3^{a}	289.0^{b}	271.5^{c}	6.6
Average leaf area (cm^2)	6	12.75^{e}	13.60^{b}	13.92^{a}	13.28^{c}	12.95^{d}	0.15
	9	10.75^{e}	11.54^{b}	11.92^{a}	11.21^{c}	11.02^{d}	0.17
Leaf area index (cm^2)	6	8.47^{e}	11.66^{b}	12.30^{a}	10.33^{c}	9.51^{d}	0.62
	9	8.70^{e}	10.81^{b}	11.44^{a}	10.28^{c}	9.50^{d}	0.54
Leaf dry weight (g)	6	11.06^{d}	15.10^{ab}	15.57^{a}	13.75^{b}	12.76^{c}	0.64
	9	11.14^{d}	15.93^{b}	16.31^{a}	15.61^{bc}	14.63^{c}	0.74
Stem dry weight (g)	6	4.30^{e}	6.64^{b}	6.93^{a}	5.52^{c}	5.03^{d}	0.20
	9	5.50^{e}	7.33^{b}	7.94^{a}	6.40^{c}	6.01^{d}	0.34
Root dry weight (g)	6	3.02^{d}	4.77^{ab}	4.85^{a}	4.07^{b}	3.65^{c}	0.11
	9	4.03^{e}	6.53^{b}	6.75^{a}	5.83^{c}	5.28^{d}	0.18

Table 3.2: Effect of five foliar concentrations of epibrassinolide (EBL) on various biochemical attributes of *Catharanthus roseus* L. at 6 and 9 MAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Biochemical attributes	EBL Concentrations (M)						
	MAP	10^{-0}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	LSD at 5%
Total chlorophyll content (mg g^{-1} FW)	6	1.206^{e}	1.362^{b}	1.533^{a}	1.307^{c}	1.269^{d}	0.032
	9	1.027^{d}	1.317^{b}	1.394^{a}	1.114^{c}	1.032^{cd}	0.037
Total carotenoids contents (mg g^{-1} FW)	6	0.194^{a}	0.220^{a}	0.222^{a}	0.202^{a}	0.202^{a}	NS
	9	0.187^{a}	0.203^{a}	0.203^{a}	0.202^{a}	0.193^{a}	NS
Nitrate reductase activity [nM NO_2^- g^{-1}(FW)h^{-1}]	6	213.9^{c}	244.1^{ab}	245.0^{a}	228.8^{b}	225.1^{bc}	4.7
	9	205.1^{c}	223.1^{ab}	226.2^{a}	216.2^{b}	207.3^{bc}	5.8
Carbonic anhydrase activity [mol (CO_2) kg^{-1} (FW) s^{-1}]	6	5.35^{d}	5.77^{ab}	5.78^{a}	5.56^{b}	5.47^{c}	0.04
	9	5.19^{d}	5.52^{ab}	5.53^{a}	5.32^{b}	5.21^{c}	0.03
Leaf nitrogen content (%)	6	3.077^{d}	3.413^{b}	3.962^{a}	3.355^{c}	3.421^{ab}	0.050
	9	2.350^{e}	3.111^{b}	3.218^{a}	2.999^{c}	2.621^{d}	0.041
Leaf phosphorus content (%)	6	0.362^{d}	0.416^{ab}	0.419^{a}	0.397^{b}	0.379^{c}	0.009
	9	0.304^{d}	0.362^{ab}	0.368^{a}	0.341^{b}	0.330^{c}	0.005
Leaf potassium content (%)	6	3.555^{c}	3.644^{ab}	3.705^{a}	3.642^{b}	3.576^{bc}	0.036
	9	2.992^{c}	3.205^{ab}	3.249^{a}	3.175^{b}	3.019^{bc}	0.035

Content and Yield attributes

The spray of EBL at the concentration of 10^{-7} M resulted in the maximum increase in the content and yield of leaf and root alkaloids. However, the effect of EBL treatments on stem alkaloid content and yield was found not significant. The EBL treatment at 10^{-7} M exceeded the control by 6.5 and 9.8% in leaf alkaloid content and by 49.4 and 60.7% in leaf alkaloid yield, as recorded at the two stages, respectively (Table 3). The treatment also increased the root alkaloid content by 6.4 and 6.2% and the root alkaloid yield by 73.9 and 76.9% at 6 and 9 MAP, respectively (Table 3).

Table 3.3: Effect of five foliar concentrations of epibrassinolide (EBL) on various yield and quality attributes of *Catharanthus roseus* L. at 6 and 9 MAP. Means within a column followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Yield and Quality Attributes	EBL Concentrations (M)						
	MAP	10^{-0}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	LSD at 5%
Leaf alkaloid content (%)	6	0.77^{c}	0.81^{ab}	0.82^{a}	0.79^{b}	0.78^{bc}	0.01
	9	0.71^{d}	0.77^{ab}	0.78^{a}	0.75^{b}	0.73^{c}	0.01
Stem alkaloid content (%)	6	0.28^{a}	0.36^{a}	0.37^{a}	0.33^{a}	0.32^{a}	NS
	9	0.26^{a}	0.35^{a}	0.35^{a}	0.33^{a}	0.27^{a}	NS
Root alkaloid content (%)	6	1.55^{c}	1.63^{ab}	1.65^{a}	1.59^{bc}	1.57^{b}	0.02
	9	1.62^{d}	1.71^{ab}	1.72^{a}	1.67^{b}	1.64^{c}	0.01
Leaf alkaloid yield per plant (µg)	6	85^{e}	123^{b}	127^{a}	108^{c}	100^{d}	2.5
	9	79^{e}	122^{b}	127^{a}	117^{c}	106^{d}	1.9
Stem alkaloid yield per plant (µg)	6	12^{a}	24^{a}	26^{a}	18^{a}	16^{a}	NS
	9	15^{a}	24^{a}	26^{a}	19^{a}	17^{a}	NS
Root alkaloid yield per plant (µg)	6	46^{e}	77^{b}	80^{a}	64^{c}	57^{d}	2.2
	9	65^{e}	110^{b}	115^{a}	97^{c}	86^{d}	2.5

4. Discussion

Brassinosteroids (BRs) are cholestane derivatives with significant growth-promoting activity. Brassinolide (BL) is considered to be the end-product of biosynthetic pathway of BRs and shows the highest biological activity among BRs (Grove *et al*., 1979) [27]. BRs are considered as hormones with pleiotropic effects, as they influence various developmental processes like plant growth, germination of seeds, rhizogenesis, flowering and senescence, etc. (Rao *et al*., 2002) [28]. Besides, BRs elicit various physiological responses and are essential for male fertility and xylem differentiation (Müssig and Altmann, 1999) [29].

In this experiment the leaves of *Catharanthus roseus* were sprayed with different molar concentrations of EBL. The treated plants were compared with the control ones at two growth stages viz. 6 MAP and 9 MAP. Compared to control plants, EBL-treated plants showed considerable improvement in growth attributes. Number of leaves and average leaf area were influenced positively at both the stages (Tables 1). Due to the foliar effect of different EBL concentrations the overall as well as discretely leaf, stem and root dry mass were augmented (Tables 1). Growth promoting effects of BRs have been observed in many crops by a number of researchers. The positive effects of EBL were also suggested in the cases of *Arachis hypogaea* (Vardhini and Rao, 1998) [30], pepper plants (Houimli, *et al.*, 2010) [31] *Pisum sativum* L. (Shahid *et al*., 2011) [32] and *Brassica juncea* L. (Arora *et al*., 2012; Fariduddin *et al.* 2015) [33, 34] Their growth promoting effect results primarily from the stimulation of cell elongation and includes induction of the expression of genes encoding proteins such as xyloglucan endotransglycosylases (XETs) (Xu *et al*., 1995) [35], which are probably involved in cell wall metabolism and loosening. Moreover, effects such as cell-wall space acidification appear to contribute to BRs-induced growth stimulation. Effects of BRs on cell division are less clear; however, the induction of CycD3 transcription by epibrassinolide (EBL) might represent a mechanism by which BRs can drive cell division (Hu *et al*., 2000) [36]. Though our understanding of the molecular mechanism of action of brassinosteroids is still in its infancy stage, few years ago, a leucine-rich protein (BRI1) from *Arabidopsis thaliana* has been identified, which is considered as the receptor of BRs (Li and Chory, 1997) [37]. Unlike in animal system, where receptors for steroid hormones are intracellular, the receptor of BRs (BRI1) is located in the plasma membrane. It functions at the cell surface and transduces extra-cellular signals (Clouse and Sasse, 1998) [8]. Further, the binding of BR molecule to the receptor causes activation of the kinase domain and the subsequent phosphorylation of additional kinases and/or phosphatases (Chow and McCourt, 2006) [38].

Foliar application of EBL significantly influenced most of the biochemical parameter (Tables 2). However, EBL application did not affect the content of total carotenoid in the leaves. Rest of the parameters, viz. leaf chlorophyll content, NR and CA activity and the content of leaf-N and -P and -K were notably enhanced by EBL application at both the growth stages (Tables 2). There are evidences, that BRs do not undergo long-distance transport (Symons *et al*., 2006) [39], however, they may influence long-distance signaling by altering auxin transport (Paponov *et al*., 2005; Wiśniewska *et al*., 2006) [40, 41]. Probably, after perception in the leaves, the signals of BRs might interfere with the activities of said parameters within the leaves.

Various EBL concentrations positively influenced the leaf and root alkaloid content significantly (Table 3). The yield of leaf and root alkaloids per plant was also affected significantly by EBL application (Table 3). However, the content and yield of stem alkaloids does not affected significantly by EBL concentrations. The biosynthesis of alkaloids is an integration of several metabolic pathways, which require linking of several steps such as continuous production of precursors, their transport and translocation to the active site of synthesis, and finally the transport of alkaloids to accumulation site. This sequence of steps depends on normal functioning of associated metabolic pathways. Any disorder in normal metabolic pathways also affects the sequence of steps in biosynthesis. Accordingly, a plant may alter/adopt its specific metabolic pathway in response to particular effect, such as nutrient imbalance, hormone application, etc. EBL is a potent growth regulator that has proved efficient in altering the metabolic pathway in the case of several medicinal plants such as lavender (Youssef and Talaat, 1998) [42], mint (Maia *et al*., 2004; Naeem *et al*., 2012) [43, 5] and geranium (Swamy and Rao, 2009) [44]. In this study, a positive correlation of growth parameters as well as metabolic enzymes viz. Nitrate reductase and Carbonic anhydrase with alkaloids yield depicts the respective contribution of these parameters to alkaloids yield in various plant parts.

5. Conclusion

Our findings give a clear impression that all the physiological, biochemical and growth parameters were significantly improved caused by EBL application. EBL at 10^{-7} M improved significantly the crop growth and yield, leaf -N, -P,-K and other physiological attributes as well as the content and yield of alkaloids. It also improved the total contents of indole alkaloids of leaves and roots of *C. roseus*. Thus, application of EBL as foliar spray could be used to enhance the crop productivity and quality including alkaloids production in *C. roseus*.

6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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