RESULTS AND DISCUSSION

A. Growth Studies

1. Effect of pre-treatment of PGRs or Dipping method

The effect of pre-treatment of stem cuttings of *Coleus forskohlii* with low and high concentrations of PGR solutions triacontanol, brassinosteroids, CCC and ethephon on various growth parameters of *Coleus forskohlii* evaluated at various growth stages such as 45 DAP, 90 DAP and (135 DAP) at the maturity stage can be evident from Fig. 1 to Fig. 18 and from plates 1(A) to 3(C).

The plant growth and development is very complex process which is significantly influenced by plant growth regulators either of natural or synthetic origin. The role of PGRs in modifying plant growth and development is well established in agriculture, horticulture and also in medicinal plant cultivation so far. PGRs are extensively used in order to get enhanced production of desired biochemical product / plant / plant parts from medicinally important plants. PGRs give desired effect either by modifying source - sink relationship or by accelerating physio-biochemical processes in plants/plant parts or by expediting dry matter accumulation. Review of literature revealed that apart from foliar spray treatment of PGRs on well grown coleus plants, seed treatment or stem cutting treatment with PGRs before plantation also gives positive results w. r. t. plant growth of medicinal plant *Coleus forskohlii*. (Swamy and Rao (2010, 2011)). So, in the present investigation an effort has been made to assess the effect of dipping / Pre-treatment of stem cuttings in PGR solutions before plantation on growth, development and dry matter partitioning of *Coleus forskohlii*.

It can be clearly noticeable from fig. 1 to fig. 18 and the plates from 1(A) to 3(C) that all the growth parameters of *Coleus forskohlii* such as average shoot length, average number of leaves, average leaf length and breadth, average leaf area, average total leaf area per plant, average number of branches, circumference of stem base, leaf fresh weight and dry weight, stem fresh weight and dry weight, average total number of tuberized roots, root length, root circumference, root fresh and dry weight evaluated at three successive growth stages with an interval of 45 days (at 45 DAP, 90 DAP and 135 DAP or maturity stage) exhibited constant increment in all values along with increase in growth or age of plant from zero growth stage to maturity of plant (at 135 DAP). It can be also seen that there is overall increment in the levels of all the growth parameters of *Coleus forskohlii* plants subjected to pre-treatment of both the
concentrations of triacontanol, brassinosteroids, CCC and ethephon as compared to control except for high concentration of ethephon which is found to be slightly inferior in increasing all the growth parameters of *Coleus forskohlii* as compared to all the other PGRs tried but certainly superior than control treatment.

To illustrate it further, it can be clearly seen from all the figures from fig. 1 to fig. 18 and plates 1(A) and 1(B) that at 45 DAP growth analysis of *Coleus forskohlii*, there is no or very slight variation in levels of all the growth parameters due to all the PGRs pre-treatment and control except for number of roots and root tuberization which is found to be slightly elevated due to all PGRs as compared to control. It can be also evident from figures and plates that both doses of triacontanol, brassinosteroids and ethephon induced early root tuberization and root length stimulation as compared to control while both doses of CCC failed to do so at 45 DAP.

It is clear from fig. 1 to fig. 18 and plates 2(A) to 2(C) that at 90 DAP growth stage analysis, both doses of triacontanol effectively increased all the growth characters mentioned earlier against control however low dose of triacontanol is found more promising.

In case of effect of BRs, it can be seen that at 90 DAP, both doses of BRs have positively influenced the growth attributes of *Coleus forskohlii* like shoot height, leaf length and breadth, average leaf area per plant, fresh and dry weights of leaf and root, root length, average root and stem-base circumference however total number of leaves, total leaf area, number of branches, number of roots, stem fresh weight and dry weight found to be decreased as compared to control. Also, high concentration of BR is more dominant than low concentration. CCC at both doses caused increase in shoot height, leaf fresh and dry mass, total number of tuberized roots, root circumference, root length, root fresh weight and dry weight however caused decrease in total number of leaves, total leaf area, number of branches, stem fresh wt. and dry wt. while there observed no variation in leaf length, breadth and average leaf area. Both doses of CCC found to cause similar level of effect. Ethephon at 90 DAP found to decrease total number of leaves, branches and stem dry weight however increased shoot height, leaf length and breadth, leaf area, total leaf area, leaf fresh weight and dry weight, stem fresh weight, total number of roots, root length, root fresh weight and dry weight, root circumference and stem base circumference in *C. forskohlii*. 
RESULTS AND DISCUSSION

Lower dose of ethephon is found to be more prominent in causing positive effect on growth as compared to control and high dose of ethephon.

It is clearly seen in fig. 1 to fig. 18 and plates 3(A) to 3(C) that at 135 DAP or at maturity stage of *C. forskohlii*, the plants subjected to pre-treatment by PGRs shows distinct increase in all growth parameters as compared to control or D. W pre-treatment. It can be clearly revealed that shoot height is increased due to all PGRs against control except for high dose of ethephon. Maximum shoot height is found due to high dosage of BRs. Growth attributes like number of leaves, leaf area, number of branches, leaf fresh weight and dry weight, stem fresh weight and dry weight are found to be enhanced due to all PGRs pre-treatment as compared to control. Elevated results are obtained in plants subjected to triacontanol, BRs and ethephon (low dose) pre-treatment. In case of root growth, parameters like total number of tuberized roots, root length, root fresh and dry weight and root circumference are increased in response to all PGRs treatment tried as compared to control. Stem base circumference is found highest in case of ethephon pre-treatment. Among all PGRs treatments tried both concentrations of CCC are found to be less effective than all other PGRs but certainly gave better results than control. Both concentrations of triacontanol, BRs and ethephon at low concentration found to be more promising as a pre-treatment causing significant increment in growth in coleus.

Regarding dry matter partitioning and determination of source-sink relationship w. r. t. PGRs application the root/total dry weight ratio at the harvest or maturity stage was taken into consideration. The effect of pre-treatment of various PGRs and control on ratio of root dry weight to whole plant dry weight is presented in Table no. 2 and from Fig. 19 to 28. It is evident from the figures that both concentrations of all the PGRs like triacontanol, BRs, CCC and ethephon caused increment in the root/ total dry weight ratio as compared to control. All the PGRs caused increase in dry matter accumulation in root part than leaf and stem part of plant against control pre-treatment and thus increase in economic yield. Though all PGRs show high level of root/total dry weight ratio there is very less variation in ratio found between low and high concentration of same PGR however, triacontanol and ethephon show maximum positive effect on root dry matter partitioning. It clearly indicates that all the PGRs tried as a pre-treatment accelerated the flow of assimilates more towards root part of plant *C. forskohlii* against control and thus influenced the
RESULTS AND DISCUSSION

source-sink relationship favourably. Number of attempts have been made by various workers to evaluate the effect pre-treatment with PGRs on various growth parameters of several plants.

Ries and Wert (1977) reported increased dry weight and leaf area of a whole plant in case of rice (*Oryza sativa* L.) seedlings subjected to triacontanol applied in nutrient culture solutions. Sumeria (2003) reported increased plant height, dry matter accumulation per plant, number of primary and secondary branches per plant and maximum yield in case of mustard plant (*Brassica juncea* L.) in response to triacontanol granules applied at a rate of 30kg/ha in soil. Swamy (2004) studied effect of triacontanol on Ashwagandha (*Withania somnifera* Dunal.). He tried three methods of triacontanol application Viz. seed soaking, root dipping of seedlings and foliar spray application. He reported that among three methods of application of triacontanol, influence of foliar spray and seed soaking method were more significant on growth of plant as compared to root dipping method. This finding is not in concert with the present findings as in case of *Coleus forskohlii* triacontanol gives positive effect due to both ways of applications, as foliar spray and also as a dipping of stem cutting. Tantos *et al.*, (1999 and 2001) and Verma *et al.*, (2011) have already proposed that the provision of triacontanol into nutrient media could increase the number of roots per plant. Pal *et al.*, (2009) also proposed that triacontanol increases leaf area index along with over all increment in growth of plant. Sitinjak and Pandiangan (2014) assessed the effect of plant growth regulator triacontanol as a pre-treatment supplied to the pot mixture on the growth of cacao seedlings (*Theobroma cacao* L.) and reported 1.0 ml/L as a best triacontanol concentration which effectively increased the advancement of cacao seedlings, height of seedlings, leaf number, length of leaves and diameter of stems against the control which is in agreement with the present findings. Review of literature reveals that there are very less number of references regarding the effect of pre-treatment/dipping of stem cuttings with triacontanol on medicinal plants though there are plenty regarding seed pre-treatment or incorporation into nutrient solution in *In vitro* studies. However, Meenakshi and Lingakumar (2011) assessed effect of soaking of stem cuttings in 2, 4-D and IAA solutions in medicinal plant *Mentha arvensis* L. and recorded increased overall vegetative growth measured in terms of shoot, root length, total fresh weight and dry
weight, leaf area and leaf numbers per plant. Similar PGRs solution soaking method was applied by Swamy and Rao (2010) in case of Coleus forskohlii stem cuttings. According to Cavusoglu (2007 and 2008) and Sitinjak and Pandiangan (2014) increment in stem base diameter due to triacontanol can be attributed to the endogenous auxin produced by actively growing leaves or shoots. Auxin synthesized in upper plant parts is transported to stem base where it stimulates cell division in vascular cambium involved in secondary growth which in turn causes stem elongation and enlargement. This results in increase in stem diameter by increasing width of xylem and phloem and thus help in better transport in between root and shoot. The increased plant height might be attributed to increased cell division and cell elongation in response to stem basal application of triacontanol. Increased number of leaves, leaf area, number of branches in response to triacontanol might be attributed to apical polar transport of auxin to basal auxiliary buds due to PGR pre-treatment as discussed earlier.

Observations made by Ronsch et al., (1993) goes in tune with the present findings as they also reported similar kind of enhancement in root initiation and root growth on stem cuttings of Norway spruce (Picea abies Karst.) in response to the application of 22,23-diepi-28-homobrassinolide. Bobrik et al., (1998) and Khrpach et al., (2000) reported that potato cuttings cultured in a medium supplied with 24-epibrassinolide or 28-homobrassinolide significantly encouraged all the important characters of growth and development and increased the yield of potato. Swamy and Rao (2006) also recorded improved rooting, increased root growth and all other shoot growth parameters in geranium (Pelargonium graveolens L.) Bourbon type plant stem cuttings subjected to 24-EBR and 28-HBR treatment. Increase in foliage growth due to BRs application results in increased photosynthesis and in turn better growth of the plant. Increased rate of photosynthesis in response to BRs treatment is also reported by Bajguz and Czerpak (1998) in green alga Chlorella vulgaris Beijerinck. Similar kind of increment in foliage growth and photosynthesis is observed by Swamy and Rao (2008) in Geranium (Pelargonium graveolens L.). This result goes in tune with the present observations. Swamy and Rao (2010) also assessed the effect of dipping of stem cuttings of Coleus forskohlii in 24-EBR and 28-HBR solutions on its growth and development. They reported increased rhizogenesis in terms of number of roots, number of leaves, total leaf area, shoot length, fresh and dry weight of leaf, root and
RESULTS AND DISCUSSION

shoot along with increased root tuber harvest. These findings are in agreement with
the present results. They also proposed that 28-HBR at 100µM concentration is best
suitable for *Coleus forskohlii*. Mussig *et al.*, (2003) proposed that there exists a
positive correlation between the concentration of endogenous BRs and root growth in
*Arabidopsis* sp. which clearly indicates that BRs play role in root formation. These
findings support the growth promoting ability of BRs and the present findings.

Tolbert (1960) found that CCC application either by soil application or by
adding in nutrient media ranging from $10^{-2}$ to $10^{-6}$M concentration was positively
effective in wheat plant. Larter *et al.*, (1965) in barley recorded higher tiller number
and higher diameter of main tiller but reduced internodal elongation causing shorter
plant height in barley Cv. Parkland but no significant decrease in plant height in
variety Cv. Hannchen in response to CCC applied both as a soil drench and as a spray.
In present investigation also CCC could not shorten the shoot height of Coleus
characteristically as a growth retardant when applied as a dipping method though
affected as a foliar spray. This might be because of plant's ability to overcome PGRs
effect in mean course of growth and might also depends on organ of plant exposed to
PGRs and exposure time. This is supported by Cathey (1964) and Larter *et al.*, (1965)
who stated that plants with a uniform growth rate are not retarded in growth in
response to CCC.

Mullins (1972) in case of *Phaseolus aureus* (Roxb.) cuttings reported
inhibition of rooting due to ethephon treatment whereas Krishnamoorthy (1972) in
moong bean hypocotyl cutting reported increased rooting due to ethephon. Mudge and
Swanson (1978) studied its efficacy on rooting in light grown mungbean cuttings
exposed to dip treatment of ethephon for 24 hr. in humidified growth chamber which
were then allowed to grow in distilled water for 5 days. They reported no effect of this
ethephon pre-treatment on rooting of mungbean cuttings. These findings are differing
with the present findings as in present study, there is observed increased root growth
measured in terms of root length, no. of adventitious and tuberized roots, root fresh
weight and dry weight of coleus due to ethephon dipping treatment. Shrivastava *et al*.,
(1998) reported increased growth and yield of dry matter in Palmarosa (*Cymbopogon
martinii*) plant 4 varieties PRC-1, Tripta, Trishna and Composite in response to
eethephon at 500µg/ml concentration which was applied by wrapping ethephon soaked
cotton around the stem of palmarosa for two times first during the vegetative stage
and later at anthesis stage. Khuankaew et al., (2009) witnessed decreased plant height but no effect on rhizome size, length and number of storage roots in *Curcuma alismatifolia* Gagnep. plant subjected to ethephon application by soil drenching method. Efficacy of ethrel pre-treatment was studied by Misra et al., (2009) in *Catharanthus roseus* L. seedlings by dipping rooted seedlings in various concentration solutions of ethrel for 24 hr. and pre-treated seedlings were then planted in earthen pots. They reported overall stunted growth in *Catharanthus roseus* plants measured in terms of shoot height, number of leaves, herb yield. This finding goes in tune with the present findings as far as effect of high concentration of ethephon on coleus is concerned.

Thus, it can be concluded that both concentrations of triacontanol, BRs, Ethephon at low dose and CCC to some extent are advantageous over control in overall growth enhancement, source-sink translocation and dry matter partitioning of *C. forskohlii* when applied as a pretreatment

2. Influence of foliar spray of PGRs

The influence of foliar application of both concentrations of plant growth regulators triacontanol, brassinosteroids, CCC and ethephon on various growth parameters of *Coleus forskohlii*, assessed at the two growth stages such as at the 2.5-month-old age of coleus plants (75 DAT (days after transplantation)) and at the maturity/harvesting stage (165 days) can be observed from Fig. 29 to Fig. 46 and from plates 4(A) to 5(D).

It can be clearly recognized from the Fig. 29 to Fig. 46 that all the growth parameters of *Coleus forskohlii* studied at two successive growth stages i. e. at the 2.5 month old age (75DAP) and at the harvesting stage or at 165 DAP such as average shoot length, average number of leaves, average leaf length and breadth, average leaf area, total leaf area per plant, root fresh weight and dry weight are positively influenced by both the concentration of triacontanol, brassinosteroids, CCC except for ethephon as only low concentration of ethephon is found to be effective in increasing all the growth parameters of Coleus as compared to control while high concentration of ethephon show negative effect on all growth characters of both aboveground and underground plant parts of coleus.

To carry out any project to increase the growth, yield and productivity of any economically important crop, it is necessary to consider source-sink relationship in
that plant. Such studies had been carried extensively in cereal crops and also in pulses but it is not true in case of non-conventional crops and especially in case of medicinal plants such as *Coleus forskohlii*. As per the opinion of Marcelis, (1996) source is broadly termed as an organ of plant which is net exporter of carbon assimilates. The source organs are but obviously leaves which are major sites of carbon assimilation. So, factors such as leaf number, leaf area, leaf thickness, canopy of plant also biochemical components such as activity of photosynthetic enzymes and products and other enzymes like sucrose phosphate synthase, phosphatases etc. determines the source strength (Sonnewald and Willmitzer, 1992, Nikam, 2007). Thus, the source strength depends upon the rate at which carbon assimilates are produced and exported to sink parts.

Sink regions are those parts of the plant to which assimilates are transported from source tissue. There are mainly two types of sink tissues such as utilization sinks and storage sinks. Utilization sinks are actively growing plant parts such as actively dividing meristematic cells which utilizes the imported assimilates for growth processes. In contrast, the storage sinks are those plant tissues where carbon assimilates are stored for long term e. g. seeds in case of cereals, pulses etc. while in case of *Coleus forskohlii*, sweet potato, sugar beet, Chlorophytum etc. tuberous roots are the main sinks. Sink strength determines the productivity of the crop. The sink strength is the competitive capacity of plant organ to receive or import assimilates (Farrar, 1993). According to Warren (1972) the sink strength is the product of sink size and sink activity. It can also be stated as the product of sink weight and relative growth rate. Wardlaw (1990) stated that sink strength depends on sink size, as increase in sink size increases sink strength. It is associated with increase in the size of the surface area across which metabolites are transported from the vascular system to the area of utilization. In case of *Coleus forskohlii* tuberous roots are major storage sink. To avoid the competition between the sink organs, it is general cultural practice followed in crops like onion, sweet potato, chlorophytum etc. is to eliminate the reproductive parts or inflorescences to avoid transport of assimilates to reproductive parts. In case of coleus plant also inflorescences are removed time to time.

It is also necessary to have knowledge about dry matter partitioning to study the effect of various growth promoting factors on yield and productivity of that plant. The term dry matter partitioning can be broadly defined as the distribution of dry
RESULTS AND DISCUSSION

matter between various organs of a plant and this is nothing but the fractions of dry matter of the whole plant (Marcelis, 1996, Nikam, 2007). In case of present investigation of *Coleus forskohlii* the root yield or dry matter production of root tissue is most important than shoot. According to Gudhate *et al.*, (2009) in *Andrographis paniculata* it confirmed that treatments of various plant growth regulators might be responsible for increased transport of assimilates from source to sink and thus yield of economically important plant parts which is found to be true in present study. The importance of plant growth regulators is increased in agriculture due to the capacity of PGRs to manipulate the source sink relationship. Several attempts have been made by many workers to study the effect of various plant growth regulators on the growth, development and dry matter production and partitioning in various plants.

It is evident from the Fig. 29 to Fig. 46 and plates 4(A) to 4(C) of 2.5 month old growth analysis and plates 5(A) to 5(D) of harvest stage growth analysis that in the present study due to both concentrations of triacontanol there is significant increment in all the growth attributes of both aboveground and underground plant parts such as average shoot length, average number of leaves, average leaf length and breadth, average leaf area, total leaf area per plant, number of branches, circumference of stem base, leaf fresh weight and dry weight, stem fresh weight and dry weight, total number of tuberized roots, root length, root circumference, root fresh weight and dry weight but high dose of triacontanol was more promotive.

It can be clearly seen from Fig. 29 to Fig. 46 and plates 4(A) to 4(C) of 2.5-month old growth analysis and plates 5(A) to 5(D) of harvest stage growth analysis that foliar sprays of both concentrations of brassinosteroids are positively effective in enhancement of all growth characters of coleus studied as compared to control. Low concentration of BRs more effectively stimulated average shoot length, average number of leaves, average leaf length and breadth, average leaf area, total leaf area per plant, leaf fresh weight and dry weight per plant while high concentration of BRs positively influenced number of branches, circumference of stem base, stem fresh weight and dry weight, total number of tuberized roots, average root length, root circumference, root fresh and dry weight. Thus, it can be seen that both doses of BRs improved source-sink transportation and increased root yield as compared to control.

From Fig. 29 to Fig. 46 it can be clearly seen that foliar application of both concentrations of CCC are found to be effective in increasing all the growth
parameters at the harvesting stage of Coleus such as number of leaves per plant, average leaf area per plant, total leaf area, number of branches, circumference of stem and root, leaf fresh weight and dry weight, stem fresh and dry weight, total number of roots, root length, root fresh weight and dry weight except shoot height as compared to control treatment.

It is evident from the figures that in the present investigation, the foliar spray of high concentration of ethephon caused negative effect on all the growth parameters of coleus except that it increased the circumference of the stem base against control and low concentration of ethephon where as low concentration of ethephon is found to enhance the total number of leaves, number of branches per plant, circumference of stem base, leaf fresh weight and dry weight, stem fresh weight and dry weight, total number of roots per plant, root length, root fresh weight and dry weight but plant height, leaf area and root circumference is found to be reduced due to low dose of ethephon as compared to control.

In the present investigation as per the definition given by Marcelis (1996) i. e. dry matter partitioning, is in fact the end result of processes acting on dry matter. In other words, dry matter partitioning is used in a relative sense referring to fractions of dry matter distributed in whole plant. In case of \( C. forskohlii \) variation in dry matter partitioning in response to PGR foliar application is evaluated by comparing the ratio of root dry weight to total dry weight in \( C. forskohlii \), both economically and medicinally. The ratio of root dry weight to total dry weight can be used as an indicator of flow of assimilates from source (leaf and stem) organ to sink (root) organ of plant and also as a measure of dry matter partitioning (Peres et al., 2001).

The influence of various concentrations of PGRs on dry matter partitioning measured in terms of ratio of root dry weight to total dry weight in \( C. forskohlii \) is represented in Table no. 4 and Fig. 47 and from Fig. 48 to 56. It can be clearly seen that the ratio of root/ total dry weight is increased due to both concentrations of triacontanol, ethephon and low concentration of CCC as compared to control. Thus, it can be put forth that the accumulation of dry matter in root part than other plant parts is accelerated due to these PGRs. Also, as compared to control, foliar spray by triacontanol, ethephon and CCC stimulates the flow of assimilates more towards sink (root) from source (leaves).
Thus, the prominent differences observed in root/total dry weight ratio among the control and all other PGR treated plants seem to be determined by the PGRs applied as a spray or endogenous plant hormone levels in shoot and root and their interaction with other factors which affects transport of assimilates either towards the shoot or the root system. This determines the suitability of that PGR as a growth promoter in enhancing the economic yield of the plant.


In case of Opium poppy (Papaver somniferum L.) plants treated with triacontanol at 0.01 mg L\(^{-1}\) concentration Srivastava and Sharma (1990) observed increase in plant height, shoot fresh and dry weight and capsule number. Misra and Srivastava (1991) in lemongrass (Cymbopogon flexuosus (Steud.) Watts.) plant recorded overall increased growth w. r. t. increased plant height, biomass yield and other yield attributes due to Miraculan, a triacontanol formulation at 0.4 µg/ml.

Srivastava and Sharma (1991), Shukla et al., (1992), Vasundhara et al., (1992) and Gupta et al., (1992) reported increased plant height, leaf number, leaf area, shoot fresh and dry matter production and economic yield as a result to triacontanol in Mentha arvensis L. plant @0.1g/ml, in Aretemissia annua L. plant @1 and 5ppm, in Marjoram (Majorana hortensis Moench.) plant @ 6 ppm and in Ocimum carnosum plant @ 10 ppm concentration respectively.


In lemon grass (Variety ckp-25) Balyan et al., (1994) witnessed significant increment in shoot length, no. of leaves, leaf length and herbage yield in response to lower concentration of triacontanol. Similar observations were made by Bhattacharya and Rao (1996) in rose scented geranium plant (Pelargonium sp.) Cv. Bourbon. They also evidenced increased plant height, number of branches, leaves, fresh and dry
weights of leaves, stem along with increased root characters such as number of roots, total root length, root yield etc due to triacontanol and mixtalol. These findings support our present results in coleus in response to both low and high concentration of triacontanol.


The results found by Muthuchelian et al., (2003) about growth and biomass production are consistent with us. They reported that triacontanol treatment improved root and shoot length, leaf density, leaf area and fresh and dry weight of Erythrina variegata plants.

Liu-Hua et al., (2002), Samui and Roy (2007) and Mallick et al., (2009) reported positive effect of foliar spray of triaontanol on potato and its tuber yield along with increase in LAI, dry matter production. Naeem et al., (2009) found stimulatory effect on growth of Hycinth bean (Lablab purpureus L.) which is rich in enzyme tyrosinase and has medicinal value. They found increased plant height, plant fresh and dry weights, leaf area and leaf number due to 10⁻⁶ M concentration of triacontanol foliar spray.

In medicinal plants like Ashwagandha (Withania somnifera L.) and Artemisia annua L. growth promoting effect of triacontanol w. r. t. shoot height, number of leaves and branches, root and shoot fresh and dry weights was also confirmed by Nasir (2009) and Aftab et. al., (2010) respectively.

Parallel results were obtained by Hashmi et al., (2011) and Naeem et al., (2011) in case of Ocimum basilicum L. and Mentha arvensis L. respectively at 10⁻⁶ M concentration of triacontanol foliar spray. Observations made by Kamble and Chavan (2011) in medicinal plant vetiver (Vetiveria zizanoides) goes in tune with present
findings. They also reported increased tiller number, root and shoot dry weight of plant in both KS1 and local variety of vetiver grass.

Singh et al., (2012) revealed stimulatory effect of triacontanol foliar spray on growth and other attributes in ginger (Zingiber officinale Rosc.) plant at most suitable concentration $10^{-6}$ M. They recorded maximum shoot height, leaf density, number of tillers per plant, fresh and dry masses of shoots and rhizome and yield but they reported negative effect due to higher doses ($5 \times 10^{-5}$ M) of triacontanol. Khan et al., (2014) also found that $10^{-6}$ M concentration of triacontanol as best suitable for augmentation of all growth parameters of lemongrass (Cymbopogon flexuosus (Steud.) Wats.) var. Krishna such as increased shoot length, root length, number of leaves, number of tillers, shoot and root fresh and dry weights as compared to control. Stimulatory influence of triacontal was also confirmed by Sitinjak and Pandiangan (2014) in cacao seedlings (Theobroma cacao L.) at 1 ml/L dose.

There are various evidences supporting the role of triacontanol in reducing the adverse effects caused due to various stresses and resuming the normal growth after exposure to many stresses. Muthuchelian et al., (1996) and (1997) reported anti stress effect of triacontanol in Erythrina variegata plant exposed to salt stress and water stress respectively. Radhakrishnan and Ranjithakumari (2008) and Perveen et al., (2014) recorded ameliorating effect of triacontanol in soybean and wheat plants respectively which were exposed to salt stress.

Apart from these, Moorthy and Kathiresan (1993) studied influence of triacontanol treatment in viviparous hypocotyls of Rhizophora apiculata Blume. They reported enhanced growth of root and shoot, root length, number of primary and secondary roots, shoot height and biomass due to triacontanol but higher concentration decreased the growth parameters which is not in harmony with present investigation as both concentrations of triacontanol are found to be positively effective in coleus plant.

Hangarter and Ries (1978) proposed that triacontanol effectively increases growth due to increase in number of cells. As proposed by Ries and Wert (1992) the positive effect of triacontanol on plant growth and biomass accumulation might be due to the effective and rapid translocation of triacontanol throughout the plant causing cascade of metabolic activities resulting in significant enhancement in growth and development. According to Taiz and Zeiger (2004) positive effect of triacontanol
on plant height may be attributed to the very well-established effect of application of triacontanol on elongation of internodes through cell division and cell expansion. The triacontanol treated plants possessed comparatively higher leaf area than control which is found to be true in present study. According to study of Srivastava and Sharma (1991) and Naeem et al., (2010, 2011) in Mentha arvensis L. this increase in leaf area could presumably be due to stimulation of cell division and cell enlargement. They also proposed that triacontanol affects the overall growth of the plant by increasing three most important growth attributes of plant such as stem length and fresh and dry weights per plant which represents the overall growth of any plant resulting in betterment of overall growth and yield of plant. These views are in line with our present findings in this regard.

Similar kind of improvement in growth and development of various plants due to application BRs was witnessed by several workers. According to Clouse and Sasse (1998) BRs application stimulated cell elongation and thus shoot elongation or epicotyl or hypocotyl growth in dicots and coleoptile elongation in monocots. Root growth promotion in response to BRs application in terms of increase in root length, root fresh weight and root dry weight was reported by Schilling et al., (1991) in sugar beet tap root mass under drought stress, Vardhini and Rao (2003) in tomato plant, by Bao et al., (2004) in Arabidopsis thaliana by promoting lateral root development.

Abd El- Wahed et al., (2004) studied the impact of stigmasterol (@100ppm) on chamomile plant (Chamomilla recutita L.). Stigmasterol is an important sterol, necessary for normal growth and development as like brassinosteroids (He et al., 2003). They reported that exogenous application of stigmasterol significantly increased growth attributes such as plant height, no. branches or tillers, fresh and dry weights during vegetative growth.

Hayat et al., (2000 and 2001) and Fariduddin et al., (2004, 2005, 2008) reported enhanced level of all growth attributes in mustard (Brassica juncea) and in mung bean (Vigna radiata) plants in response to application of 28-Homobrassinolide foliar spray respectively.

Observations made by Ali et al., (2006) in two wheat cultivars subjected to salt stress and normal conditions along with BRs treatment supports present results. They reported improved growth and increased shoot and root fresh weight in two wheat cultivars (S-24) and (MH-97) due to BRs treatments under both normal and
RESULTS AND DISCUSSION

Saline condition. BRs treatment also ameliorated the adverse effects of salinity stress but higher concentration of BRs failed to do so.

Swamy and Rao (2006, 2008, 2009) assessed the positive outcome of 24-epiBL and 28-homoBL on plant growth, rooting, root growth and shoot growth of geranium plant (*Pelargonium graveolense* (L). Herit.) bourbon type, an important medicinal plant. They reported increased shoot length, number of branches and leaves, total leaf area, leaf fresh weight, shoot and root fresh weight and dry weight along with increase in doses of 24-epibrassinolide (EBR) and 3µM concentration of 28-Homobrassinolide (HBR) was reported as highly effective in improving growth of geranium. These evidences goes in agreement with present findings in case of coleus. In Barley plant also, enhanced root growth was reported by Kartal et al., (2009) due to homobrassinolide along with enlarged root tip.

The analysis performed by Swamy and Rao (2010) with BRs application in same plant *Coleus forskohlii* also goes in harmony with the present work. They investigated the effect of 28-HBR and 24-EBR on coleus and recorded that 28-homobrassinolide treatment at 100 µM concentration was strongly effective in induction of roots on coleus stem cuttings and root growth. They also observed enhanced fresh and dry weight of shoot, leaf area and thus overall improved plant growth.

Results obtained by Eskandari (2011) in case of Savory herb (*Satureja bachtiarica*) subjected to various levels of drought stress and various dosages of 28-homobrassinolide goes in tune with the results of present investigation. He reported that exogenous application of BRs improved growth of savory plant by increasing herbage yield, leaf number, dry weight of shoot, plant height, diameter of shoot, number of sub branches, total dry weight of roots. Same is found to be true in case of coleus plants due to both doses of BRs. He also proposed that BRs improved the adverse effect of drought stress on savory plants and improved all growth parameters most significantly at 10⁻⁸M concentration of 28-HBR. Swamy and Rao (2011) witnessed significantly stimulatory effect foliar spray of 24-EBR and 28-HBR on coleus (*Coleus forskohlii*) plant most suitably at 3µM concentration. They reported improved growth of coleus in terms of plant length, leaf number, leaf area and dry weights of leaves and shoot and about 2-fold increment in the tuberous root yield.
RESULTS AND DISCUSSION

Naeem et al., (2012b) evidenced best performance of HBR at $10^{-7}$M concentration on all growth parameters and herbage yield of medicinally important mint (*Mentha arvensis* L.) plant. They also reported that higher concentration of HBR exhibited slightly inferior results on growth of mint than $10^{-7}$M dose, but superior than control treatment. This is also found true in present investigation. Results obtained by Alam et al., (2012) regarding the growth attributes of Rosea and Alba varieties of *Catharanthus roseus* L. in response to homobrassinolide treatment at $10^{-7}$M concentration in terms of increase in shoot and root length, shoot and root fresh and dry weight, leaf area index etc. supports present results.

Root growth promotion by BRs application was also reported by Vardhini et al., (2012) in radish (*Raphanus sativus*) storage roots. They recorded enlarged root length, root fresh and dry weight due to all concentrations of 28-HBR and 24-EBR (@ 0.5 µM, 1 µM, 3 µM) tried as compared to control treatment in radish. However, 28-HBR at 3 µM concentration was found to be most effective.


The increased shoot length, number of branches, number of leaves and leaf area can be because of an expression of induced cell division and/or cell enlargement by BRs. Supportive evidences provided by various workers such as Steffens (1991), Sairam (1994), Pipattanawong et al., (1996), Clouse and Sasse (1998), Sakurai et al., (1999) and Hayat et al., (2012) reported that BRs affect both cell division and cell enlargement. According to Kalinich et al., (1985), Sasse (1985) and Sairam (1994) BRs induced growth in plant tissue might be as a result of increased cell division, RNA and DNA synthesis, protein synthesis and increased dark CO$_2$ fixation (Braun and Wild 1984). Clouse and Zurek (1991) have already observed stimulation of cell division in cultured cells of parenchyma of *Helianthus tuberosus* and Chinese cabbage and Petunia protoplasts of by Nakajima et al., (1996) and Oh and Clouse
RESULTS AND DISCUSSION

(1998) resp. due to BRs. According to Szekere and Koncz (1998) BRs play a significant and a unique role in the regulation of cell wall biosynthesis and its role in other physiological functions interdependent or additive with other PGRs like auxins, gibberellins and cytokinins. Observations made by Mussig et al., (2003) in Arabidopsis plant regarding root growth parameters also goes in agreement with present results in coleus plant such as root length, root diameter and number of roots. According to them low concentration of BRs promoted root elongation in Arabidopsis and root growth induction by BRs is independent of auxin and GA in Arabidopsis. Thus, overall increase in growth parameters of Coleus in present work might be attributed to collective effects of brassinosteroids on stimulation of cell division, cell elongation and/or cell enlargement, cell wall biosynthesis and other physiological functions.

Similar evidences regarding CCC effect were recorded by many workers. Radwan et al. (1971) in potato plant reported increased dry matter of leaves, total foliage and tubers and yield characters. They reported decreased shoot height along with increase in concentration of CCC which goes in line with present observations. Bhattacharjee et al., (1974) also reported that CCC at 2500-5000 ppm concentration pointedly improved the number and biomass of tuberous roots in *Dahlia variabilis*. This also goes in tune with present findings.

El-Antably et al., (1975a) and El-Antably et al., (1975b) recorded increased dry weights of stem, leaves and whole plant but decreased plant height in *Origanum majorana* L. and in *Solanum laciniatum* aiton. resp. due to foliar spray of CCC at 2000 ppm concentration. Niimi (1979) studied CCC effect on grape wine and reported increased dry weight of root but no change in shoot length and shoot dry weight. Influence of CCC on geranium plant was analysed by Mohandas and Sampath (1985) and they revealed that at higher concentration of CCC (400ppm), plant height was reduced against control but the foliage yield was improved.

Observations made by El-Khateeb and Selim (1988) in Peperomia plant in response to CCC (@2000ppm) application also supports present findings. They also reported decreased plant height but increased stem diameter, number of leaves per plant, number of branches per plant, maximum fresh and dry weights of leaves and darker green leaves as compared to control. Similar suppression in plant height was also recorded by Bhat et al., (1989) in davana (*Artemisia pallens* Wall.) plant in
RESULTS AND DISCUSSION

response to CCC at 2000ppm and 4000ppm doses. Shah and Prathapasenan, (1991) also observed increase in dry matter of leaves and pods in mungbean (Vigna radiate (L.) Wilczek var. Guj-2 due to CCC application at 1000ppm. Evidences given by Umesha (1988) in Clocium (Ocimum gratissimum L.) and Vasundhara et al., (1992) in Marjoram plants don't go in agreement with present findings except for reduced plant height as they reported decreased internodal length, reduced leaf area, number of branches and leaves and also reduced dry mass accumulation in both plants as compared to control. Abo-El- Kheir et al., (1994) also reported negative effect of CCC application on number of leaves, LAI and dry mass accumulation in soybean plant.

Reduced plant height due to CCC application was also reported by Pando and Srivastava (1985) in sunflower, by Grewal et al., (1993) in Brassica napus, by Mangalprasad and Rajendraprasad (1994) in cotton, by Rajput et al., (1996) in Indian mustard when sprayed at flower initiation stage, by Lone (2001) in Brassica juncea, by Koler (2008) in cotton at 40ppm, 60ppm and 80ppm at various growth stages. There are so many evidences which goes in harmony with present findings. Bhattacharya et al., (1995) also found increased number and yield of leaves and shoot in geranium plant due to CCC foliar application @2000ppm. Similar supportive observations were made by Chaudhary and Gupta (1996) in Catharanthus roseus L. who also reported increased number of leaves, increased leaf and root fresh and dry matter and dwarfism along with increase in CCC concentration. However, Borse and Dhumal (2001) recorded differing effect of CCC on plant height of Solanum kharianum as they reported enhanced plant height but they also reported increased number of branches and leaves per plant in response to CCC in Solanum kharianum.

Enhanced root yield was reported in medicinally important plant Withania somnifera (L.) due to combined effect of phosphobacteria and CCC application at 2000ppm concentration by Bharathkumar et al., (2001). Lone (2001) reported significant increase in dry mass of Brassica juncea cultivars due to CCC application at 400ppm. Results obtained by Bharad (2005) also proved the stimulatory effect of CCC on various growth aspects of Okra (Abelmoscus esculentus L.) plant such as number of branches, number of leaves, leaf area per plant, dry weight of leaves, stem and roots but negative effect on internodel length, root elongation and plant height. This is also found true in present work. Sridhar (2006) found retarded growth,
RESULTS AND DISCUSSION

reduced internode length but increased branching in jasmine (*Jasminum auriculatum* L.) plant in response to CCC.

Ameliorating effect of CCC was also reported by Khan *et al.*, (2008) in vetiver (*Vetiveria zizanoides*) plant under drought stress and UV-stress. They revealed that application CCC amended the negative effects of drought and UV-stress on vetiver plant w. r. t. plant height, root yield. They also reported enhanced root yield of vetiver in unstressed condition. Increased tuber yield and vegetative growth of potato var. Zhongshu-3 was reported in Southern China by Wang *et al.*, (2010) in response to foliar application of CCC at 1.5 and 2 g/l. Kamble and Chavan (2011) proved that CCC foliar sprays increased dry matter accumulation in shoot and root tissues of vetiver grass local variety. Shukla and Shukla (2012) also investigated the effect of foliar sprays of CCC at 2000ppm and 3000ppm on medicinal plant *Withania somnifera* Dubal. Ashwagandha plant Var. Poshita and JA 20. They revealed that both concentrations of CCC remarkably enhanced the weight of fresh and dry roots per plant but reduced plant height with short and dark green leaves which is also found true in present investigation. In studies performed by Hashemabadi *et al.*, (2012) to find effect of various foliar sprays of various combinations of CCC and B9 plant growth retardants on medicinally important plant *Calendula officinalis* L., they reported that 500mg/L CCC + 4500mg/L B9 and 500mg/L CCC + 1500mg/L B9 produced lowest plant height and highest number of leaves respectively. In cassava plant, foliar application of CCC 45 and 90mg of a. i. (active ingredient) per plant effectively controlled excessive vegetative growth by reducing plant height. It reduced tuberous root number but enhanced tuberous root fresh weight and thus increased harvest index and root dry mass (Medina *et al.*, 2012). Same is found true in present investigation. According to Zheng *et al.*, (2012) in Lilium oriental hybrid 'Sorborne', the plants showed enhanced level of biomass of leaves and stem along with increased number of leaves and its leaf area, stem height and bulb weight in response to CCC at 300ppm and thus produced more photoassimilates for transportation and utilization into bulb of plant. Dey (2013) noted that in *Stevia reboudiana* Bertoni there was remarkably reduced plant height and internodal length but increased total number of leaves, branches per plant and leaf area per plant over control. Though these results are in consonant with present findings there are some reports which do not go in agreement with present results regarding number of leaves.
and leaf area increment in coleus such as Pando and Srivastava (1985), Abo-El-Kheir et al., (1994), Lone (2001) who found reduced number of leaves and leaf area in sunflower, soybean, and Brassica juncea respectively in response to CCC. Anath and Kumar (2012) also found negative effect of CCC application on overall growth and yield of Nerium.

The decrease in plant height of coleus in present study due to both doses of CCC as compared to control appears to be due to decreased rate of cell division and reduced cell elongation and expansion as CCC is an antagibberellin dwarfing agent (Rademacher and Jung, 1986; kar et al., 1989; Choudhary and Gupta et al., 1996; Lone 2001). As it has been already stated by Moore (1980) that CCC inhibits gibberellin biosynthesis by hindering the conversion of geranyl pyrophosphate to copalyl pyrophosphate that is the primary step of gibberellin biosynthesis. This causes deficiency of gibberellin growth hormone causing dwarfing in plant. Grossman (1990) also reported that decrease in plant height is due to slowing down of transverse cell division specifically in cambium region at the base of the internode which is also the zone of high meristematic activity. The remarkable upsurge in sum of branches per plant due to CCC application especially due to high concentration of CCC in the present analysis might be due to inhibition of apical dominance as an output of increased auxin activity due to CCC. This might have caused diversion of polar transport of auxin towards the basal bud leading to excessive branching as compared to control treatment.

Both concentrations of CCC also increased both fresh and dry mass of root which is the most economically important part of coleus. This enhancement in root parameters might be attributed to dwarfing effect of CCC which resulted in saving of metabolites from root and thus accumulation of more metabolites in root tissue. Plant growth retardants like CCC usually increase the partitioning of assimilates to roots and improve root yield through the inhibition of gibberellin biosynthesis or function. In addition, growth retardants can be used to manage extra vegetative growth (Fletcher et al., 2000; Rademacher, 2000; Ghosh et al., 2010), and to increase quality characteristics such as dry matter and starch content of the economical plant part (Tsegaw & Hammes, 2005) and Coleus forskohlii plant is no exception for that. This is also supported by Choudhary and Gupta (1996), Bharathkumar et al., (2001), Medina et al., (2012) and Shukla and Shukla (2012).
RESULTS AND DISCUSSION

There are some reports which are in line with the present findings regarding the effect of ethephon on coleus growth. Mangal et al., (1981) reported reduced shoot length and increased no. of branches in response to ethrel treatment @ 500ppm in bitter gourd plant. Mohandas and Sampath (1985) also reported reduced plant height and foliage yield in geranium plant treated with ethrel spray at 4000ppm. It is also supported by Rafeekher et al., (2002) who also found reduced plant height but improved number of branches due to ethrel application at 200ppm in cucumber plant. But in case of geranium plant Cv. Bourbon, Rao et al., (1994) reported significant increase in growth parameters like plant length, number of branches, biomass production w. r. t. yield of leaves, stem and branches in response to foliar application of ethephon at 0.062%. These finding goes in agreement with the present results found in case of low concentration of ethephon. As per the observations made by Sridhar (2006) ethephon at 200ppm caused excessive dwarfism, reduced growth of lateral branches, number of internodes, number of leaves, its area and reduced vegetative growth in Jasminum auriculatum Vahl., jasmine plant. Similar negative effect is found in coleus due to high dose of ethephon in present work. Anti-stress or ameliorating effect of ethephon application on vetiver grass (Vetiveria zizanoides) subjected to drought stress was recorded by Khan et al., (2008). They also reported that in unstressed vetiver plants also there was reduction in plant height and increase in root yield as compared to control. Same is true in present case. Misra et al., (2009) reported significant decrease in all the growth attributes of Catharanthus roseus L. studied under the influence of ethrel application as compared to control. In case of Curcuma alismatifolia Gagnep the results obtained by Khuankaew et al., (2009) in response to ethephon drenching application goes in tune with present findings. They reported decreased plant height along with increase in concentration of ethrel in curcuma but rhizome size i.e.; length and width of rhizome, length and number of storage roots, dry weight of both aboveground and underground plant parts, number of leaves per shoot and number of shoots per plant remained unaffected due to ethephon.

Chaturvedi et al., (2009) found increased root length and increased dry weight of root but decreased leaf area of an endangered medicinal plant, Saussurea costus (Falc.) Lipsch. subjected to foliar application of ethephon. These results goes in agreement with present findings especially in case of low concentration of ethephon.
in coleus plant. Maximum number of primary branches were recorded by Hilli et al., (2010) in case of ridge gourd (*Luffa acutangula* L. Roxb.) plant subjected to ethrel spray at 500ppm. Similar result is found in coleus due to low concentration of ethephon. Joshi et al., (2011) also found that in case of *Jatropha curcas* plant, ethrel at all concentrations (50, 100, 150ppm) especially at higher concentration arrested the plant growth, tree spread and tree volume. They also reported increased collar diameter or diameter of shoot due to ethephon in jatropa. These findings goes in tune with the present findings as in coleus also there is increased stem diameter in response to both concentrations of ethephon. Similar increase in stem diameter and reduced plant height was also reported by Bowen (2012) in periwinkle (*Catharanthus roseus*) plant due to ethephon treatment. This increase in stem diameter might be due to ethylene released from ethephon and endogenous auxin which might had promoted xylem production and cambial growth and it also might be due to induction of enzymes associated with lignification by ethylene (Joshi et al., 2011). Ananth and Kumar (2012) investigated the effect of three concentrations of ethrel (1000ppm, 1500ppm and 2000ppm) as a foliar spray on Nerium plant. They revealed that due to ethrel treatment there was decrease in shoot length, number of leaves, inter nodal length but increase in number of branches but of shorter length, fresh weight and dry weight of plants as compared to control treatment. These results goes in compliance with present findings but only in case of effect of low concentration of ethephon on coleus. However, high concentration of ethephon caused significantly negative effect on all the growth parameters studied in coleus in present work. This complete reduction in growth of coleus due to high dose of ethephon might be because of increased rate of respiration and decreased rate of carbon accumulation induced due to ethylene released from ethephon. As a result of increased respiration, glucose is broken down and loss of ATP takes place which is important for plant metabolic processes like photosynthesis, amino acid synthesis, protein synthesis etc. Consequently, plant growth is inhibited. According to Stepanova and Alonso (2005) in Arabidopsis, high ethylene concentration inhibited growth due to reduction of cell expansion. A higher concentration of ethylene may play a role in the decrease of cell division and cell expansion. This might be responsible for decreased level of all growth parameters of coleus in response to high concentration of ethephon in present investigation because according to Khuankaew *et al.*, (2009) plant growth at cellular
RESULTS AND DISCUSSION

level requires a coordinated balance of both cell division and cell expansion. As already stated by them it can be assumed that the reduction of plant size in coleus by the highest concentration of ethephon (500 ppm) might be caused by low absorption, translocation and reduced accumulation of nutrients. There are also several evidences which support that ethylene had an interaction with other growth hormones such as ABA, GA and auxin (Pierik et al., 2006). Bidwell (1979) has already proved that effect of auxin and ethylene are inseparable as IAA causes ethylene production in tissue and many of IAA effects are actually secondary effects caused by ethylene which is produced due to IAA stimulation. Ethephon caused inhibition of stem elongation or dwarfism found in case of coleus might be because of ethylene and IAA interaction as Koch and Moore (1990) proposed that ethylene caused inhibition of stem elongation in whole green plants either by inhibiting basipetal IAA translocation or by affecting IAA metabolism in some manner or some other, auxin dependent reaction.

Partitioning of photosynthetic metabolites between leaf, stem and root is an important factor in yield determination (Srivastava and Luthra, 1991; Misra et al., 2009). Regarding effect of low concentration of ethephon on coleus it is evident that as compared to dry matter accumulation in aboveground plant parts like leaf and shoot more dry matter accumulation in underground plant part that is tuberous roots and in stem base which was not observed in case of control and high concentration of ethephon treatment. From these observations it can be seen that coleus responded differently to both concentration of ethephon. At low concentration, ethephon was found to be little beneficial while at high concentration it is found to cause inhibition of growth of coleus so it can be concluded that the effect of ethephon on plant growth and development is concentration dependant. This is also supported by Kidd and James (1991). According to them the result of the application of exogenous gaseous ethylene or ethephon solution differs with plant species, chemical concentrations, timing and duration of application and site of its application.

Thus, the triacontanol triggered significant increment in all the growth attributes of both aboveground and underground plant parts in *Coleus forskohlii* and also root yield however high dose of triacontanol was more promotive. The brassinosteroids found to be positively effective in enhancement of all growth characters of coleus and also improved source-sink transportation and increased root
RESULTS AND DISCUSSION

yield as compared to control. Also, CCC effectively increased all the growth parameters at the harvesting stage of Coleus except shoot length as compared to control treatment. The foliar spray of high concentration of ethephon caused negative effect on all the growth parameters of coleus where as low concentration of ethephon was found to enhance the growth characters as compared to control.

B. Photosynthetic pigments

Influence of foliar spray of PGRs on Photosynthetic Pigments

1. Chlorophylls

The influence of both concentrations of triacontanol, brassinosteroids, CCC and ethephon on chlorophyll content of *Coleus forskohlii* leaves is shown in Fig. 57 and Table No. 5. It can be clearly revealed from figure that the chlorophyll contents like chlorophyll a, chlorophyll b and total chlorophylls are significantly increased due to both, low and high concentrations of tiacontanol and ethephon as compared to control where as in case of both concentrations of brassinosteroids and CCC, all the values of chlorophyll a, chlorophyll b and total chlorophyll are at par with the control or slightly increased due to low doses only against control. However, there is no variation found in chlorophyll content values between low and high concentration of PGRs w. r. t. Chl a, Chl b and total chlorophylls. So, the Chl a/Chl b ratio due to all PGRs is equal or unchanged including control. This indicates that both doses of PGR influenced Chl a and Chl b level equally. The maximum enhancement in total chlorophylls is found due to ethephon low concentration while BRs and CCC effect on chlorophyll content is not significant in Coleus.

In higher plants, Chlorophylls is a class of green pigments which have a fundamental role to perform in the unique process of photosynthesis. The process of photosynthesis vitally depends upon harvesting of solar energy which is performed by Chlorophyll pigments. Chlorophyll pigments converts light energy into chemical energy by process of photolysis of water and thus act as electron gun of all the autotrophic plants. Chlorophylls occupies a unique position among the various plant pigments and it is situated in the thyllakoids of photosynthetic apparatus, chloroplast. Chlorophyll is basically a porphyrin molecule which has four pyrrole rings, ligated into a tetrapyrrrole ring with magnesium atom at the centre. A long chain aliphatic alcohol, the phytolate tail is esterified to a propionic acid residue on one of the tetrapyrrrole rings which makes chlorophyll extremely non-polar. Thus, chlorophyll
RESULTS AND DISCUSSION

molecule resembles a tadpole with porphyrin head and phytol tail. Chlorophyll absorbs light in the 430 nm (blue) and 680 nm (red) wavelengths of visible spectrum (Malkin and Niyogi, 2000). Along with chlorophyll a, photosynthesizing eukaryotes contains chlorophyll b, Chl c, or Chl d. All oxygen evolving photosynthetic organisms contain chlorophyll a. Chl a plays leading role in photosynthesis while chl b has a secondary role. A leaf with 70 million cells occupies equivalent to $5 \times 10^9$ chloroplasts, each containing about 600 million molecules of chlorophyll (Simpson and Knoetzel, 1995). Chlorophyll a has a methyl group but higher plants and algae also use an additional form of chlorophyll for light harvesting, chlorophyll b, that has a formyl group instead of a methyl group. The porphyrin ring of chlorophyll with its conjugated double bonds is assembled in the chloroplast from eight molecules of 5-aminolevulinic acid, which is a highly reactive non-protein amino acid (5-amino, 4-keto pentanoic acid). The biosynthetic pathway of chlorophyll a from 5-aminolevulinic acid to uroporphyrinogen III and beyond to protoporphyrin IX is a multistep complex pathway catalyzed by 5-aminolevulinic acid dehydratase, porphorobilinogen deaminase, Uroporphyrinogen III synthase, Decarboxylase, Oxidase, Mg chelatase, Methyl transferase, Cyclase, Vinyl reductase, Reductase and chlorophyll synthetase (Wettstein et al., 1995). Chlorophyll b is synthesized from chlorophyll a through the action of oxygenase enzyme that converts a methyl to a formyl side group. All chlorophyll molecules in the chloroplast are bound non-covalently to proteins in the photosynthetic membrane. Photosystems are functional and structural units of protein complexes working in photosynthesis that collectively bring about the primary photochemistry of photosynthesis that is the absorption of light and the transfer of energy and electrons. Photosystems are found in the thylakoid membranes of plants, algae and cyanobacteria. At the centre of a photosystem exists the reaction centre actually an enzyme that reduces molecules (provide with electrons) by using sunlight. This reaction centre is bounded by light-harvesting complexes which boost the light absorption. In photosystems two families of reaction centres exists. Type I reaction centre (such as photosystem I (P700) in chloroplasts and in green-sulphur bacteria and type II reaction centres (such as photosystem (P680) in chloroplasts and in non-sulphur purple bacteria. Each photosystem is more reactive to certain wavelength of light. For PS I and PS II it is 700 and 680 nm respectively in chloroplasts depending upon the amount and type of light-harvesting complexes.
RESULTS AND DISCUSSION

present. PS- I and PS- II are two photosynthetic complexes present in every oxygen evolving plants. The PS- I reaction centre is a light driven membrane bound pigment-protein complex - plastocyanin: ferrodoxin oxidoreductase in the thylakoid membranes of higher plants. Photosystem II (PSII) is a specialized protein complex that uses light energy to drive the transfer of electrons from water to plastoquinone, resulting in the production of oxygen and the release of reduced plastoquinone into the photosynthetic membrane. The reaction centre of photosystem II is possessed of two proteins such as D1 and D2 and occupies six chlorophylls, two pheophytins, two \( \beta \)-carotenes, two quinones and one or two haems of cytochrome-\( b_{559} \) (Zinth and Kaiser, 1993). Antenna region of each reaction center contains approximately 250 chlorophyll molecules. Chlorophyll a is found in all reaction center complexes as well as in antennae, whereas chlorophyll b is found only in antenna complexes. Each photosystem has its own complement of chlorophyll binding antenna proteins, and these pigment protein complex have properties to optimize the reaction for particular reaction center. Thus, antenna protein of reaction centre PS I have property to absorb maximum wavelength at 700nm and PS II have at 680 nm (Buchanan et al., 2000).

The amount and phase of chlorophyll pigments from leaf, is thus most important aspect which determine the overall photosynthetic efficiency of the plants and thus productivity. The chlorophyll level is regulated by both endogenous factors and environmental factors. There are several evidences which suggested that plant growth regulators influence chlorophyll level and contents. Srivastava and Sharma (1990) found enhanced content of total chlorophyll in opium poppy (Papaver somniferum) leaves in response to 0.01mg/L concentration of triacontanol while higher concentration (4mg/L) of triacontanol reduced chlorophyll was noticed by them. Muthuchelian et al., (1990) also reported improved chlorophyll content most significantly at 2mg/L. Srivastava and Sharma (1991) in their studies on Mentha arevensis, recorded promoted net photosynthetic rate along with increased chlorophylls content and Chl a/Chl b ratio due to triacontanol application at low dose (0.1mg/L). Similar increment in total chlorophylls content was also reported by Misra and Srivastava, (1991) in lemongrass (Cymbopogon flexuosus Steud. Watts.) leaves but with decreased chl a/ chl b ratio by triacontanol. In mangrove also, Rhizophora apiculata leaves triacontanol treatment induced high total chlorophyll content (Moorthy and Kathiresan, 1993). Muthuchelian et al., (1995; 1997; 2003) studied the
RESULTS AND DISCUSSION

effect of triacontanol foliar application on *Erythrina variegata* plant subjected to various stresses like flooding stress, water stress and chilling stress respectively. They reported that all kind of stresses negatively affected the rate of photosynthesis, contents of Chl a, Chl b and total chlorophylls and decreased activity of RuBISCO in *Erythrina variegata* plants however foliar spray with triacontanol encouraged process of photosynthesis, increased Chl a, Chl b pigment level along with total chlorophylls under both stressed and normal growth conditions. Similar enhancement in Chlorophyll a, b and total chlorophyll content was reported by many workers such as Kumaravelu *et al*., (2000) in green gram, Jirali (2001) in turmeric, Chen *et al*., (2002) in rice, Anilkumar, (2005) in patchouli plant, by Ganapathi (2006) in carrot leaves, Pothalkar (2007) in pigeon pea due to foliar application of triacontanol in various forms and concentrations. Khan *et al*., (2007), Khan *et al*., (2009) and Naeem *et al*., (2009) reported increment in level of Chl a, b and total chlorophylls in response to foliar spray of triacontanol in opium poppy, tomato and Hyacinth bean plants respectively. Ameliorating effect of triacontanol was reported by Borowski and Blamouski (2009) in *Ocimum basilicum* under chilling stress. They observed that triacontanol alleviated the negative effects of stress in sweet basil and noted maximum quantum efficiency along with increase content of Chl a and Chl b prominently at 0.1mg/L concentration. In corianer (*Coriandrum sativum*) leaves also Idrees *et al*., (2010) noticed positive effect of triacontanol and GA synergistically on total chlorophyll contents.

There are several evidences regarding the enhancement of Chlorophyll content of leaves in various medicinally important plants in response to triacontanl foliar spray such as in *Artemissia annua* (Aftab *et al*., 2010), in Coffee senna (*Senna occidentalis* L.) by (Naeem *et al*., 2010), in *Ocimum basilicum* (L.) by (Hasmi *et al*., 2011), in *Mentha arvensis* (Naeem *et al*., 2011), in vetiver (*Chrysopogon zizanoids*) grass by (Kamble and Chavan, 2011), in *Catharanthus roseus* (L.) leaves by (Alam *et al*., 2012), in ginger (*Zingiber officinale* Rosc.) leaves by (Singh *et al*., 2012) and in lemongrass (*Cymbopogon flexuosus* Steud. Watts) by Khan *et al*., (2014). All these evidences are in agreement with the present findings in Coleus. Shahbaz *et al*., (2013) recorded increased photosynthesis, the Chl a/Chl b ratio, total chlorophylls and electron transport rate in canola (*Brassica napus*) under saline stress because of triacontanol application. Thus, there are plenty evidences supporting the present
findings of Chlorophylls contents. As suggested by Ivano and Angelov (1997), Chen et al., (2003), Naeem et al., (2010) and Hashmi et al., (2011) the increased contents of chlorophylls, Chl a, Chl b and total chlorophylls in response to triacontanol most apparently be due to growth in number and size of chloroplasts. This might be also attributed to cascade of biochemical events induced by exogenous application of triacontanol. It has also been suggested that the use of growth regulators improved the availability of assimilates which in turn resulted in prolonged chlorophyll synthesis (Stoddart 1965). Decreased rate of chlorophyll degradation and increased chlorophyll synthesis and the increase in total chlorophyll content can also be attributed to involvement of growth regulators in promoting the synthesis of chlorophyll as well as development of chloroplast (Fletcher and Mc Cullagh, 1971).

Braun and Wild (1984) in wheat and mustard recorded enhanced soluble reducing sugars but chlorophyll content was hardly affected due to BRs. Similar trend is found true in present investigation however low concentration of BRs caused increase in chlorophyll pigment level. This is also supported by Sairam, (1994) in wheat grown under water stressed condition. They reported improved level of chlorophylls and net photosynthetic rate due to BRs foliar application at low concentration 0.1 and 1 ppm in both normal and stressed condition. Talaat and Youssef (1998) in Hibiscus sabdariffa noticed increased level of total photosynthetic pigments by BRs application whereas Yu et al., (2004) in cucumber reported very little effect of EBR foliar application on total chlorophyll content but increased leaf area along with increased RuBISCO and CO₂ assimilation. Bajguz and Asami (2005) in Wolffia arrhiza noticed enhanced photosynthetic pigments chlorophyll content due to BRs application at 10⁻⁹M concentration. In Vigna radiata Fariduddin et al., (2006) reported increased photosynthesis along with higher total chlorophyll content and delayed senescence due to HBR foliar application, most significantly at 10⁻⁹M concentration. Increment in total chlorophylls of excised cotyledons of red cabbage was reported by Cag et al., (2007) due to incubation in epiBL at 0.001 µM concentration against control. Hayat et al., (2000) in mustard, Brassica juncea L. found increased level of total chlorophylls by foliar spray of HBR. Alam et al., (2007) in Brassica juncea L. also reported enhanced total chlorophylls and its contents in response to 28-HBR. Cevahir et al., (2008) reported stimulated level of Chl a Chl b and total chlorophylls by EBR foliar spray in soybean seedlings grown in light and
RESULTS AND DISCUSSION

dark condition depending upon low concentration of BR. In *Pelargonium graveolense* L. Herit, a rose scented geranium, application of 28-HBR and 24-EBR @ 0.5, 1 and 3 μM resulted in increased level of photosynthetic pigments chlorophylls and CO₂ fixation with improved growth (Swamy and Rao, 2008; Swamy and Rao, 2009). Ameliorating effect of 24-EBR was reported by Houimli *et al.*, (2010) in salinity stressed pepper (*Capsicum annua*) plant. They reported improved level of total chlorophylls, Chl a and Chl a/Chl b ratio under both stressed and normal conditions. Susila *et al.*, (2011) applied foliar spray of BRs at 0.1 ppm on water melon during 2nd and 4th leaf stage and found significantly enhanced Chl a, Chl b and total chlorophylls content along with improved dry matter. Similar positive influence of 24-EBR and 28-HBR foliar application at 3 μM concentration on photosynthetic pigments was recorded by Swamy and Rao, (2011) in *Coleus forskohlii*. They also noticed increased chlorophylls content with improved growth. Karlidag *et al.*, (2011) carried out studies to assess the effect of 24-EBR foliar spray on strawberry plants under salinity stress. They recorded elevated level of chlorophylls measured in terms of leaf chlorophyll reading values (LVRV) and increased dry weight under both normal and stressed condition. In tomato plants grown under Cd-stress Hayat *et al.*, (2012) recorded improved chlorophylls content due to BRs application under both stressed and normal conditions as compared to control. In response to HBR foliar application at 10⁻⁷ M concentration there was increased contents of total chlorophylls in leaves of *Mentha arvensis* as reported by Naeem *et al.*, (2012b). Choudhari *et al.*, (2012) in Radish (*Raphanus sativus* L.) evaluated the efficiency of BRs in Chromium stress mitigation. They reported that reduced contents of chlorophylls a, b and total chlorophylls under Cr-stress were improved by the application of EBR to the radish plant as compared to control proving anti-stress ability of BRs in radish. In *Catharanthus roseus* L. Alam *et al.*, (2012) recorded significantly improved level of total chlorophylls in response to foliar spray of HBR at 10⁻⁶M concentration as compared to control. Increased level of total chlorophylls was also reported by Bera and Kalipada (2013) in lentil (*Lens culinaris* Medik.) plant due to twice spraying of HBR application @ pre-flowering +pod developing stage. In the present investigation BRs caused slight increase in the level of chlorophylls which can be attributed to increased leaf area and increased RuBISCO activity and CO₂ assimilation as suggested by Yu *et al.*, 2004. Thus, it can be concluded that BRs might have
RESULTS AND DISCUSSION

contributed differently in the process of photosynthesis and growth of plant as at low concentration it improved the chlorophyll contents while at high concentration it was found to be less effective.

There are divisive evidences regarding the effect of CCC on photosynthetic pigments. Wahdan et al., (1985) in *Atropa belladonna* plants recorded reduced Chl a, Chl b due to foliar spray of CCC at 2000ppm. Favourable effects of growth regulators on chlorophyll content have been reported by number of workers. Cyocel at 250 and 500 ppm significantly improved the chlorophyll contents in leaves of Brassica napus (Grewal et al., 1993). The beneficial effects of cyocel on chlorophyll content have also been reported in various plant systems, like safflower (Kar et al., 1989), sesamum (Bashist, 1990), wheat (Sairam et al., 1991), mungbean (Shah and Prathapasesan, 1991), soybean (Abo El-Kheir et al., 1994), green gram (Mandal et al., 1997), Brassica juncea (Lone, 2001). Jayakumar and Thangaraj (1998) reported increased activity of chlorophylls in groundnut in response to CCC application at 120ppm. Similar positive response was reported by Garai and Datta (2002) due to CCC in leaves of green gram var. B-105(Panna). Observations made by Anilkumar (2005) in *Pogostemon cablin* Benth. plant goes in agreement with the present studies. They reported reduced level of chlorophylls only at 90 DAP stage due to CCC in patchouli plant where as at all the growth stages they found chlorophyll contents at par with the control treatment. Same trend is noticed in Coleus. In contrast to this Sridhar (2006) and Koler (2008) found maximum total chlorophylls content in jasmine and cotton due to spray of CCC at 500 and 80 ppm and Mepiquat chloride at 50 and 100 ppm respectively. Similarly, Xu et al., (2011) also observed enhanced total chlorophyll content and net photosynthetic rate in *Ginkgo biloba* in response to CCC foliar spray at all the concentrations 0.5, 1 and 2 g/L. Zheng et al., (2012) also reported increased level of chl a, chl b and total chlorophylls in Lily plant in response to CCC and paclobutrazol as compared to control. Generally leaves treated with growth retardants are deep green coloured as a result of higher synthesis of chlorophyll and its content which is found true in present study due to low concentration of CCC but due to high concentration it is at par with control. Effect of CCC on photosynthesis is still unclear. CCC found to increase or decrease chlorophylls and photosynthetic rate however retardants are found to delay leaf senescence by maintaining chlorophyll stability.
Wahdan et al., (1985) in *Atropa belladonna* plant, noticed decreased Chl a in leaves at 100 and 200 ppm concentration of ethrel. Shahine et al., (1992) in *Trigonella foenum* fenugreek plant recorded increased leaf chlorophylls by ethrel application. Elevated level of chlorophylls content was also recorded by Singh and Misra (2001) in *Mentha spicata* var. MSS-5 in response to ethrel treatment at 1000 µg/L. Misra et al., (2009) in *Catharanthus roseus* detected lowered contents of Chl (a+b), chl a and decreased photosynthetic efficiency in response to ethrel treatment. El-Sherbeny et al., (2009) performed comparative analysis of effect of ethrel application by seed treatment and foliar spray application on growth of *Foenicum vulgare* Mill. They reported that at 250 mg/L concentration ethrel foliar application enhanced leaf chlorophyll pigments while at highest concentration (1000mg/L) pigment level decreased. In case of *Jatropa curcas* L., Joshi et al., (2011) studied combined effect of auxin and ethylene on physiology and growth of jatropa. They reported stimulated level of total chlorophylls and increased photosynthetic efficiency along with increased concentration of ethrel. Mansouri et al., (2013) studied effect of ethephon spray on photosynthetic pigments in *Cannabis sativa* at productive stage. They reported that at low concentration of ethephon (1 µM) significant enhancement in Chl a, Chl b and total Chlorophylls was seen in Cannabis. These findings goes in tune with the present findings.

In the present investigation there is significant enhancement in the chlorophyll content of Coleus due to ethephon which might be attributed to decreased rate of chlorophyll degradation and increased rate of chlorophyll synthesis and stimulated development of chloroplast (Fletcher and Mc Cullagh, 1971) induced due to exogenous application of ethephon. It has also been suggested that the application of growth regulators increased the availability of assimilates which in turn resulted in prolonged chlorophyll synthesis (Stoddart 1965).

### 2. Carotenoids

Impact of foliar treatment of triacontanol, brassinosteroids, CCC and ethephon on carotenoid content of leaves in *Coleus forskohlii* is depicted in Table No. 6 and depicted in Figure 58. It is revealed from the Table and figure that the carotenoids content in leaves is increased due to both concentrations of triacontanol (Vipul), ethephon and BRs except for high dosage of BRs it is at par with control treatment.
RESULTS AND DISCUSSION

whereas it is decreased due to both doses of CCC as compared to control and all other PGR treatments.

Carotenoids are generally C\textsubscript{40} terpenoids. A group of hydrocarbons that contribute in various life processes in plants, such as photosynthesis, photomorphogenesis, photoprotection, and development. Carotenoids acts as precursors for two plant hormones and a varied set of apocarotenoids. Carotenoids are the second most abundant naturally occurring pigments on the earth, with more than 750 members classified mainly into hydrocarbon carotenes like lycopene and β-carotene or xanthophylls (Nisar \textit{et al}., 2015). Carotenoids are associated with the process of light harvesting reaction center complexes (Mc Donald, 2003). Coloured carotenoids exists in fruits, flowers probably act as attractants for pollination and seed dispersing (Cunningham and Gantt, 1998). Gill and Tuteja (2010) noticed that carotenoids absorb light at the wavelength from 400-500 nm and transfer it in to the chlorophyll reaction centres. Carotenoids are having conjugated double bonds in the isoprene residues, which allow excess of energy as heat (Non-photochemical quenching) (Mittler, 2002). As suggested by Siefermann-Harms, (1987) and Cunningham and Gantt, (1998), (Lopez-Juez and Pyke, 2005) in all plants, algae and cyanobacteria carotenoids are synthesized and its metabolites are accumulated in plastids, chloroplasts and chromoplasts, as a vital and integral part of photosynthetic apparatus, in which they efficiently quench triplet chlorophyll, singlet oxygen and superoxide radicals. Plants synthesize carotenoids via 1-deoxy-D-xylose-5-phosphate (DOXP) pathway rather than the mevalonic acid pathway (Lichtenthaler,1999). Carotenoids are derived from two isoprene isomers, isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP). There are two main classes of carotenoids: the carotenes that are cyclized or uncyclized hydrocarbons (β carotene) and the xanthophylls that are oxygenated derivatives of carotenes (lutein, violaxanthin, and neoxanthin). Chloroplasts of higher plants showed accumulation of lutein, β carotene, violaxanthin, and neoxanthin in order of their abundance (Peter and Thornber, 1991 and Ryberg \textit{et al}., 1993). Lutein occupies more than 50% of total carotenoid pool. It is located in the crystallized structure of the LHC (Light Harvesting Complex) and is the only xanthophyll detected in the photosystem (II) core (Bassi \textit{et al}., 1993 and Kuhlbrandt \textit{et al}., 1994).
As suggested by Olson, (1989), Krinsky, (1989) some carotenoids also serve as precursor for vitamin A, which plays an essential role in human and animal diets and also as an antioxidant, and can be helpful in reducing the risk of certain forms of cancer, heart disease and degenerative eye disease. According to Young, (1991) carotenoids are lipid soluble antioxidants having capacity to detoxify various forms of ROS. According to Verma and Misra (2005), carotenoids detoxify the adverse effect of ROS in plants. Cazzonelli, (2011) proposed that in plants along with the photoprotection, carotenoids are also involved in production of phytohormones, including ABA and strigolactone. Application of various chemicals to plant causes alteration in carotenoid formation leading to photo destruction of chlorophyll (Anderson and Robertson, 1960). Favourable effects of growth regulators on carotenoids content have been reported by number of workers.

Moorthy and Kathiresan, (1993) reported increased Carotenoids in response to triacontanol in mangrove species Rhizophora apiculata. Kumaravelu et al., (2000) in green gram recorded no significant variation in carotenoids content at low concentration of triacontanol however at high concentration observed reduction in carotenoids. Similar observations were made by Anilkumar (2005) in Patchouli (Pogostemon cablin Benth.) due to triacontanol. Naeem et al., (2009) recorded enhanced carotenoids due to triacontanol spray prominently at $10^{-6}$M concentration in Hyacinth bean leaves however at high concentration ($10^{-5}$M) of triacontanol level of carotenoids decreased. Ganapathi (2006) also found more carotenoids in carrot by triacontanol spray at 1000 and 2000ppm. In tomato plants Khan et al., (2006; 2009) recorded increased content carotenoids and $\beta$-carotene in tomato leaves and fruit due to triacontanol spray. Khan et al., (2007) in opium poppy found low level of carotenoids. In medicinal plants triacontanol is found to cause positive effect on Carotenoids. Naeem et al., (2010), Aftab et al., (2010), Idrees et al., (2010), Hashmi et al., (2011) and Naeem et al., (2011) reported improved carotenoids in Coffee senna, Artemisia annua, coriander, Ocimum basilicum and in Mentha arvensis respectively, mostly at $10^{-6}$M concentration of triacontanol. Observations made by Kamble and Chavan (2011) in vetiver grass also agreeing with present investigation as they also reported enhanced carotenoids due to triacontanol foliar application. Similar supportive observations were made by Alam et al., (2012). They reported
increased carotenoids in *Catharanthus roseus* in response to triacontanol at $10^{-7}$M concentration which was found beneficial in plant.

In the present study foliar application of Triacontanol result in the elevation of carotenoids. This increased level of carotenoid in response to PGRs application help to maintain chlorophyll stability, as well as it helps to prevent the degradation of chlorophyll protein complex of thylakoid membrane as indicated by Besford *et al.* (1993).

According to studies made by many workers in various plants brassinosteroids are also found to influence level of carotenoids pigments in plant along with increased chlorophylls and positive effect on carotenoid level was more prominent in stressed plants. Talaat and Youssef (1998) in *Hibiscus sabdariffa* noticed increased level of total photosynthetic pigments by BRs application. Bajguz and Asami (2005) in *Wolfia arrhiza* noticed enhanced level of carotenoid content due to BRs application at $10^{-9}$M concentration. In *Vigna radiata* Fariduddin *et al.*, (2006) reported increased photosynthesis along with higher total Carotenoids content and delayed senescence due to HBR foliar application, most significantly at $10^{-9}$M concentration. Cevahir *et al.*, (2008) reported stimulated level of carotenoids by EBR foliar spray in soybean seedlings grown in light and dark condition depending upon low concentration of BR. In tomato plants grown under Cd-stress, Hayat *et al.*, (2012) recorded improved chlorophylls content in leaves and carotenoids content in fruits in the form of β-carotene due to BRs application under both stressed and normal conditions as compared to control. Naeem *et al.*, (2012b) in response to HBR foliar application at $10^{-7}$ M concentration found increased contents of total carotenoids in leaves of *Mentha arvensis*. Choudhari *et al.*, (2012) in Radish (*Raphanus sativus* L.) proved the efficiency of BRs in Cromium stress mitigation by reporting enhanced level of photoprotective pigments carotenoids under both stressed and unstressed condition in radish. In *Catharanthus roseus* L., Alam *et al.*, (2012) recorded significantly stimulated level of total carotenoids in response to foliar spray of HBR at $10^{-6}$M concentration as compared to control. All these evidences support present investigation in Coleus. Thus increased level of carotenoids in Coleus due to BRs is helpful in chlorophyll stability, to maintain photosynthetic efficiency and avoid photooxidation effects caused due to elevated photosynthetic rate.
Wahdan *et al.*, (1985) in *Atropa belladonna* plants recorded reduced carotenoids due to foliar spray of CCC at 2000ppm. Anilkumar (2005) in *Pogostemon cablin* Benth. plant reported no significant change in carotenoids content due to any concentration of CCC in patchouli plant as compared to control at all the growth stages. Xu *et al.*, (2011) found beneficial effect of CCC spray at 0.5, 1 and 2g/L concentration in *Ginkgo biloba* leaves. As reported by Kamienska and Chominski (1971), CCC was applied to ripening tomato and red pepper fruits. They reported that CCC inhibited chlorophyll degradation and carotenoid formation and thus CCC inhibited fruit ripening. This might be responsible for equivalent to control or slightly increased values of chlorophylls and decreased values of carotenoids in leaves of Coleus plant treated with CCC.

Shahine *et al.*, (1992) in *Trigonella foenum* fenugreek plant recorded increased leaf carotenoids by ethrel application. Mansouri *et al.*, (2013) reported significant enhancement in carotenoids content in leaves of *Cannabis sativa* as a response to ethephon spray most significantly due to low concentration of ethephon (1 µM) at productive stage.

Thus, in the present investigation increased level of chlorophylls and carotenoids due to triacontanol, brassinosteroids, ethephon and to some extent by CCC might be beneficial to increase photosynthetic efficiency, enhanced carbon metabolism leading to improved growth and yield of *Coleus forskohlii* while increase in carotenoids content as compared to plant will be beneficial to plant to sustain through adverse stress conditions. Increased carotenoids also play beneficial role in protection of cell from photodestruction and maintaining chlorophyll stability.

C. Carbohydrate metabolism

**Influence of foliar spray of PGRs on carbohydrate metabolism**

1. Carbohydrates

The influence of foliar spray of plant growth regulators on carbohydrate fractions (Reducing sugars, starch and soluble sugar content) from leaf, stem and root part of *C. forskohlii* is shown in Table No. 7, 8 and 9 and in Fig. 59, 60 and 61. It is evident from the results that in *C. forskohlii* leaf part, reducing sugars are increased due to growth promoters like triacontanol and BRs but decreased due to growth retardants like CCC and ethephon whereas the starch content is increased in leaves.
RESULTS AND DISCUSSION

due to all PGRs. The soluble sugar fraction is decreased due to all PGRs except for CCC it is at par as compared to control.

In case of Coleus stem, there is overall enhancement in reducing sugars and starch content because of all PGRs treatments while the soluble sugar content is decreased due to triacontanol and BRs but increased due to CCC and ethephon.

In coleus roots, there is overall enhancement in reducing sugar content and starch content due to all PGRs except for high dose of BRs which caused significant decrease in reducing sugar content in root whereas soluble sugar content of root is significantly decreased due to all PGRs as compared to control. Thus, it can be clearly seen that there is overall enhancement in reducing sugars and starch content in both stem and root part of Coleus due to all PGRs with exception of BRs high dose while in leaf tissue the starch content is increased but soluble sugar is decreased due to all PGRs.

Being major products of photosynthetic carbon assimilation and the substrates for chief catabolic energy generating process, respiration, Carbohydrates are of utmost importance in all green plants. So, the carbohydrates status in the plant tissue gives an indirect idea about the metabolic status of the plant tissue as well as the energy content of the plant tissue. Carbohydrates provide carbon skeletons for extensive range of carbon compounds present in the plant tissue including amino acids, organic acids and other secondary metabolites. Some of these secondary metabolites, have a characteristic medicinal or defensive value such as phenols, flavonoids, alkaloids and terpenoids etc. In higher plants, the protective cell wall has carbohydrate components in the form of sugar polymers like cellulose and pectin. As cell wall comprises a very complex structure, the complete degradation of these polysaccharides is very difficult because of the limitation of enzymatic attack upon them. Starch is a steady state storage product of photosynthetic carbon reduction cycle most commonly found in seeds and tubers, rhizomes, bulbs for long term storage basis and found in leaf chloroplast on short term basis. Apart from starch, hemicelluloses amyloids and the raffinose series oligosaccharides may also present and may sometimes form major carbohydrate reserve (Bewley and Black, 1994). Chloroplast is the site of starch biosynthesis in leaf cells however the non-photosynthetic tissues in plants also synthesize starch from sucrose and it is deposited in amyloplast. Starch is a complex polymer made up of large number of glucose units joined together by glycosidic
bonds. Depending on the plant type starch is composed of about 20 to 25% amylose and 75 to 80% amylopectin (Brown and Poon, 2005). Enzymes ADP glucose pyrophosphorylase plays a key role in starch biosynthesis (Zeeman et al., 2010). Starch synthase and branching enzymes are also important enzymes in the starch biosynthesis. ADP glucose pyrophosphorylase is the key allosteric enzyme in the process of starch biosynthesis and the activity of this enzyme is regulated by 3PGA and iP. The assimilatory starch stored in leaf chloroplast during daytime subsequently serves as respiratory substrate through liberation of glucose units following its degradation. Similarly, the starch stored on long term basis in storage organs such as seeds, tubers, rhizomes is degraded during germination or sprouting and regrowth. In higher plants, sucrose is the most important non-reducing type of sugar, produced during photosynthetic carbon assimilation reaction and plays significant role as the most suitable source of carbon, plays a vital role as a transport sugar and provide energy for plant growth processes (George, 1993). Sucrose is the major carbohydrate which is transported from leaf to other plant parts through phloem. Sucrose biosynthesis occurs in the cytoplasm, as a result of photosynthetic CO$_2$ fixation. The sugar nucleotide UDP- Glucose plays important role in sucrose biosynthesis which is mainly catalysed by two enzymes, sucrose phosphate synthase and sucrose phosphatase. Sucrose phosphate synthase is a most important regulatory enzyme in sucrose biosynthesis and its activity is regulated by number of endogenous and environmental factors. As sucrose is a form of transport sugar, growth of sink tissue is regulated by sucrose (Farrar, 1996). However, sucrose cannot be used directly for metabolic processes, but must be hydrolysed into hexose sugars (glucose and fructose) by enzyme invertase (β-fructosidase, EC. 3.2.1.26) or sucrose synthase UDP-D-glucose: D-fructose-2-α-D-glucosyl-transferase, EC.2.4.1.13 sucrose synthase) before entering into carbohydrate metabolism. Glucose and fructose are hexose sugars produced by sucrose breakdown are major reducing sugars in plants tissue which acts as a substrate for respiration and starch biosynthesis in storage organs such as seeds, tubers etc. Apart from these, the pentose sugars such as ribose are binding constituents of nucleic acids while tetrose sugars like erythrose serves as a precursor for biosynthesis of aromatic compounds. Sugars also play an important role as an osmoregulator. The sugars are also reported to play a role of signalling molecules (Rolland et al., 2002). The sugar galactose is a constituent of galactolipids.
RESULTS AND DISCUSSION

component of chloroplast membranes. Thus, being an integral part almost all metabolic processes, the level of various carbohydrate fractions such as starch, sucrose, reducing sugars in the plant tissue is highly important in evaluation of overall metabolic status of the plant tissue.

As stated by Roitsch et al., (2003), physiological mosaic i.e., carbohydrate partitioning between the synthesizing source tissues and various sink tissues contending for a mutual pool of carbohydrates is a highly active process which is consistent with each stage of growth and development in higher plant. With respect to this it is necessary to have idea about status of carbohydrate metabolism under the influence of PGRs application. There are several evidences about effect of plant growth regulators on carbohydrate metabolism directly or indirectly by influencing various metabolic processes and source-sink transport. Many researchers have reported the positive role of TRIA in enhancing growth, yield, photosynthesis, enzymes activities along with carbohydrate metabolism (Ries 1991; Ries et al. 1993; Nagoshi and Kawashima 1996; Borowski et al. 2000; Naeem et al. 2009; Aftab et al. 2010; Idrees et al. 2010; Naeem et al. 2011). Ries, (1978; 1985), have noticed enhanced level of soluble reducing sugars and starch in the leaves of rice and maize along with improved dry weight. Kim et al., (1989) also recorded positive effect of triacontanol on carbon metabolism w. r. t. elevated soluble sugars and starch content in rice and maize leaves. Misra and Srivastava (1991) in lemongrass Cymbopogon flexuosus Steud.) and Ivanov and Angelov (1997) and Blamowski et al., (1998) recorded enhanced intensity of CO2 binding. According to Srivastava and Sharma (1991) the CO2 exchange rate, total chl. and shoot fresh and dry weight were higher due to triacontanol treatment at (0.01 mg/L.) in opium poppy (Papaver somniferum L.) plant. Thakur and Thakur (1992) and Muthuchelian et al., (1995) recorded increased level of sugars and starch in case of Acacia catechu and Erythrina variegata leaves respectively. Kumaravelu et al., (2000) noted that triacontanol foliar spray at 0.5 mg/L significantly promoted saccharides or sugars level along with increased starch content in green gram. Sharma et al., (2002) found increased reducing sugar content in non pareil almond. Muthuchelian et al., (1997; 2003) recorded that triacontanol treatment increased CO2 assimilation along with enhanced starch and sugar content level in Erythrina variegata seedlings grown under different stress conditions. Ganapathli (2006) observed increased total sugars in carrot (Daucus carota L.) due to
combined effect of triacontanol (@2000ppm) and fertilizers. Enhanced level of total carbohydrates was found by Singh et al., (2008) in (Zingiber officinale Rosc.), ginger and in turmeric (Curcuma longa) rhizome part due to triacontanol treatment. Ries and Stutte (2008) also suggested that triacontanol application stimulated activity of various enzymes associated with carbohydrate metabolism. Krishnan and Kumari (2008) also reported increased soluble sugar in salt stressed soybean plant treated with triacontanol. Naeem et al., (2009) studied influence of triacontanol on hyacinth bean (Lablab purpureous) and recorded raised level of total carbohydrate fraction at $10^{-6}$ M concentration significantly however at high concentration of triacontanol ($10^{-5}$ M) its decreased. In case of medicinal plant vetiver grass (Chrysopogon zizanoids L.), Kamble and Chavan (2011) detected increased reducing sugars in leaf and raised starch content in roots. Hashmi et al., (2011) also found enhanced percent leaf carbohydrate contents due to foliar spray of triacontanol at $10^{-6}$ M dosage against control in sweet basil (Ocimum basilicum L.). Singh et al., (2014) carried out studies to find effect of foliar spray of triacontanol in ginger (Zingiber officinale Rosc.) plant and detected significant increment in various fractions of carbohydrate in rhizome part particularly at $10^{-6}$ M concentration. In Bougainvillia glabra, the amount of reducing sugar in leaves was found stimulated along with enhanced sucrose accumulation and sucrose phosphate synthase (SPS) enzyme activity in response to triacontanol foliar application, more significantly at 2.5mg/L dose. All these evidences are in agreement with the present investigation as in case of Coleus forskohlii also we found increased reducing sugars and starch content in leaf, stem and root parts due to triacontanol spray treatment as compared to control. Several evidences have proved a triacontanol triggered enhancement in the amount of both CO₂ fixation and photosynthesis in Mentha arvensis L. (Srivastava and Sharma 1991) as well as in other plants (Misra and Srivastava 1991; Ivanov and Angelov 1997; Kumaravelu et al. 2000; Chen et al. 2002; Muthuchelian et al. 2003; Naeem et al. 2009; 2010).

Thus, triacontanol has been found to increase the crop production by enhancement in photosynthetic activity, pigments, enhanced nutrient uptake and rapid increase in various carbohydrate fractions, soluble protein, succinate and changes in permeability of membrane. The elevated levels and activities of photosynthesis-related enzymes due to triacontanol may have increased the sugar content of the
RESULTS AND DISCUSSION

leaves. This ability of triacontanol as a growth promoter might be responsible for improved carbohydrate metabolism in Coleus. Triacontanol also found to augment source-sink relationship which reflected in improved level of carbohydrate contents in root of Coleus in present study which represents better qualitative growth of plant.

Braun and Wild (1984a; 1984b) after their studies on mustard and wheat reported increased soluble reducing sugars with promoted rate of photosynthesis, RuBISCO activity, CO₂ fixation and growth due to BRs treatment significantly at 10⁻⁶M concentration. Schilling et al., (1991) analysed the efficiency of BRs, (22s,23s)-homobrassinonide/SSHb) in removing adverse effects of drought stress in sugar beet plant. They reported enhancement in sucrose content only in roots of sugar beet plants exposed to stress supplied with BRs but not in normal or unstressed plant. This indicates the role of BRs in induction of Sucrose synthase enzyme activity and as a stress reliever. Khripach et al., (1996) found 20% increase in productivity of Potato crop along with enhanced starch content by BRs. In Arachis hypogaea groundnut plant application of 24-EBR resulted in enhancement in level of various carbohydrate fractions and soluble proteins is reported by Vardhini and Rao, (1998). Enhanced level of soluble sugars, sucrose and starch content under the influence of BRs along with increased activity of enzymes related with carbohydrate metabolism such as SPS enzyme, Sucrose synthase and acid invertase enzyme was witnessed by Yu, et al., (2004) in cucumber - Cucumis sativus plant. Vardhini and Rao (2003) also recorded enhanced level of total carbohydrate fraction leading to promoted growth in tomato due to BRs application. Bajguz and Asami (2005) in their studies regarding the effect of 24-EBR on growth and other parameters of Wolffia arrhiza reported that addition of epiBL to culture, promoted growth of plant along with increased amount of photosynthetic pigment, sugar and protein significantly at 10⁻⁹M concentration of EBR. Hayat et al., (2007) analysed the performance of 28-HBL in Brassica juncea under the condition of Cd- stress. They recorded that spraying of Cd stressed seedlings of Brassica with HBR resulted in improved level of various carbohydrates fractions in both root and leaf under both normal and stressed condition which was previously found to be decreased due to stress. In geranium (Pelargonium graveolense L. Herit) plant, Swamy and Rao (2008) and Swamy and Rao (2009) observed enhanced growth with sharp increase in the amount of reducing and non-reducing sugars and starch content in leaves by application of 28-HBR (@3 µM) and


RESULTS AND DISCUSSION

24-EBR (@0.5, 1, 3µM) as compared to control respectively. Talaat and Abdallah (2010) noted that application of 0.1mg/L of EBR on faba bean varieties Sakha 1 and Giza 40 resulted in better quality of yield w. r. t enhanced total carbohydrate content. Swamy and Rao (2011) in same plant Coleus forskohlii witnessed that 24-EBR and 28-HBR caused improved growth and dry weight along with increased level of various carbohydrate fractions most prominently at 3µM concentration. Similar rise in carbohydrate content is observed in present investigation due to both concentrations of BRs w. r. t. reducing sugars and starch content in all plant parts except that at high concentration of BRs decreased level of reducing sugars found in all plant parts. Eleiwa and Ibrahim (2011) noticed ameliorating effect of 28-HBR on wheat (Triticum aestivum L.) plants subjected to saline stress condition. They reported significant increment in total carbohydrates such as reducing and non-reducing sugars by BRs treatment in wheat grains and straw parts under both normal and saline stress condition. Similar kind of ameliorating effect of BRs was also noticed by Choudhari et al., (2012) in case of radish root plant subjected to Cr-stress. They also recorded enhanced amount of total soluble sugars due to EBL application alone or in combination with polyamine spermidine in both stressed and unstressed condition.

Vardhini et al., (2011;2012) evaluated the effect of 24-EBR and 28-HBR on the qualitative growth of radish root (Raphanus sativus). They noticed root growth stimulation with enhanced levels of non-reducing sugars and starch with increased quality of radish root due to all concentrations of BRs against control however 3µM concentration of 28-HBR exhibited maximum amount of reducing sugars as starch. Serna et al., (2013) noticed increase in carbon, organic acid and sugar content in Cichorium endivia vegetable due to application of BRs analogues, DI-31 and DI-100.

As noted by Biesaga-Koscielniak et al., (2014) in pea and lupin plants, increased level of carbohydrate metabolism and accumulation of soluble sugar in pea and lupin seeds was noticed due to 24-EBR foliar spray. In support of all these evidences and results of present investigation Schluter et al., (2002) reported decreased level of starch and sugar contents in BR-deficient mutant of Arabidopsis thaliana emphasizing the important role of BRs in carbohydrate metabolism induction. The enhancement in content of carbohydrate fractions as a response to BRs application might be presumably due to increased photosynthetic efficiency of plant and or stimulated source sink translocation triggered by endogenous or exogenous BRs treatment.
RESULTS AND DISCUSSION

According to Prusakova et al., (1999) the most characteristic feature of BRs is their ability to increase not only the growth and yield of crop plant but also the quality of crop plant by diminishing harmful contents like nitrate and other heavy mineral elements and increasing positive factors such as sugars and starch. This is also found true in case of Coleus in present investigation.

The efficiency of BRs in enhancement of growth and metabolism of the shoot system is a very well-known fact, but the present study states the capability of BRs in monitoring the source – sink partitioning in root system which is reflected in the form of enhanced level of metabolites like carbohydrate fractions and soluble proteins in the root system of coleus.

The evidences regarding the effect on CCC application on carbohydrate metabolism are limited. Cathey and Stuart (1961) proposed that application growth retardants lead to production of dwarf plants with short and dark green leaves with increased photosynthetic rate which is the most important factor for storage of carbohydrates in underground plant parts. This is also found true in present study. Chemical composition of soybeans from plants treated with growth retardants was investigated by El-Fouly et al. (1970). They observed CCC treatment enhances total carbohydrate content. Higher concentrations of reducing sugars were observed in carnation after CCC treatment (El-Fouly et al., 1977). Sharma et al., (1998a and 1998b) reported increased starch content in tubers of potato and improved tuberization due to CCC foliar spray. Xu et al., (2011) in Ginkgo biloba observed increased soluble sugars in leaves due to CCC @ 0.5, 1, 2 g/L concentration. These findings are in tune with the present findings. Zheng et al., (2012) studied influence of CCC and Paclobutrazol growth retardants on the carbohydrate accumulation in bulbs of Lilium orientale hybrid 'Sorbonne'. They reported improved biomass of leaves and stem which contributed to transportation of photo assimilates and its utilization. CCC and PBZ foliar spray both caused substantial enhancement in sucrose content in leaves and starch content accumulation in bulbs of Lily. In present study also starch content is increased in root, stem and leaves while reducing sugars increased in stem and root part of Coleus due to CCC.

According to Mares et al., (1981) several evidences proved that GA causes a remarkable reduction in the activity of adenosine diphosphate-glucose pyrophosphorylase (AGPase) enzyme activity, which is a key rate-limiting enzyme in
starch biosynthesis leading to reduced starch accumulation however treatment with CCC may work against this reduction in starch synthesis by blocking GA synthesis. While according to Zheng et al., (2012) CCC treatment could have a long-term impact on plant growth by changing endogenous hormone contents. Effect of CCC on increased sugar and starch content in coleus might be most presumably due to one of these factors.

There is scarcity of records regarding the effect of ethephon on carbohydrate metabolism. Lukaszewska, (1995) found increased sugar accumulation in tepals and pistils of Tulip Cv. Apeldoorn due do ethephon treatment along with reduced stem elongation and pistil growth. Vadigeri et al., (2001) observed that ethrel treatment at 200ppm and 400ppm was beneficial in increasing quality of cucumber (Cucumis sativa) fruits w. r. t enhanced level of reducing and soluble sugars in fruit. In Catharanthus roseus, Misra et al., (2009) recorded improved carbon assimilation rate due to ethrel application depending upon incorporation of 14CO₂ and 14C sucrose fed to system. They reported that when 14CO₂ was fed to plant system, treatment with ethrel resulted in increased total accommodation of 14CO₂ into the leaves while decreased total integration of 14C sucrose.

In the present investigation ethephon treatment caused reduction in reducing sugar content in leaves but increase in stem and root while starch content is elevated in all plant parts and soluble sugars are decreased in all plant parts. The effect is found to be significantly positive due to low concentration than high concentration of ethephon. so low dose of ethephon may be beneficial to coleus as far as quality of root is considered.

Thus, it can be concluded that among all PGRs both doses of triacontanol, brassinosteroids and lower doses of CCC and ethephon are found to be beneficial for the coleus plant as far as carbon metabolism and accumulation of carbohydrate fraction in root, stem and leaf along with quality of coleus root is considered.

2. Enzyme α -amylase activity (EC 3.2.1.1)

Effect of foliar spray of PGRs on the enzyme activity of the root of Coleus forskohlii is shown in Table no. 10 and Fig. 62. It can be clearly seen in figure that in root tissue α -amylase enzyme activity is increased due to both concentrations of both triacontanol and CCC whereas decreased due to both doses of BRs and ethephon.
Amylase is a class of endo-amylolytic enzymes which includes α amylase ((E.C.3.2.1.2.) and β amylase ((E.C.3.2.1.2.) enzymes. Enzyme α amylase is most important enzyme in carbohydrate metabolism responsible for starch hydrolysis. Starch is a major product of photosynthetic CO₂ assimilation and it accumulates in the chloroplast during daytime. α amylase and β amylase break down starch resulting into amylose and amylopectin (Gallagher et al., 1997). α amylase hydrolyses α (1-4) bond in amylose and amylopectin which results into production of fragments that can be broken down by β amylase and glucoside de branching enzymes. During process of starch break down α amylase acts as regulatory enzyme and can attack and degraded intact starch granules, whereas β amylase, can hydrolyses the oligosaccharide products of α amylase activity. Thus, play a secondary part in the regulation of starch hydrolysis. Assimilated starch degraded during night hours and products of this degradation that is glucose molecule is utilized as respiratory substrates. Alpha amylase enzyme which contributes one of the key enzymes of carbohydrate metabolism has been extensively studied during germination as its activity is increased extensively during seed germination to fulfil increasing demand of energy of growing seedling. So, it is found to be exclusively active in cotyledons in case of dicot seeds (Kozlowski, 1972). Enzyme has a binding site for calcium which is essential for enzyme activity.

The evidences regarding effect of triacontanol on amylase enzyme activity are scarce however Sivakumar et al., (2002) reported increased soluble sugar content in grains of pearl millet indicating the role of triacontanol in source - sink translocation which indirectly depend upon amylase activity. As reported by Sircar and Kundu (1960) plant growth regulators influence the source-sink translocation of soluble sugars. Effect of triacontanol on root amylase activity is unclear.

Sairam et al., (1996) reported positive effect of BRs application on enzyme amylase activity during wheat seed germination under moisture stress. Opposite is found true in present case as in case of coleus root BRs influenced amylase activity negatively.

In contrast to present observations opposite trend is reported by few workers. The effect of GA, CCC on morphophysiological and yield attributes of kidney beans (Phaseolus vulgaris) was studied by El- Fouly et al. (1988). They noticed GA increased amylase activity while CCC decreased amylase activity 30 days after
RESULTS AND DISCUSSION

spraying. Abdul Jaleel et al., (2007a) in white yam tubers reported suppression of GA which in turn resulted in inhibited α-amylase and β-amylase activities. However foliar spray of CCC (10 to 100 ppm) on carnation cultivar Hanancy, grown in pot showed enhancement of floral initiation by CCC which was coincided with increased amylase activity (El- Fouly et al., 1977). Shah and Prathapasenan (1991) stated foliar spray of CCC (1000 ppm) increased amylase activity in leaf tissue of mung bean (Vigna radiata L.). El-Fouly and Garas (1974) reported CCC treatment increases amylase activity in leaves correlated with earlier tuberization in potato. These findings are in agreement with the present results. Consequence of ethephon and gibberellin A3 application on Amaranthus caudatus, seed germination and alpha- and beta-amylase activity under salinity stress was studied by Bialecka and Kepczynski, (2009). They reported positive effect of ethephon on stress affected amylase activity during seed germination in all seeds exposed to all salinity treatments as compared to GA3.

The role of PGRs in influencing the root alpha amylase activity is unclear. However, it can be concluded that activity of alpha amylase is influenced by PGRs differentially. As amylases play a significant role in carbohydrate metabolism and also in regulation of source - sink translocation, change in its activity under the influence of PGRs will certainly influence growth and quality of roots in coleus plant.

3. Enzyme Invertase activity (EC 3.2.1.26)

Influence of foliar spray of both doses triacontanol, BRs, CCC and ethephon on the activity of enzyme invertase from root tissue of C. forskohlii can be seen in Table no. 11 and Fig.63. It is revealed from the results that the activity of enzyme invertase in root tissue of C. forskohlii is decreased due to all doses of all PGRs as compared to control. Among all PGRs lowest activity of invertase is found due to both doses of ethephon spray.

As suggested by Farrar, (1996) the supply of the transport sugar, sucrose is a regulating factor for the development of sink tissue as sucrose is the most vital transport form of sugar as discussed earlier. However, Sucrose cannot be utilized directly for metabolic activities, so it must be hydrolysed into hexoses such as glucose and fructose by enzyme invertase (β-fructosidase) before participating into carbohydrates metabolism. Enzyme invertase can be detected in many organs of plants as in multiple forms. So, these enzymes are grouped depending upon its optimum pH (as acid and neutral/alkaline invertase), based on solubility properties (as
RESULTS AND DISCUSSION

soluble and insoluble invertase) and according to its cellular locations. According to Kim et al., (2000) cell wall bound invertases are acidic and insoluble whereas vacuolar invertases are also acidic but soluble. While cytoplasmic invertases are both neutral alkaline and soluble. As proposed by Nguyen-Quoc and Foyer, (2001), extracellular invertase is associated with carriage of sucrose into the apoplast and vacuole and hexose transport across the plasmamembrane while vacuolar invertase links the transport of sucrose into the apoplast and vacuole and hexose transport across the tonoplast. Extracellular invertase is a cell-wall bound enzyme associated with the unalterable breakage of sucrose released into the apoplast through sucrose transporters while the resulting hexose monomers (glucose and fructose) are then transferred into the sink by monosaccharides transporters. As reported by Roitsch et al., (2003) the extracellular invertase is mainly suited as a significant regulator of apoplastic phloem unloading because of its enzymological nature. Thus, invertases participates in sucrose partitioning between source and sink organs. In young sink tissues, cell wall-bound invertases can boost carbohydrate transport by maintaining a sucrose concentration gradient between phloem and sink tissues (Cheng et al., 1996) while in mature tissues they can alter sucrose and starch balance (Huber, 1983). Particularly, in source organs such as leaf the enzyme invertase may regulate carbohydrate export rate by hydrolyzing sucrose, thus delaying sucrose loading into phloem while in sink organs its activity regulates phloem unloading. According to Hawker (1985), the acid invertase is involved in the supply of hexoses for respiration, the establishment of sucrose gradients and the vacuolar osmotic-turgor related cell expansion.

As opined by Pollock, (1986) invertase also perform other minor catalytic activities such as hydrolysis of raffinose, hydrolysis and synthesis of fructan. It also catalyses the synthesis of sucrose from raffinose and glucose as stated by Nadkarni et al., (1992). Apart from various factors like pH, photosynthetic rate, availability of substrate and products accumulation there are various evidences supporting the role of PGRs in regulation of enzyme invertase. Several evidences backs the theory that gibberellic acid performs a major role in regulation of invertase levels (Tymowska and Kreis, 1998). Gibberellic acid promoted cell elongation is significant for flower induction and it increases invertase activity in many plant organs such as sugar cane stem (Sacher et al., 1963). The activity of extracellular invertase is triggered by auxin (Weil and Rauch, 1990). The experiments of Trouverie et al., (2003) showed that
vacuolar invertase activity seems to be correlated with ABA concentration in xylem sap. Yu et al., (2004) assessed the impact of 24-epibrassinolide (EBR) spray application on Rubisco enzyme and carbohydrate metabolism in cucumber (Cucumis sativus L.). They reported enhanced level of carbohydrate contents followed by increased activity of acid invertase activity in leaves along with stimulated activity of SPS and SS enzyme after EBR treatment. In agreement with this, Schluter et al., (2002) also reported decreased starch and sucrose contents, and reduced activities of invertase in BR-deficient Arabidopsis mutant as compared with the wild type indicating role of BRs in influencing invertase enzyme activity. There are limited studies on effect of BRs on root invertase enzyme.

Fong Chong et al., (2010) investigated the effect of hormone ethylene on the connection between growth and early sucrose accumulation in sugarcane. The sugarcane variety KQ228 was exposed to a low concentration of the ethylene-forming compound 2-chloroethylphosphonic acid (CEPA). They reported that higher reducing sugar level in the apical region of the culm might have boosted faster internode development. This concurred with elevated vacuolar and cell wall acid invertase gene expression which resulted in increased sucrose production in the vacuole and raised apoplastic uptake of reducing sugars. Biomass enhancement can be correlated with increase in acid invertase activity in young leaves, which probably provided additional assimilates to the plant because of their larger sizes (Schilling et al., 1991)

Several evidences support involvement of gibberellic acid in regulating invertase levels. Gibberellic acid stimulated cell elongation is significant for flower induction and causes increase in invertase activity in several plant organs (Sacher et al., 1963; Tymowska and Kreis, 1998). So as CCC is an antigibberllins, its application might have hindered the GA induced stimulation of invertase resulting in low invertase enzyme activity in tuberous root of coleus in present investigation. As mentioned earlier invertase activity is also influenced by substrate and product concentration in cell sap (Nikam, 2007) which might be the factor behind reduced invertase activity in root of coleus. Or it may be due to altered source - sink translocation process induced by PGRs.

It can be concluded that as activity of enzyme invertase in root tissue is reduced due to all PGRs as compared to control may result in increased accumulation
RESULTS AND DISCUSSION

of sucrose or sugars in root tissue which will be beneficial to quality and yield of coleus root.

D. Organic acids

Influence of foliar spray of PGRs on organic acids (TAN)

Influence of foliar spray of both concentrations of triacontanol, BRs, CCC and ethephon on the organic acid level (as indicated by TAN values) in leaf tissue in Coleus forskohlii is recorded in Table no. 12 and Fig. 64. It is clear from the figure that the amount of organic acids in the leaf tissue is slightly increased due to both concentrations of triacontanol, high concentration of BRs and due to low concentration of CCC and ethephon. However, there was no significant increase or decrease observed in organic acids by other PGRs doses as compared to control. Thus, it can be said that organic acid level is not influenced by PGRs significantly.

Organic acids are the most important intermediates of a central metabolic pathway, TCA cycle. These are perhaps the most common metabolites present in all aerobic organisms. Malic acid is one of the common organic acid which is involved in CAM and C_4 pathway while isocitric acid and succinic acid are important intermediates of glyoxylate cycle. The carbon skeletons for the synthesis of number of important metabolites are provided by these compounds. It is now very well established that accumulation of organic acids in plant is a measure of the adjustment of the cation-anion balance in plant sap and the transport of metabolic cations in plant (Popp and Kinzel, 1971; Triplett et al., 1980). Osmond, (1963) have reported that oxalic acid participates in osmoregulation in halophytes such as Atriplex. The level of organic acid in plant tissue can be altered by the relative levels of available anions (Cl^-, SO_4^{2-}, NO_3^-) or a cation (NH_4^+) which can be metabolized by the plants. As mentioned by Nikam, (2007) the physiological role and significance of organic acids in the metabolic processes of plant, which are involved in the respiratory or photosynthetic chain has not yet been explained, apart from that of particular tissues. In the present investigation, in case of Coleus leaves the organic acid level is slightly increased due to some of the PGRs like triacontanol and BRs up to some extent there was no significant difference observed organic acid content in between all the treatments and control. The level of organic acid is at par with the control due to all other PGRs tried so this does not indicate any major shift in the organic acid metabolism of coleus under the influence of PGRs.
Serna et al., (2013) studied effect of two BRs analogues DI 100 and DI 31 in *Cichorium endivia* L. and recorded that All treatments with DI-100 and DI-31 resulted in the highest production increase however the chemical variables related to endive quality, such as moisture, carbon and nitrogen content and organic acid content were similar in the control and treated endives.

According to very recent studies by Ramana and Chaitanya (2015) *Coleus forskohlii* is a CAM plant showing high NAD-Me type enzyme activity. Organic acids being most important intermediates of a central metabolic pathway, TCA cycle, are perhaps the most common metabolites present in all aerobic organisms. Increased level of organic acids measured in terms of TAN, due to foliar application of triacontanol, BRs and low doses of CCC and ethephon indicates positive effect of PGRs on metabolic activities. It might also stimulate carbon metabolism as organic acids provide basic carbon skeleton for several metabolites.

**E. Antioxidant Status**

**Influence of foliar spray of PGRs on enzymatic antioxidants and non-enzymatic antioxidants**

Oxidative stress can arise from an imbalance between the formation and elimination of reactive oxygen species (ROS), leading to excess ROS levels, that causes damage to almost all biomolecules, which results in various physiological disorders, various diseases and even cell death. Reactive oxygen species can be eradicated by a number of enzymatic and non-enzymatic antioxidant defense mechanisms (Scandalios, 2005).

1. **Enzymatic antioxidants**

As mentioned by Ahmed et al., (2002) and Sairam, et al., 1998 and 2002) to counteract the hazardous effects of ROS, plants have evolved a complex antioxidative defense system composed of both antioxidant enzymes and metabolites such as ascorbate peroxidase, Catalase, Superoxide dismutase, glutathione reductase, ascorbic acid, reduced glutathione, Oxidized glutathione and vitamin E.

a. **Catalase enzyme (CAT) (EC 1.11.1.6)**

The influence of foliar application of triacontanol(Vipul), Brassinosteroids (BRs), CCC and Ethephon on the activity of enzyme catalase in leaf and root tissue of *Coleus forskohlii* is shown in Table no. 13 and Fig. 65. It is revealed from the figure that the activity of enzyme catalase is stimulated due to both concentrations of all the
PGRs in leaf tissue where as in root tissue it is positively influenced only by triacontanol and ethephon and decreased due to BRs and CCC. It can be also revealed that catalase enzyme activity in root tissue is elevated than leaf tissue in Coleus in control and PGR treatments except CCC. Khatun et al., (2011) also made similar observations in same plant Coleus forskohlii. They also reported higher catalase activity in root tubers than leaf and stem part.

Catalase is a hydrogen peroxide oxido-reductase enzyme and consists of a dumbbell shaped tetramer of four identical protein subunit having iron centered porphyrin ring, homo-tetrameric heme protein, this heme group is responsible for catalysis. It catalyses the decomposition of hydrogen peroxide into water and oxygen and acts as an antioxidant.

Peroxides are very reactive compounds that create an oxidation hazard to cells when they accumulate to higher concentrations in cells. The H₂O₂ (Hydrogen peroxide) is formed during photorespiration in C₃ species which is removed by enzyme catalase located predominantly in leaf peroxisomes (Dat et al., 2000). As stated by Asada, (1994) the electron transfer chain of the chloroplast is the best-documented source of H₂O₂. According to Pang et al., (2005) Hydrogen peroxide acts as a precursor of more cytotoxic or highly reactive oxygen derivatives such as peroxynitrite or OH. So, it is very important to scavenge excess H₂O₂. Various abiotic and biotic stresses also stimulate the generation and accumulation of Hydrogen peroxide. Hydrogen peroxide is also a part of the reactive oxygen regulatory network (Mittler et al., 2004). Therefore, it is important that H₂O₂ is scavenged rapidly and systematically by the antioxidative defence system. As proposed by Guo et al., (2006) catalase is one of the primary H₂O₂ scavenging enzymes. Catalase catalyses decomposition of H₂O₂ into water and oxygen, which does not require reducing substrate for its activity (Mittler, 2002).

According to Dat et al., (2000), Catalase is known to present principally in leaf peroxisomes, where it mainly carries out removal of H₂O₂, formed during photorespiration in C₃ species. However, there are also few reports of its localization in the mitochondrion of maize and in the apoplast. Plant catalases are haem enzymes which operates as tetrameric proteins with four protoporphyrin IX moieties and show a high molecular efficiency but a very low Km values ranging from 10 and 140 mM H₂O₂ (Feierabend, 2005). In general, hydrogen peroxide is considered as less harmful
than other reactive oxygen species (ROS) like superoxide anion radical and hydroxyl radical (Dietz et al; 2006). H$_2$O$_2$ also serves as a signalling function in cellular communication because of its capacity to diffuse over substantial distances within and between cells (Apel and Hirt, 2004). Also, a long-distance signalling function has been attributed to H$_2$O$_2$ w.r.t systemic acclimation to surplus excitation energy as stated by Karpinski et al., (1999) in Arabidopsis. This dual role of H$_2$O$_2$ as a potentially damaging compound and as a messenger requires a balanced defence system. So, it is important to study the effect of various PGR application on antioxidant status of the plant w. r. t. increased productivity and sustainability of plant through adverse conditions or PGR induced photosynthesis stimulation. There are various evidences regarding the effect of PGRs on Catalases.

Khandaker et al., (2013) recorded increased level of antioxidant activities in Bougainvillea glabra in response to triacontanol spray. Karam et al., (2016) found enhanced activity of catalase due to triacontanol in Arsenic-stressed coriander (Coriandrum sativum) plant at 5, 10, 20 µmol L$^{-1}$ along with increased SOD and Peroxidase activity.

Anuradha and Rao (2007) noticed decreased activity of catalase (CAT) in radish seedlings due to Cd toxicity but addition of BRs increased the activity of catalase indicating alleviation of adverse stress effects by HBL (3µM) of the heavy metal. A similar increment in catalase activity under water stress in sorghum seedlings caused by the application of brassinosteroids was reported by Vardhini and Rao, (2003). Thus, activity of catalase can be positively correlated with increase in growth of the plant (Anuradha and Rao, 2007). Hayat et al., (2007) noticed that foliar spraying of HBL improved the Cd-tolerance in Brassica juncea by raising activity of antioxidative enzymes and the content of osmolyte (such as proline). Yu et al., (2009) studied the effect of 24-EBR on cucumber roots infected by Fusarium wilt w. r. t its antioxidant status and they reported that BRs treatments reduced the pathogen induced accumulation of reactive oxygen species (ROS) and activities of defence-related and ROS-scavenging enzymes like catalase. Behnamnia et al., (2009) in tomato plants under drought stress reported highly increased activity of catalase enzyme so as to sustain adverse effects of drought stress which was reduced by BRs application. Sharma et al., (2010) and Hasan et al. (2008) also reported HBL-mediated enhanced activity of antioxidant enzymes and the protection of Raphanus
RESULTS AND DISCUSSION

*sativus* and *Cicer arietinum* against Cd- stress. As observed by Kumar et al., (2010) in chilling (4°C) stress subjected *Brassica juncea* seedlings, exogenously applied 24-EBR diminished the lethal effect of H$_2$O$_2$ by elevating various enzymes activities like CAT and SOD which are involved in antioxidant defence system.

Wang et al., (2010) tried to study the effects of CCC, on mineral nutrition, antioxidant enzyme system, and tuber yield of potato (cv. Zhongshu 3). They recorded increased activity of catalase (CAT) in the leaves of potato at all concentration.

El-Kady et al, (1980b) studied effect of various growth regulators on some enzyme activities and phenolic compounds in datura *Datura stramonium* plant. They reported that catalase activity was highest in plants treated with ethephon at 50 ppm.

As suggested by Pang et al., (2005) an increased activity of catalase under salt stressed condition in leaf tissue of *Suaeda salsa* might be attributed to lowering of H$_2$O$_2$ and thus increased level of catalase due to PGRs under stressed conditions might be playing an important role in lowering H$_2$O$_2$, resulting in the protection of plant tissue from oxidative stress created due to H$_2$O$_2$.

In the present investigation increased leaf catalase due to PGRs might be an indicator of increased rate of photosynthesis and growth (Anuradha and Rao, 2007), or altered metabolic reactions, changed membrane permeability triggered due to PGR application and an indicator of strong antioxidant system of plant induced by PGR (Kumar et al., 2010). Thus, increased catalase activity due to PGRs will be helpful to coleus plant to boost its antioxidant system.

b. Peroxidase enzyme (EC 1.11.1.7)

The influence of foliar spray of triacontanol (Vipul), brassinosteroids (BRs), CCC and ethephon on the activity of enzyme peroxidase in leaf and root tissue of *Coleus forskohlii* is shown in Table no. 14 and Fig. 66. In case of triacontanol treated plants, peroxidase is decreased in leaf and slightly increased or at par with control in root tissue due to both doses of triacontanol. Whereas in case of BRs only high concentration shows stimulated peroxidase activity in leaf while in root both doses of BRs decreased the activity of peroxidase. In CCC treated plants, only high dose of CCC stimulated leaf and root peroxidase while opposite trend is seen due to low concentration of CCC. It can be seen that both doses of ethephon shows significant enhancement in leaf peroxidase activity while in root tissue only high dose of...
etephon caused high peroxidase activity in Coleus. Significantly increased activity in root tissue is found only at high concentrations of CCC and etephon. Highest activity of peroxidase is recorded due to high dose of etephon in both leaf and root tissue while lowest due to BRs.

Peroxidases are oxido-reductase (Vamos-Vigyazo, 1981) enzymes which play an important role in ROS removal, as an antioxidant and occurs in majority of plant tissues. These enzymes are heme containing glycoproteins which contribute in a large number of physiological processes like biosynthesis of lignin and ethylene, defence against pathogens and wounding, auxin metabolism and stress response (Halbrock and Gricebach, 1979; Lagrimini and Rothstein, 1987 and Kim et al., 1999; Nikam, 2007; patil, 2011; Jadhav, 2016). According to Haard, (1973) and Mc Dougall, (1993) number of peroxidase isozymes have been detected in soluble, ionically-bound and covalently-bound forms. The soluble forms are cytoplasmic, whereas bound forms are generally thought to be associated with particulate components such as plant cell walls as proposed by Hepler et al., (1972) and some cell organelles such as in Mitochondria (Haard, 1973).

As suggested by Prasad et al., (1994) and Vianello et al., (1997) peroxidase catalyses the dehydrogenation of structurally diverse phenolic and indole substrates by \( \text{H}_2\text{O}_2 \) and are often considered as antioxidant enzymes, protecting cells from the damaging influence of \( \text{H}_2\text{O}_2 \) and derived oxygen species. Cell wall-located peroxidase activity has a role to perform in cell wall formation (Ingham et al., 1998). It is thought to play a key role in controlling the deposition of lignin in vascular tissue and this has been supported by the cytochemical localization of peroxidase activity in the reticulate secondary wall and adjacent primary wall of Coleus. According to Van Huystee, (1987), the peroxidases belong to a family of glycoproteins containing iron atoms as a prosthetic group and different quantities of carbohydrate residues. Peroxidases are classified as class I enzymes (from mitochondria, chloroplast and bacteria), class II enzymes (from fungi) and class III enzymes (classical plants peroxidases). Van Huystee (1987) and Van Huystee and Esnault (1992) studied the structure and function of enzyme peroxidase after the extensive work on peanut. There are various evidences regarding the effect of PGRs on peroxidases.

Henry and Gordon, (1980) reported that triacontanol application caused an increase in peroxidase activity in ‘Little Marvel dwarf’ (LM) and ‘Alaska’ peas (AP)
plants as compared to the untreated controls. They also stated that the effects of triacontanol on root and stem growth, peroxidase activity, and auxin-destruction appeared to be cultivar-specific, with respect to LM and AP varieties of peas. Lesniak and Ries (1983) studied effect triacontanol on various enzyme activities and they recorded that peroxidase activity remained relatively constant on a per plant basis and decreased slightly on a per mg protein basis in corn seedlings. Similar decrease in leaf tissue peroxidase is observed in present studies due to triacontanol. Karam et al., (2016) found enhanced activity of peroxidase due to triacontanol in Arsenic-stressed coriander (*Coriandrum sativum*) plant at 5, 10, 20 µmol L\(^{-1}\) along with increased SOD activity.

Anuradha and Rao (2007) noticed decreased activity of catalase (CAT) in radish seedlings due to Cd toxicity but increased activity of peroxidase enzyme in response to stress however they reported that addition of BRs to Cd-stressed plants reduces the activity of peroxidase indicating alleviation of adverse stress effects. According to Hayat *et al.*, (2007) foliar application of HBL was reported to improve Cd-stress tolerance in *Brassica juncea* through increasing activity of antioxidative enzymes like peroxidases. In chilling (4°C) stressed *Brassica juncea* seedlings, application of 24-EBR removed the toxic effect of \(H_2O_2\) by increasing the activities of enzymes of antioxidants (Kumar *et al.*, 2010). Rady, (2011) opined that Cd tolerance noticed in *Phaseolus vulgaris* was as a result of 24-EBL mediated enhanced activity of antioxidative enzymes and proline content and subsequent improvements in the membrane stability index (MSI), relative leaf water content (RLWC). Alleviation of the damaging effect of Cd was reported in tomato cultivars (K-25 and Sarvodya) by 28-HBR or 24-EBR stimulated improvement in photosynthetic apparatus and antioxidant defense system (Hasan *et al.*, 2011). Similar amelioration effect of BRs application at \(10^{-8}\) M was found in *Solanum lycopersicum* plants, BRs enhanced antioxidant system activity and improved fruit yield and quality under Cd Stress (Hayat *et al.*, 2012). Zhang *et al.*, (2008) noticed that foliar application of BRs elevated the activities of POD and SOD, increased in the leaves of drought exposed soybean (*Glycine max*). There are several reports regarding the ameliorating ability of BRs in various abiotic and biotic stresses by improving catalase and reducing peroxidase activity and other defence enzymes in plants which is reviewed by Vardhini and Anjum (2015). Choudhari *et al.*, (2012) found that of co-applications of
EBL and Spd modulated more remarkably the constituents of antioxidants (glutathione, ascorbic acid, proline, glycine betaine and total phenol and activities of antioxidant enzymes (guaiacol peroxidase, catalase, superoxide dismutase and glutathione reductase) in Cr-stressed *Raphanus sativus* plants than their individual applications and control. Yu *et al.*, (2009) reported decreased activity of ROS scavenging enzymes like peroxidase due to EBL treatment in cucumber roots which was increased due to infection with Fusarium wilt. Cag *et al.*, (2007) studied the effect of cotyledon growth by incubation in 3 ml distilled water (control group) and in epiBL of various concentrations (0.001, 0.1 and 10 μM) for 3 days in excised red cabbage cotyledons of *Brassica oleracea* seedlings. They noticed that peroxidase activity, as a result of epiBL application in excised red cabbage cotyledons show difference related to concentration. At high concentration (10 μM) it was decreased while at low dosage it remained unchanged as compared to control. Similar concentration dependent change in peroxidase activity is also noticed in present investigation in both leaf and root tissue of Coleus. Behnamnia *et al.*, (2009) in tomato plants under drought stress reported reduced activity of peroxidase enzyme as an adverse effects of drought stress which was found to be increased by BRs application.

Wang *et al.*, (2010) noticed the effects of CCC treatment on antioxidant enzyme system, and tuber yield of potato (cv. Zhongshu 3) and reported increased activity of superoxide dismutase (SOD), peroxidases (POD) enzymes in the leaves of potato due to CCC. Farooqi *et al.*, (2005) studied the responses of *Cymbopogon martini* and *C. winterianus* subjected to drought stress and chlormequat chloride (CCC) and IAA application. They observed increased peroxidase activity in *C. martini* due to drought stress however ameliorative effects of chlormequat chloride and IAA were observed in drought stressed plants of both species. Khan, *et al.*, (2008) in *Vetiveria zizanioides* analyzed the impact of water stress and plant growth retardants (Chlormequat chloride, paclobutrazol, ethrel) on root yield, oil content, oil yield and oil composition. Ameliorating effect of CCC and ethrel was reported in drought stressed plant. They found increased peroxidase activity by application of plant growth retardants both in unstressed and stressed plants. Similar increment is visible in both leaf and root tissue of coleus due to high dose of CCC in present study.
Singh and Misra (2001) detected enhanced peroxidase activity in *Mentha spicata* L. var. MSS-5 due to ethephon. Plantlets were cultured in nutrient solution were treated with gibberellin (GA) and ethrel at high concentrations (1000 mg/m). El-Kady *et al.*, (1980b) studied effect of various growth regulators on some enzyme activities and phenolic compounds in datura (*Datura stramonium*) plant. They reported increased peroxidase activity in the leaves was highest in plants treated with ethephon 4000 ppm.

No consensus emerges from the literature regarding the influence of PGRs on peroxidase activity. There are two views regarding the activity of peroxidases such as activity of peroxidase is low under normal healthy growing conditions and increases in response to certain stress conditions in plant and again there are many evidences which prove that its activity gets decreased due to many stresses and PGRs application helps in increasing its activity. So, in case of present study at par values of leaf peroxidase due to growth promoters like triacontanol and BRs indicate healthy state of plant where as high peroxidase activity in roots indicate strong antioxidant system which will help plant to face stress due to abiotic factor. High peroxidase activity due to CCC and ethephon at high dose indicates altered physiological or biochemical processes due growth retarding nature of these PGRs.

Thus, it can be concluded that activity of peroxidase was found to be influenced differentially based on concentration and part of plant. However overall enhancement in peroxidase activity due to triacontanol, CCC and ethephon in leaf and more prominently in root exhibit positive influence of PGRs on antioxidant defence system of Coleus.

2. Non enzymatic Antioxidant

a. Ascorbic Acid content

The variation in the level of ascorbic acid content from leaf and root tissue of *Coleus forskohlii* in response to foliar application of triacontanol, BRs, CCC and ethephon is depicted in Table no. 15 and Fig. 67. It can be revealed from the results that in leaf tissue the ascorbic acid content is reduced due to both concentrations of all the PGRs tried except due to CCC lower dosage it is significantly increased in leaf tissue as compared to control While in case of root tissue Ascorbic acid level is increased by all PGRs applications except for BRs high and CCC low dosage it is at par with the control treatment.
Ascorbic acid or Vitamin C is multifunctional molecule essentially required by plants as well as by human beings. It is a water-soluble antioxidant that mainly performs a protective role in both plants and animals. Humans are unable to make vitamin C (Chatterjee, 1973; Gallie, 2013). So, we are completely dependent upon plant based ascorbic acid for our Vitamin C needs so several attempts have been made to study this important plant molecule and its properties. However, in plant system, ascorbic acid plays several roles such as it is a major redox buffer (Pignocchi and Foyer, 2003) and also acts as an obligatory co-factor for many enzymes and as a major antioxidant (Smirnoff and Wheeler, 2000). It also regulates cell division and growth (Kerk and Feldman, 1995) and is involved in signal transduction (Pignocchi and Foyer, 2003). Ascorbic acid acts as a redox buffer and as a co-factor for enzymes involved in photosynthesis, hormone biosynthesis and producing other antioxidants (Gallie, 2013).

In plants, ascorbic acid is synthesized through the Smirnoff-Wheeler pathway from L-galactose (Wheeler, et al., 1998). L-galactose is formed from mannose-1-phosphate by the conversion of guanosine diphosphate (GDP)-mannose to GDP-L-galactose catalysed by enzyme GDP-mannose 3′, 5′-epimerase (Wolucka et al., 2001). GDP-L-galactose is further transformed into L-galactose. This is further get oxidized in the presence of enzyme NAD-dependent L-galactose dehydrogenase to synthesize L-Galactono-1,4-lactone. This L-Galactono-1,4-lactone acts as an immediate precursor of ascorbic acid and it get oxidized to form ascorbic acid which is catalysed by enzyme L-galactono-1,4-lactone dehydrogenase found located on the outer side of the inner membrane of mitochondria (Bartoli et al., 2000; Siendones et al., 1999). Preliminary steps of the ascorbic acid biosynthesis are traced in the cytoplasm whereas the oxidation of L-galactono-1,4- lactone through cytochrome c takes place in mitochondria which indicates incorporation of ascorbic biosynthesis with cellular oxido-reduction state and energy metabolism (Gallie, 2013). Along with this well-known Smirnoff-Wheeler pathway in plants there are some recent studies which have proposed presence of additional ascorbic acid biosynthetic pathway in plants. This biosynthetic pathway was putforth by Loewus and Kelly (1961) using detached ripening strawberry fruit in which L-galacturonic acid-1-14C was metabolized to L-ascorbic acid-6-14C by an inversion pathway. It is proposed that in this pathway, D-galacturonic acid, formed from the breakdown of pectin in the
RESULTS AND DISCUSSION

Ripening fruit, is reduced to L-galactonic acid by the action of an enzyme NADPH-dependent D-galacturonic acid reductase (GalUR), and the L-galactonic acid then spontaneously converts to L-galactono-1,4 lactone (Valpuesta and Botella (2004). However, additional studies indicated, that the generation of L-ascorbic acid from GalUR could be responsible for only a little portion of the total ascorbic acid produced in strawberry fruit suggesting that this pathway make an insignificant contribution to Ascorbic acid biosynthesis or may be specific to certain organs under specific conditions (Loewus, (1963). Recent studies with Arabidopsis mutants also confirmed that the Smirnoff-Wheeler pathway is probably the most responsible for the majority of foliar Ascorbic acid biosynthesis in Arabidopsis and perhaps in other plant species also.

Ascorbic Acid is present and transported throughout the plant so its level in one part or tissue of the plant affects level of ascorbic acid in other part. Ascorbic acid uptake in chloroplasts utilizes a specific transporter (Beck et al., (1983), Foyer and Lelandais (1996) whereas, in mitochondria, it shows low affinity and therefore probably crosses the mitochondrial membrane in its oxidized form (DHA- an oxidized form of ascorbic acid) (Szarka et al., (2004). Long distance transport of ascorbic acid takes place through phloem as indicated by the accumulation of radiolabelled ascorbic acid in phloem and its transport to root tips, shoots and floral organs, but not to mature leaves (Franceschi, and Tarlyn, (2002). There are various factors which regulates the ascorbic acid content in plant as reviewed by Gallie, (2013) such as light intensity, biotic and abiotic stresses and it is highly essential to study the effect of PGRs on this indispensable antioxidant molecule which is deeply integrated in almost all plant life processes directly or indirectly. There are some attempts made by some workers to study this.

Khan et al., (2009) reported enhanced content of fruit ascorbic acid and lycopene contents in tomato (Lycopersicon esculentum Mill.) in response to triacontanol.

Anuradha and Rao (2007) observed increased activity of enzyme ascorbic acid oxidase (AAO) in radish seedlings under the Cd- stress. The activity of AAO is inversely proportional to content of ascorbic acid so, increased activity caused decreased ascorbic acid content. However, they noticed that increased activity of AAO under heavy metal stress, was decreased due to supplementation of
RESULTS AND DISCUSSION

brassinosteroids causing accumulation of ascorbic acid. The depressed activity of AAO could be an adaptive feature found in flooding-tolerant cultivars of rice, which shows enhanced amounts of ascorbic acid and this elevation in ascorbic acid is very important to advance defense system against abiotic stress (Sarkar and Das 2000). Yu et al., (2009) also found increased activity of ascorbate peroxidase in cucumber plant due to Fusarium wilt disease as a pathogen related response of plant however they reported that EBR application reversed the same and decreased AAO activity resulting in stability of ascorbic acid in EBL treated wilt infected cucumber plant. Vardhini et al., (2012) reported increased ascorbic acid and niacin contents in the roots of radish plants by the foliar application of BRs. According to them, 28-HBL at 3µm concentration was more dominant in increasing the ascorbic acid and niacin contents compared to 24-EBL treatments and untreated control plants. Biesaga-Kościelniak et al., (2014) in pea and lupine after application of brassinosteroid (24-epibrassinolide via spraying recorded accumulation of antioxidants (vitamins). Ascorbic acid (as vitamin C), tocopherols and tocotrienols (as vitamin E) and β-carotene (as provitamin A) are known as substances that affect the nutritional value of plants. Raghu and Rao (2016) found enhanced level of the antioxidant potential by means of DPPH, H$_2$O$_2$ and OH radical scavenging activity and ferric reducing power and also antioxidant content (phenols and flavonoids) in the leaves, stems and roots of Tinospora cordifolia supplemented with BRs as well as brassinosteroids caused improved radical scavenging activity compared to untreated control plants.

In cucumber plants, ethrel foliar application at 200 and 400ppm resulted in increased growth along with increased ascorbic acid content in fruit (Vadigeri et al., 2001).

In present study also, ascorbic acid content is increased in root tissue which is major sink tissue in coleus plants due to application of PGRs. As plant-based foods make the major source of vitamin C in human diets. The opportunity of increasing the ascorbic acid content of plants to improve their nutritious value has received substantial attention in recent years (Gallie 2013). Thus, in the present investigation, increased accumulation of ascorbic acid is beneficial for healthy root growth and protection of plant from oxidative stress by maintaining growth and ultimately better yield and quality of crop.
F. Secondary metabolism

Influence of foliar spray of PGRs on various secondary metabolites and enzymes of secondary metabolism

1. Total polyphenols

Influence of foliar spray of triacontanol, BRs, CCC and ethephon on total polyphenols content of the leaf and root tissue of *Coleus forskohlii* is shown in Table No. 16 and depicted in Figure 68. It is evident from the results that in both leaf and root tissue of *Coleus forskohlii* similar trend of increment or decrease due to PGRs is seen. Total polyphenol content is increased due to both concentrations of ethephon, low concentration of CCC and high concentration of BRs in both leaf and root. In case of triacontanol treated plant, in leaf and root tissue total polyphenols are at par with the control due to low dosage of triacontanol while reduced due to high concentration. Lowest content of total polyphenols is seen in plants treated with low dose of BRs and high dose of CCC. Thus, it can be revealed that various PGRs affected the contents of polyphenols differently at different concentration in leaf and root tissue. However, leaf and root tissue behaved similarly to same concentration of PGR.

Phenolics are most abundant secondary metabolites in plants, possessing one or more aromatic rings with one or more hydroxyl functional groups. These form a chemically heterogeneous group of nearly 10,000 individual compounds. Polyphenolic compounds are present in most of the phanerogamic species. Phenolics group constitutes a group of compounds from simple molecules like phenolic acids, flavonoids, anthocyanidins and other related substances with an unsophisticated structure, to highly polymerised substances like tannins. Phenolics are widely distributed in plant., with more than 8,000 phenolic structures currently known. According to Taiz and Zeiger, (2006) some phenols are soluble only in organic solvents while others are water soluble. Carboxylic acids and glycosides and others are large, insoluble polymers. Phenolics have been considered powerful antioxidants *in vitro* and have proved to be more potent antioxidants than vitamin C (ascorbic acid) and E and carotenoid as suggested by Rice-Evans et al., (1997). As illustrated by Luque- Rodriguez et al., (2007) antioxidant properties of phenolic compounds can be mediated by the following 3 mechanisms such as: 1) scavenging radical species such as ROS/RNS 2) suppressing ROS/RNS formation by inhibiting some enzymes or
chelating trace metals involved in free radical production; 3) up-regulating or protecting antioxidant defence. Plant phenolics include phenolic acid, flavonoids, tannins and less common stibenes and lignins. Phenolic compounds act as free radical acceptors and chain breakers. They interfere with the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals. Phenolic compounds acquire ideal structure chemistry for free radical scavenging activities because they have: 1) phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; 2) extended conjugated aromatic system to delocalize an unpaired electron (Dai and Mumper, 2010). Polyphenolic compounds are also reported to cause stimulation of protein synthesis (Greppin and Horn, 1969) and promotion of ammonia elimination (Letan, 1967). In plant cell, polyphenols are also involved in hydrogen peroxide scavenging cascade (Takahama and Oniki, 1997). Heller and Forkman, (1988) reported that in leguminous plants, both root and shoot are two sites of polyphenol synthesis through phenyl propanoid /shikimic acid pathways. The simple phenolic acids such as cinnamic acid, p-coumaric acid and caffeic acid are common to the chlorogenic acid biosynthetic pathway and differ only in the extent of hydroxylation of the aromatic ring (Grace et al., 1998). Plant phenolics are generally involved in defence against ultraviolet radiation or attack by pathogens, parasites and predators as well as contributing to plant colours. Some phenolic compounds are known to interact with phytohormones and influence the growth processes (Jacobs and Rubery, 1988). Turgorins are new class of phytohormones that regulates the turgor of the plants all leaf-movements (nyctinastic and seismonastic movements). There are many reports regarding the role of PGRs in manipulation of the phenolic contents in a plant apart from abiotic and biotic stresses. Secondary metabolites like phenols, indoles, quinones, diterpenes etc are actually playing role in medicinal properties of medicinal plants such as artemisinin, vinblastine, catharanthine, diterpenoids like forskolin, coleonol, withanolide etc. Triacontanol has been reported to enhance the production of secondary metabolites in Artemisia annua (Yaseen and Tajuddin 1998). Kumaravelu et al. (2000) reported the positive effect of TRIA applied at 0.5 mg dm-3 on total phenols in green gram (Vigna radiata L.) Grzegorczyk et al. (2006) observed a positive effect of TRIA on shoot multiplication, production of biochemical compounds, and antioxidant capacity of S. officinalis. Moreover, TRIA also increased carnosol content in sage shoots, with a little influence
on carnosic acid proving the ability of TRIA to increase diterpenoid production. Naeem et al. (2010) recorded a TRIA-mediated improvement in the level of anthraquinone and sennoside content in *coffee senna* which are phenolic compounds. Naeem *et al.*, (2011) recorded that triacontanol, applied at 10-6 M, increased the total phenolic content maximally in mint plant. They observed that TRIA application resulted in an increase in phenolic content by 6.7 and 10.3% over the control at 100 and 120 DAP, respectively however thereafter, at 10-5 M TRIA, the content of phenols decreased significantly in Japanese mint, *Mentha arvensis* L. Kamble and Chavan (2011) reported increased level of total polyphenol content in vetiver grass due to triacontanol (vipul) foliar treatment. Khandaker *et al.*, (2013) recorded increased level of Phenol contents in *Bougainvillea glabra* in response to triacontanol spray. Anilkumar (2005) reported no significant variation or at par with control level of total phenolic contents in leaves of Patchouli plant *Pogostemon cablin* Benth. L. due to application of triacontanol (Miraculan) at 1000 and 2000ppm. Same is found true in present study.

As mentioned by Ries and Houtz, (1983) triacontanol like other PGRs also influence enzyme activities and triggers the cascade of various effects resulting in increased metabolism and accumulate various critical intermediates of metabolic processes. However, in coleus leaf and root the polyphenol contents are less affected due to triacontanol or decreased due to high dose indicating altered mode of secondary metabolism.  

Khripach *et al.*, (1996) suggested that the augmented resistance to sprouting and diseases in BR-treated potato tubers was related with enhanced synthesis of abscisic acid (ABA) as well as phenolics and terpenoids. As proposed by Khripach *et al.*, (2000) in cucumber plants, increased activities of peroxidase and polyphenol oxidase (PPO) enzymes, involved in the metabolism of polyphenols, might be a contributing factor to BR-induced disease resistance. Abd El-Wahed *et al.*, (2003) in rice recorded increased level of phenolic compounds in rice plant due to sitosterol. Abd El-Wahed *et al.*, (2004) reported effect of stigmasterol on chamomile plant. They reported increased number of phenols and indoles at 75mg/L concentration. Yu *et al.*, (2009) noticed that EBL treatments significantly lowered pathogen induced accumulation of reactive oxygen species (ROS) and phenolic compounds. El-Bassiony *et al.*, (2012) found increased total free amino acids (FAA) in leaves and
total phenolic acids in the pod in Snap bean plant by BRs at 25 ppm in comparison to control-treatment. Similar increment in phenols is seen due to high dose of BRs in both leaf and root in Coleus. Naeem et al., (2012b) in Mentha arvensis found significant increase in total leaf phenol content and other active constituents in response to HBR, applied at 10⁻⁷ M concentration, however at 10⁻⁶ M concentration, the application of HBR could no more enhance the total phenolic content. Choudhari et al., (2012) found that co-applications of EBL and Spd modulated more remarkably the constituents of antioxidants (glutathione, ascorbic acid, proline, glycine betaine and total phenol in Cr-stressed Raphanus sativus plants than their individual applications and control. Serna et al., (2013) studied effect of two BRs analogues DI 100 and DI 31 in Cichorium endivia L. and recorded that total antioxidant activity and total phenols increased significantly in endive treated with brassinosteroid analogues. Biesaga-Koscielniak et al., (2014) in pea and lupine after application of brassinosteroid 24-epibrassinolide via spraying recorded accumulation of antioxidants (vitamins). Ascorbic acid (as vitamin C), tocopherols and tocotrienols (as vitamin E) and β-carotene (as provitamin A) are known as substances that affect the nutritional value of plants. Significant increase in total phenols in all the three parts of the plant T. cordifolia due to 1µM application of 28-homobrassinolide was recorded by Raghu and Rao (2016). Highest phenol content was observed in leaves. Similar increment is also reported in leaf and root tissue of Coleus in present investigation due to high dosage of BRs.

The leaf phenolic composition imitates the free radical scavenging capability of the plants that are expected to aid the plants to keep the normal growth at later growth stages, at which frequent production of free radicals takes place, causing bad effects of aging (Naeem et al., 2012b). So increased polyphenol content due to BRs indicates the positive role of HBR under stress conditions, due to which the free radicals are frequently generated in plants (Clouse and Sasse, 1998; Khripach et al., 2000).

Effect of CCC on polyphenol content in present study is seen concentration dependent as low dose of CCC shows high polyphenols and Vice-versa. Anilkumar (2005) reported slightly enhanced level of total phenolic contents in leaves of Patchouli plant Pogostemon sp. due to application of CCC/mepiquat chloride at 1000 and 2000ppm as compared to control.
El-Kady *et al.*, (1980b) studied effect of various growth regulators on some enzyme activities and phenolic compounds in Datura, *Datura stramonium* plant. They reported that total and free phenols were highest in leaves of plants treated with ethephon (100 ppm). Similar kind of increased polyphenols is seen in coleus leaves and roots due to ethephon treatment in present investigation. Joaquim *et al.*, (2008) reported no significant effect of ethephon application in *Echinodorus grandiflorus* plant with respect to synthesis of total phenol, flavonoids and tannin content in leaves. Xu *et al.*, (2012b) recorded enhanced phenolic compound monoterpene production in *Houttuynia cordata* Thunb. a medicinal plant due to ethephon spray. They found two peaks of total monoterpene content at 0.1 and 100 mM ethephon applications. However, high dose of ethephon (100 mM) also caused oxidative damage increasing malondialdehyde and antioxidative enzyme activities and thus harmful so, 0.1 mM ethephon application was found most appropriate for enhanced monoterpene production. In the present investigation also ethephon treatment enhanced polyphenol content in both coleus leaf and root tissue. In medicinal plants, it is actually the quantity and quality of secondary metabolites which impart medicinal value to the plant.

The increased level of phenolic compounds in a leaf and root tissue due to PGRs is an indicator of plants capacity of free radical scavenging or a measure of plants ability to resist any kind of abiotic or biotic stresses and also its medicinal efficiency as coleus leaves are also used against some diseases. So, it might help the plant to sustain through adverse conditions. It may be an indicator of increased or altered rate of secondary metabolism in plants which is induced due to respective PGR application which might improve its medicinal properties and its efficacy against various ailments.

2. Total Flavonoids

The influence of foliar application of low and high dose of triacontanol, BRs, CCC and ethephon on total flavonoids content in leaf and root tissue of *Coleus forskohlii* is depicted in Table no. 17 and Fig. 69. It is revealed from the figure that the content of flavonoids is decreased due to both concentrations of all PGRs in root tissue where as in leaf tissue it is increased only by low concentrations of BRs and ethephon while triacontanol and CCC treatments exerted negative effect on flavonoids
content in leaf as compared to control. The level of content of flavonoids is dominant in leaf tissue than root tissue as depicted by all treatments including control.

Among various phenolic secondary metabolites, the flavonoids are polyphenolic compounds resulting from the expression of two key enzymes, phenylalanine ammonia lyase and chalcone synthase. Flavonoid subgroups such as chalcones, flavones, flavanones, aurones and isoflavonoids occur in most legume tissues (Wollenweber and Jay, 1988). As mentioned by Haslam, (1998) more than 4000 flavonoids have been identified which form a large family of low molecular weight polyphenolic compounds, which occur naturally in plant tissue and these include the flavonols, flavones, flavanones, catechins, anthocyanins, isoflavonoids, dihydroflavonols and stilbenes. Halton and Cornish, (1995) suggested that in most plant families, the initial product of Chalcone synthase is a tetra hydroxylchalcone which is further converted to other flavonoid classes, such as flavones, flavanones, flavanols, anthocyanins. The basic carbon skeleton of a flavonoid contains 15 carbons arranged in two aromatic rings connected by a three-carbon bridge compounds which can be characterized as C₆-C₃-C₆ compounds in which each C₆ portion is a benzene ring (Bogorad (1958). There are four main types of flavonoids, primarily on the basis of the degree of oxidation of the three carbon bridge, namely anthocyanins (intact aromatic rings), flavanols (keto and alcohol substitutions in the ring), flavanol monomers (mostly saturated ring with an alcohol substitution), proanthocyanidins (formed by association of flavanol monomers). The basis for classification of flavonoids into major groups is the configuration and state of oxidation of the connecting C3 portion of the molecule. The characterization of individual flavonoids within each major group is based on analyses of the pattern of substitution and on the nature of the substituents and frequently one or more of them are utilized in the formation of ethers. Glycosides of anthocyanidins are anthocyanins. Flavones and flavanols are also present in the leaves of all green plants, apart from its most obvious presence in flowers. In plants, the flavonoids are considered to have many roles including protection against UV-B radiation, defense against pathogen attack, attractants to pollinating insects and as signal compounds, for the initiation of symbiotic relationship (Parr and Bolwell, 2000). Certain flavonoids have been shown to act as powerful one-electron scavengers of free radicals (Rice-Evans et al., 1997) as well as two-electron donors to the H₂O₂-scavenging peroxidases of plant cells. As
documented by Taiz and Zeiger, (2006) flavonoids (flavones and flavonols) gather in the epidermal layers of leaves and stems and absorb light strongly in the UV-B region, allowing the visible wavelengths to pass through uninterrupted and thus protecting cells from excessive UV-B radiation (280-320 nm). Flavonoids also occur naturally in fruits, vegetables, nuts, seeds and flowers and therefore form an integral part of the human diet. Khatun et al., (2011) analysed the antioxidant status of various parts of Coleus forskohlii and reported that total phenol, flavonoids and β-carotene on dry weight basis were significantly higher in tubers than in the leaves, roots and stem, respectively. However opposite trend is noticed in present investigation on local variety of Coleus on fresh weight basis. According to Luo et al., (2002), recent studies have shown that many flavonoids and related polyphenols, secondary metabolites contribute significantly to the total antioxidant activity of many plants. There are few evidences regarding the influence of abiotic and biotic stresses on flavonoid contents in plants. Flavonoids and phenolic compounds also works as scavengers of ROS (Heim et al., 2002).

Khandaker et al., (2013) recorded increased level of flavonoid contents in Bougainvillea glabra in response to triacontanol spray.

Yu et al., (2009) noticed that EBL treatments significantly reduced pathogen induced accumulation of reactive oxygen species (ROS), flavonoids and phenolic compounds. Raghu and Rao (2016) determined the effect of BRs on the antioxidant contents such as phenols and flavonoids of the leaves, stems and roots of Tinospora cordifolia. They recorded increased content of phenols and flavonoids due to brassinosteroid supplementation. Similar increase in the content of phenols, flavonoids was noticed in tomato plants due to brassinosteroid supplementation (Ahammed et al., 2013)

Exogenous ethylene application increased flavonoid, anthocyanin and stilbenoid production in grape cell cultures (El-Kereamy et al. 2003). Joaquim et al., (2008) reported no significant effect of ethephon application in Echinodorus grandiflorus plant with respect to synthesis of total phenol and flavonoids content in leaves. Kim et al., (2009) studied the effects of gibberellic acid (GA3), kinetin (Kn), salicylic acid (SA) and ethephon (2- chloroethylphosphonic acid) on growth, total flavonoid, gibberellins (GA) and salicylic acid (SA) contents of Taraxacum officinale (dandelion), a widely used medicinal plant in Korea. The flavonoid content of
RESULTS AND DISCUSSION

dandelion was not altered with the application of ethephon. As reported by Linden et al., (2001) small quantities of 2 chloroethyl phosphonic acid /Ethephon were added to suspension cultures of Camellia sinensis reported growth reduction by 20% but production of phenolics and flavones increased by 90 and 75%, respectively.

In the present investigation, flavonoids content was decreased in root and less affected in leaf tissue due to majority of PGRs as compared to control which can be most presumably due to altered path of secondary metabolism due to PGR or low level of flavonoids might be because of low level of flavonoid synthesis.

3. Total Alkaloids

Influence of foliar spray of triacontanol, BRs, CCC and ethephon on alkaloids content of the leaf and root tissue of Coleus forskohlii is shown in Table No. 18 and depicted in Figure 70. It is evident from the result that in leaf tissue total alkaloids content are very slightly decreased due to all PGRs or in other words there is very less impact of all the PGRs on leaf alkaloid content. Whereas in root tissue it is increased due to high dosage of ethephon and low dose of CCC while there is no variation found due to other treatments as compared to control.

Alkaloids are the nitrogen containing secondary metabolites which are the pharmacologically active ‘basic principles’ of predominantly found in flowering plants. As mentioned by Taiz and Zeiger, (2006); Nikam 2007) the term alkaloid is linguistically derived from the Arabic word al-qali, the plant from which soda was first obtained. Since the identification of the first alkaloid, morphine from the opium poppy, Papaver somniferum in 1806, about 15000 alkaloids have been isolated from 20% of vascular plants and their structures elucidated till the time. In many plants alkaloids are regarded as a part of constitutive chemical defence system (Croteau et al., 2000). Alkaloids are present mainly in three different types that are true alkaloids, proto alkaloids and pseudo alkaloids. As name suggests most alkaloids are alkaline in nature, commonly found in the cytosol at (pH 7.2) or in the vacuole at (pH 5-6), the nitrogen atom is protonated; hence, alkaloids are positively charged and are generally water soluble. Medicinal plants have healing properties due to the presence of several complex chemical compounds of different chemical nature, which are produced as secondary plant metabolites in plant organs. These plant metabolites, according to their composition, are grouped as glycosides, alkaloids, corticosteroids, essential oils etc. Among them, alkaloids form the largest group; including, quinine (Cinchona),
RESULTS AND DISCUSSION

reserpine (Rauvolfia), coleonol (Coleus), aconitine (Aconite) etc. and glycosides form another important group. In fact, plants provided safe and effective drugs and has no harmful side effects unlike modern synthetic drugs and antibiotics (Aswal and Goel, 1996); Gupta et al., (2010).

As suggested by Croteau et al. (2000), alkaloids are synthesized from some common amino acids specifically lysine, tyrosine, phenylalanine, tryptophan and arginine either alone or in combination with a steroidal, secoiridoids or other terpenoid moiety. About all alkaloids are poisonous to humans when consumed in excess quantity. While at lower doses, many are useful pharmacologically. According to Katzung, (1994) the pharmaceutical properties of alkaloids are mainly due to indole alkaloids, which have been extensively used as active ingredients in pharmaceutical preparations. The alkaloid level in plants is found to be influenced by number of environmental factors such as light, temperature, water and supply of mineral elements like nitrogen and potassium (Waller and Nowacki, 1978). There also some evidences of influence of PGRs on alkaloid content in plants.

Srivastava and Sharma (1990) also examined the effect of TRIA on alkaloid biosynthesis as well as on the relationship between alkaloid production and physiological parameters in opium poppy. They reported a significant increase in capsule number and morphine content of the plant owing to foliar application of TRIA at concentration of 0.01 mg L1; whereas, there was no effect of TRIA on thebaine and codeine contents of opium poppy. Total alkaloid content in withania (Withania somnifera L.) and datura (Datura innoxia Mill.) by (Nasir, 2009) and curcumin content of turmeric (Curcuma longa L.) by (Singh 2008) was also effectively improved by foliar application of TRIA. According to Mishra and Kumar (2000), distribution and accumulation of alkaloids in plant parts may vary in roots, stems, and leaves of Catharanthus roseus. Khan et al. (2007) found a significant positive effect of cumulative application of TRIA and GA3 on the yield of opium and its morphine content. The crude opium production of opium poppy (Papaver somniferum L.) was also enhanced due to the combined application of TRIA and GA3. As opined by Srivastava and Srivastava, (2007) PGRs have, in fact, been reported to affect the tissue specific secondary metabolism and manipulate the alkaloid accumulation at particular sites. Similar results are reported in present study. Alam et al., (2012) noticed slight increment in alkaloid content, along with vincristine
RESULTS AND DISCUSSION

and vinblastine alkaloids due to triacontanol in *Catharanthus roseus* Var. Rosea and alba than control at $10^{-7}$M concentration of foliar spray as compared to control. Though there are various evidences regarding the positive effect of triacontanol on alkaloid contents, in present study triacontanol do not show any prominent effect on alkaloids content.

Significantly enhanced level of total alkaloids along with vincristine and vinblastine in *Catharanthus roseus* Var. Rosea and alba was noticed by Alam *et al.* (2012) due to HBR foliar spray at $10^{-7}$M concentration as compared to control. HBR was found to induce both total leaf-alkaloids and root-alkaloids more specifically in Rosea variety. Raghu and Rao (2016) recorded increased content of alkaloids from their studies on the effect of BRs on the leaves, stems and roots of *Tinospora cordifolia*.

Awad and Kamel (1983) reported decreased total alkaloidal content due to salinity stress in Datura *Datura innoxia* plants which was improved due to the combined effect of CCC or ethephon or kinetin, at different concentrations in salinity stressed as well as unstressed plants. El-Antably *et al.* (1975a) in *Solanum laciniatum* Aiton detected highest content of alkaloids in leaves and whole plants followed by increased yield due to early application of 2000 ppm of CCC. The increment in alkaloid contents was also observed due to combined application of GA3 and CCC and their combinations at early and late stages of growth. Wahdan *et al.* (1985) *Atropa belladonna* plants, detected increased content of alkaloid hyocyamine as a result of 2000 ppm CCC treatment. Zeng *et al.* (2012) assessed the effect of chlormequat chloride (CCC), choline chloride on the accumulation of the alkaloid camptothecin (CPT) and its analogue 10-hydroxycamptothecin (HCPT) in tender leaves of *Camptotheca acuminata* saplings. They noticed enhanced production of alkaloids due to treatments of chlormequat chloride (CCC) and choline chloride. Treatment by 40 mg/L CCC dramatically enhanced HCPT production by 308 % in pre-harvest, treatment by 60 mg/L CCC enhanced HCPT production by 100 % in postharvest. In *Catharanthus roseus* which is a great source of vinblastine, vindoline and catharanthine which belongs to terpenoid indole alkaloids, Pan *et al.* (2010) reported that Chlormequat chloride highly enhanced the accumulation of vinblastine but greatly decreased the contents of vindoline and catharanthine Whereas ethylene (ethephon) treatments resulted in a significant increase of vinblastine, vindoline and
catharanthine. In *Withania somnifera* Dunal., Ashwagandha plant, CCC application at 3000ppm with 30X20 cm² spacing was reported to be best by Shukla and Shukla (2012) for best root yield and higher alkaloid contents. Bharathkumar *et al.*, (2001) also found increased level of total alkaloid and withanolide content in Ashwagandha plant, by CCC application at 2000ppm. Xing *et al.*, (2011) carried out studies on effect of PGRs like CCC, ethephon and salicylic acid in combinations or alone as a spray on the alkaloids contents vindoline, catharanthine and vinblastine of medicinal plant *Catharanthus roseus* (L.). They confirmed that among the combination treatments, salicylic acid (0.1 mM) + ethylene (0.1 mM), ethylene (0.1 mM) + chlormequat chloride (0.1 mM) and salicylic acid (0.1 mM) + ethylene (0.1 mM) + chlormequat chloride (0.1 mM) could significantly increase the vinblastine content by 209% at 48 h, 246% at 48 h and 213% at 24 h respectively. Thus, according to them as compared to the single PGRs treatments, the combination treatments increase alkaloids accumulation more effectively.

Misra *et al.*, (2009) from their studies on effect of ethrel application and fed 14 CO2 and 14 C- sucrose treatment in a *Catharanthus roseus* recorded that When 14CO2 was fed, the incorporation into the ethanol soluble fraction, sugars, organic acids, and essential oil was significantly higher in ethrel treated leaves than in the control. They further confirmed that ethrel and GA influence the partitioning of primary photosynthetic metabolites and thus modify plant growth and alkaloid accumulation. Cho *et al.*, (1988) noticed increased production of purine alkaloids by *Coffea arabica* cells by addition of ethrel. There are lacunae in knowledge regarding the effect of ethephon on secondary metabolism.

In the present investigation there was no significant variation observed in alkaloid contents due to PGRs except for high dose of ethephon and low doses of CCC in root tissue which indicates that alkaloids metabolism was least affected due to PGRs tried. According to Srivastava and Srivastava, (2007) PGRs have, in fact, been reported to affect the tissue specific secondary metabolism and manipulate the alkaloid accumulation at particular sites and it may be species specific.

### 4. Forskolin/ Forskohlin

For the qualitative and quantitative estimation of forskolin HPTLC technique was used. The influence of foliar spray of plant growth regulators on the forskolin content from root of *C. forskohlii* is shown in Table No. 19 and Fig. No.71. It is
evident from the figure that in root tissue increased level of forskolin can be seen due to all PGRs except for high dose of ethephon which shows decreased forskolin level as compared to all the other treatments. Both concentrations of triacontanol, BRs, CCC and low dose of ethephon promoted content of forskolin in root tissue.

Forskolin is a diterpenoid found exclusively in *C. forskohlii* (Shah *et al.* (1980). Apart from all the other diverse phytochemicals found in *C. forskohlii* tuberous roots, forskolin is the major chemical constituent of the tuber. The herbal preparations of it act on various multiple pharmacological mechanisms. Dubey *et al.* (1974) reported the blood pressure lowering and antispasmodic effects of extracts of *C. forskohlii* roots. Various aspects of forskolin and its medicinal uses and pharmacological development of plant regarding forskolin extraction, its properties and its medicinal importance is already discussed earlier in this thesis under the heading review of literature in coleus review part.

Various methods have been developed and used for extraction and quantification of forskolin from plant *C. forskohlii*. However, there are very few evidences about the effect plant growth regulators on content of forskolin. Balasubramanya *et al.*, (2011) studied effect of plant growth regulators on forskolin production in *Plectranthus barbatus*. They tried different concentrations of plant growth regulators individually and in combination to induce roots in vitro. They reported that morphogenic responses and forskolin production varied depending on the concentrations of plant growth regulators added to the medium. The maximum forskolin content of 1,178 mg kg⁻¹ dry weight was found in root cultures initiated on Gamborg’s B 5 medium supplemented with 0.5 mg l⁻¹ NAA by using rhizogenic cultures. Swamy and Rao (2011) studied the effect id 24-EBR and 28-HBR foliar application on *C. forskohlii* at 3 µM concentration. They reported that tuberous roots obtained from coleus plants treated with 28-homobrassinolide contained 0.17% forskolin as compared to 0.098% in the roots of untreated control plants.

The effect of triacontanol, CCC and ethephon on content of forskolin is unclear. According to Swamy and Rao (2011) the increased tuberous root growth might have increased forskolin in *C. forskohlii* however exact role of BRs in increasing forskolin or in the process of forskolin biosynthesis is unknown. Similarly, in present investigation triacontanol, BRs caused increase in forskolin content which might be attributed to overall increment in all growth parameters and increased rate of
RESULTS AND DISCUSSION

metabolic processes triggered due to PGRs. This might also be due increased accumulation of primary product which might have stimulated secondary metabolism.

5. Polyphenol oxidase enzyme (EC 1.10.3.2)

The effect foliar sprays of triacontanol (Vipul), brassinosteroids (BRs), CCC and Ethephon on the activity of enzyme Polyphenol oxidase in leaf and root tissue of *Coleus forskohlii* is shown in Table no. 20 and Fig. 72. It is revealed from the figure that in leaf and root tissue, the activity of enzyme polyphenol oxidase is increased in both tissue due to both concentrations of triacontanol, BRs and ethephon except that in root tissue due to high dose of ethephon PPO activity is lowered as compared to control. Whereas CCC treated plants exhibit lowered activity of polyphenol oxidase in root tissue and slightly increased or at par with control activity in leaf tissue. The polyphenol oxidase is higher in roots of Coleus as compared to leaf. Same trend is reported by Khatun *et al.*, (2011) in *Coleus forskohlii*.

Polyphenol oxidases (PPOs) are copper containing metalloenzymes which bring about the oxidation of hydroxyphenols to their quinine derivatives, which then spontaneously polymerize. These enzymes catalyses the oxidation of aromatic compounds by oxygen. These enzymes are broadly distributed from bacteria to mammals (Robb, 1984). In higher plants Catechol oxidase is most common kind of nuclear coded (Lax *et al.*, 1984) intracellular polyphenol oxidase enzyme which is specific for o-hydroxylated phenols and it is located in variety of cell fractions both in organelles as a tightly membrane bound as well as in soluble fractions of the cell. Mayer and Harel, (1979) have well documented the wide occurrence of this enzyme in membrane bound form, in the chloroplast. Paul and Gowada, (2000) reported that enzyme polyphenol oxidase purified from field beans (*Dolichos lablab*) has a molecular weight of 120 ± 1-3 kDa and it is a tetramer of 30 ± 1.5 kDa, with optimum pH 4.0. Catechol, 4-methyl catechol, and L-3, 4 dihydroxy-phenylalanine are substrates for this enzyme while activity is not detected towards chlorogenic acid, catechin, caffeic acid and monophenols. Hernandez-Romero (2005), classified enzyme PPOs into two main types laccases (EC. 1.10.3.2) and tyrosinases (EC. 1.14.18.1). Laccases oxidize mainly p-diphenols and methoxysubstitued phenols, such as 2, 6-dimethoxyphenols (Thurston, 1994) while Tyrosinases catalyse two kinds of reactions: firstly ortho-hydroxylation of monophenols such as L-tyrosine (cresolase activity), yielding L-3, 4-dihydroxyphenylalanine; and the oxidation of this and other
0-diphenols to 0-quinones (catechol oxidase activity E.C.1.10.3.1). As suggested by Yelena and Brudvig (1996), there are three types of proteins related to PPOs such as catechol oxidase, laccase and cresolase. They catalyse two reactions: the hydroxylation of monophenols to 0-diphenols (monophenolase activity) and the oxidation of 0-diphenols to 0-quinones (diphenolase activity) (Shi et al., 2001). According to Vaughn et al., (1988), Polyphenol oxidases have a function in the Mehler reaction, photoreduction of molecular oxygen by PS I. The mode of action proposed for PPO is based on its capacity to oxidize phenolic compounds when the tissue is damaged (Melo et al., 2006). In this situation, the rupture of plastids, the cellular compartment where PPO is located, leads to enzyme coming into contact with phenolic compounds released by rupture of the vacuole which is the main storage organelle of these compounds (Mayer and Harel, 1981). According to Vamos-Vigyazo (1981), PPO activity may be different according to plant species and variety, tissues and even organelles of cell. According to Yoruk and Marshall (2003) even though oxidation of phenols and formation of melanins are normal physiological processes of PPO, its significance in living intact plant tissues is not fully understood. PPO is found in a variety of subcellular fractions such as peroxisomes, mitochondria and microsomes (Shomer et al. 1979; Mayer and Harel 1979; Martinez-Cayuela er al. 1989) so their subcellular localisation has to be considered.

As mentioned by Yoruk and Marshall, (2003) there is a general agreement that during normal growth and development, PPO activity is much higher in unripe fruits and young leaves than in mature fruits and mature leaves suggesting its possible role in protection of growing plants against infection or injury. The general decrease in PPO activity with tissue age may be because of conformational changes, degradation by proteases, a decline in concentration of latent enzyme activators or of phenolic substrate biosynthesis, (Barrett et al. 1991; Murata et al. 1995; Laveda et al., 2000). There are also some evidences regarding effect of PGRs on PPO enzyme activities.

As reported by Henry and Primo (1979), they observed a greater polyphenol oxidase activity in the leaf tissue of lettuce treated with foliar spray of TRIA in comparison to the control. Yu et al., (2009) noticed that EBL treatments reduced the pathogen induced accumulation of reactive oxygen species (ROS), flavonoids, and phenolic compounds, activities of defence-related and ROS-scavenging enzymes. The enzymes included superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase,
catalase as well as phenylalanine ammonia-lyase and polyphenol oxidase. El-Kady et al., (1980b) studied effect of various growth regulators on some enzyme activities and phenolic compounds in datura *Datura stramonium* plant. They reported increased phenoloxidase activity in the leaves was highest in plants treated with ethephon 4000 ppm.

Increased enzyme activity in leaf and root due to triacontanol and BRs may be attributable to appearance of new multiple forms of enzyme. Cleavage of an active PPO protein to a smaller and still active PPO form, resistant to further proteolysis and exhibiting a broad optimum pH and stimulation of PPO activity by phytohormones associated with formation of new multiple forms, as a consequence of the association of preformed low molecular weight multiple forms are considered as an alternative defence mechanisms (Mari *et al.* 1998: Saluja and Sachar 1982). However, according to Yoruk and Marshall (2003) there are other factors, beside disruption of membrane integrity, that are involved in formation of such active enzymes capable of synthesizing or oxidizing polyphenols or that affect the amount of active enzyme form and the level of enzyme activity during senescence, developmental stages or injury. Increased peroxidase activity is a common response to oxidative and abiotic stresses. Therefore, peroxidase could be part of the enzymatic system connected with the increase in ethylene formation in plants like spinach.

In the present investigation triacontanol, BRs and ethephon induced activity of PPO is an indicator of plants increased ability to survive through adverse condition via oxidation or synthesis of polyphenols which confirms growth promoting ability of PGRs to boost resistance in plant which will be helpful for healthy growth and maximum yield of plants with reference to quality of the produce that is root which has high concentration of medicinally important products produced from secondary metabolism.

6. Enzyme Phenylalanine ammonia lyase (PAL)

The effect of Plant growth regulators triacontanol, BRs, CCC and ethephon on the activity of enzyme phenylalanine ammonia lyase from the leaf and root tissue of *Coleus forskohlii* is depicted in Table no.21 and Fig. 73. It is clear from the results that the activity of enzyme phenylalanine ammonia lyase is enhanced in both leaf and root tissue due to both doses of triacontanol and BRs and low dosage of CCC where as in case of ethephon PAL activity is increased only in leaf tissue, not in root as
compared to control. It is also evident that PAL enzyme activity in root tissue is positively influenced due to all plant growth regulators. Highest activity of enzyme PAL is found in root due to CCC high concentration as compared to all treatments.

In plants, Phenylalanine ammonia lyase (PAL) is considered as one of the most critical first step enzyme in the process of secondary metabolism because the most abundant classes of secondary compounds in plants are derived from phenylalanine through the action of enzyme phenylalanine ammonia lyase (PAL). Thus, enzyme PAL catalyses the formation of trans-cinnamate by the elimination of ammonia from the pro 3 S hydrogen from L-phenylalanine and it was first detected in barley seedlings (Koukol and Conn, 1961). PAL is a central enzyme in phenylpropanoid metabolism, is the branch point enzyme between primary (shikimate pathway) (Herrmann, 1995) and secondary (phenylpropanoid) metabolism (Harborne, 1988). The reaction catalysed by PAL is considered as the first step of phenylpropanoid skeleton biosynthesis in higher plants. PAL catalyses the non-oxidative deamination of phenylalanine to cinnamic acid (El-Shora, 2002). Hugh Jones, (1984) have very well acknowledged the role of PAL in cytodifferentiation and xylogenesis. Tanaka and Uritani, (1977) reported that Phenylalnine ammonia lyase purified from injured sweet potato has molecular weight 285,000 to 320,000 which is composed of four subunits of molecular weight 80,000. Alibert et al., (1971) have reported that enzyme PAL is localized mainly in the cytoplasm and in some membranous cell organelles. According to Jahnen and Hahlbrock, (1988) the existence of PAL can be detected in all cell types immunohistochemically. The epidermal and oil-duct epithelial cells, where flavonoids and furanocoumarins are respectively synthesized at high rates, the presence and activity of enzyme PAL is at higher side. Dickerson et al. (1984) described that activity of PAL was determined at the rate of conversion of L-phenylalanine to transfer cinnamic acid. According to Cann and Towers, (1977) the activity of PAL is found to be very sensitive to the physiological state of the plant. The activity of PAL enzyme can be induced during growth or it may get influenced due to treatments or pathological events, dilution of suspension culture or the action of light as stated by Hugh Jones, (1984). Harper et al., (1970) mentioned that the PAL catalysed reaction that produces cinnamic acid is light sensitive step in flavonoid synthesis. Ghasemzadeh and Jaafar (2012) have pointed that higher content of phenolic compound such as cinnamic acid can inhibit
flavonoid biosynthesis with inhibition of PAL activity. The PAL can be also detected at high level at the site of fungal or any other infection, in the tissue surrounding the site of hypersensitive cell death. As suggested by Jahnen and Hahlbrock, (1988) high PAL enzyme levels are also detected in the both the undamaged epidermis and an apparent, re-differentiating cell layer replacing the destroyed epidermis around the necrotic spot. PAL is found to be induced at the transcriptional level by UV-radiation (Hahlbrock and Scheel, 1989). Activity of PAL increased under various biotic and abiotic stresses i.e. wounding, chilling, heavy metal stress, water stress, salinity stress and infection of various bacteria and Fungi as reported by MacDonald and D’Cunha, (2007); Nikam, (2007); Jadhav, (2015). Apart from these factors the activity of PAL is also found to be influenced by various endogenous and exogenous plant growth regulators.

Kumaravelu et al. (2000) also reported that foliar TRIA application, at a dose of 0.5mgdm−3, significantly elevated the phenol contents in green gram plants. TRIA may be responsible for the activation of PAL, chalcone synthase, and stilbene synthase gene expression. Yu et al., (2009) noticed significantly reduced pathogen induced accumulation of reactive oxygen species (ROS), flavonoids, and phenolic compounds, activities of defence-related and ROS-scavenging enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, catalase as well as phenylalanine ammonia-lyase and polyphenol oxidase due to EBL. There is limited information regarding effect of ethephon on secondary metabolism.

The increased activity of PAL due to triacontanol, BRs and CCC low dose indicates enhanced level of secondary metabolism. Specifically, being tuberous root as a most important plant part in coleus high activity of PAL in root might be beneficial to increase medicinal yield of plant w. r. t increased level of secondary metabolites in present investigation.

G. Nitrogen Metabolism

Influence of foliar spray of PGRs on nitrogen metabolism

1. Total Nitrogen

Effect of low and high concentrations of plant growth regulators triacontanol, BRs, CCC and ethephon on the total nitrogen content in leaf, stem and tuberous roots of C. forskohlii is depicted in Table no. 22 and Fig. 74. It is evident from figure that the total nitrogen content is increased in all plant parts of C. forskohlii in response to
RESULTS AND DISCUSSION

high concentration of both triacontanol and ethephon whereas it is decreased in all plant parts due to both concentrations of all the other PGRs concentrations except for leaf tissue in which N content is increased due to low concentration of ethephon. Among all the spray treatments, the highest nitrogen content is visible in all plant parts leaf, stem, root due to ethephon at high concentration while lowest is observed due to BRs at low concentration. It can be also clearly seen from the fig. that the nitrogen content is higher in leaf tissue followed by stem and root tissue. Similar trend is noticed in all plants treated with PGRs and control treatment.

Nitrogen is a basic constituent of nitrate, nitrite, amino acids, amide, ammonia, hexacosamine, quaternary ammonium compounds and proteins and nucleic acids (Salisbury and Ross, 1991). It is also an essential component of plant growth hormones like IAA, cytokinins, polyamines and many vitamins. It is also an important component of chlorophyll (Crawford et al., 2000). Being most important constituent of various biomolecules, nitrogen is involved in most of biochemical and physiological life processes. As stated by Addiscott et al., (1992) Nitrogen performs a key role in growth and development of many crop plants by influencing yield. It affects leaf area expansion, dry matter partitioning, crop development, crop quality and susceptibility to lodging. Atmospheric nitrogen is present in very inert N\(_2\) form which is not readily available for plant absorption so it must be transformed into nitrate (NO\(_3^-\)) and ammonium (NH\(_4^+\)) in the soil to get absorbed by the plants. This depends upon organic matter in the soil such as manure, residue of legume plants etc. which make nitrogen absorbable by plant. According to Lam et al., (1996) nitrogen is assimilated by plants only after the incorporation of inorganic forms of nitrogen into the carbon skeleton leading to amino acid synthesis.

In soil, nitrogen is mainly present in oxidized forms such as nitrate, nitrite and dinitrogen so plants have to use energy to reduce these nitrogen forms (Iglesias et al., 2004) where as in leguminous plants the biological nitrogen fixation helps in nitrogen nutrition. Being most important part of Chlorophyll architecture and RuBISCO enzyme (Murata, 1969); Delgado et al., (1994) nitrogen is an indicator of photosynthesis. As per the opinion of Lawlor (2002), in agricultural production role of nitrogen is closely associated with photosynthesis and carbon metabolism. According to Gastal and Lamaire (2001) amount of nitrogen absorbed by plant decides the crop growth rate. Nitrogen is an essential part of purine, pyrimidin which are parts of
RESULTS AND DISCUSSION

Nucleotide bases and thus constituent of ATP and co-enzyme NAD and NADP and sugar nucleotides which play a role in carbohydrate metabolism. As opined by Bhagwat (2004) there exists an interaction among the mitochondrial respiration, nitrogen metabolism and photosynthesis which involves recycling of carbon, nitrogen and reducing equivalents. Nitrogen in the form of IAA and cytokinin play important role in cell division and enlargement (Chapin, 1980).

Nitrogen constitutes about 0.5 to 5% of the plant dry weight being one of the most abundant element in plant (Lindblad and Guerrero, 1995). As mentioned by Marschner, (1986) the requirement of nitrogen ranges between 2 to 5% of plant dry weight for optimal plant growth which depends upon plant species, developmental stage and organ of the plant. So as to improve the overall crop growth and productivity, it is necessary to have idea about the nitrogen uptake and partitioning in the plant. Draycott, (1972) reported highest nitrogen content in leaf tissue and minimum in root tissue in sugar beet plant at harvest stage. Nikam (2007) also reported similar trend in tuberous plant Cholrophytum borivillianum. These findings go in tune with the present investigation. According to Tinker (1979) nitrogen accumulation in plant depends upon nitrogen supply, environmental factors and genotype. Nitrogen is a highly mobile element and transported very efficiently to actively growing meristematic zone or metabolically active centres like leaf. Nitrogen is translocated to other parts in the form of amino acids, amides from senescing leaves. In most of the higher plants, two major nitrogen transport forms found in phloem sap are Glutamine and Asparagine. Many reports support that plant growth hormone molecules modify various physiological processes such as photosynthesis, photorespiration, partitioning of photoassimilates, mineral ion uptake and transport, nitrogen metabolism and all these processes are linked with each other by several interactions finally affecting plant productivity. Being one of most critical life process affecting plant productivity it is necessary to assess the effect of plant growth regulators on nitrogen content. Several attempts have been made by various workers to evaluate the relationship between PGRs application and nitrogen nutrition. In present investigation, triacontanol treatment increased the total nitrogen content in leaf, stem and root of C. forskohlii at high dosage whereas at low dosage triacontanol nitrogen content is reduced in all plant parts as compared to control. Also, nitrogen content is at higher side in leaf than stem and less in root part. Similar increment in
RESULTS AND DISCUSSION


Hashmi et al., (2011) also reported increased leaf nitrogen content due to foliar spray of triacontanol in Ocimum basilicum L. at 10^{-6}M concentration. In studies carried out by Alam et al., (2012) in Catharanthus roseus L. they recorded highest nitrogen content in leaf in response to triacontanol against control and other PGRs tried. Singh et al., (2012) witnessed enhanced nitrogen content in both leaf and rhizome tissue of Zingiber officinale Rosc., ginger plant due to triacontanol foliar spray at 10^{-6}M concentration. Khan et al., (2014) also recorded increased nitrogen content in leaves of Cymbopogon flexuosus Steud. Wats. Cv. Krishna commonly known as lemon grass due to foliar spray of triacontanol. These findings are in agreement with the present findings in C. forskohlii due to high dose of triacontanol.

As opined by Knowles and Ries (1981), Ries et al., (1993), Aftab et al., (2010) and Naeem et al., (2011 and 2012a) alterations in nitrogen content under the influence of triacontanol application could be attributed most presumably to the compositional and or chemical changes in plant like increased uptake of water and nutrients, alterations in membrane permeability, altered enzyme activities, free amino acid content and soluble proteins etc brought about by triacontanol leading to changes in nitrogen concentration in plant and various plant parts resulting in improved growth and productivity.

Purbey and Sen (2007) and Balbaa et al., (2008) reported increased uptake and content of nitrogen in straw part of fenugreek and in leaves marigold plant subjected to BRs spray respectively. Similar increment in nitrogen content in response to BRs spray is reported by Fariduddin et al., (2006), Talaat and Abdullah (2010), Alam et al., (2012), Bera and Kalipada (2013) in Vigna radiata, in faba bean (Vicia faba L.), in
periwinkle *Catharanthus roseus* L. and in lentil (*Lens culinaris* Medik) plants respectively. However, studies made by Serna *et al.*, (2013) on effect of BRs (DI-31 and DI-100) on *Cichorium endivia*, a vegetable salad recorded no change in nitrogen content as compared to control. On the contrary to these in present investigation all plant parts of *C. forskohlii* shows reduced content of nitrogen in response to foliar spray of BRs. There is scarcity in the knowledge about effect of BRs on nitrogen content in root and leaf tissue. However according to Naeem *et al.*, (2010, 2011, 2012b) altered nitrogen content in various plant parts due to PGRs might be due to altered permeability of membrane because of PGR’s action. So most probable reason for reduced nitrogen content due to BRs in *C. forskohlii* is uncertain.

In case of effect of foliar treatment of CCC on nitrogen content of *C. forskohlii* in present studies, there was found reduced level of total nitrogen content in leaf, stem and root tissues as compared to control. There are several evidences which are differing to present finding such as Castro *et al.*, (1978) in *Zinnia elegans* found enhanced nitrogen content in stem and leaf part due to 5000ppm of Chlormequat (CCC). Misra and Reddy (1984) also found increase in nitrogen content in wheat under rain fed conditions. Shende *et al.*, (1987) in pea plant, Singh *et al.*, (1987) in soybean plants, Bashist *et al.*, (1990) in case of sesamum, Shah and Prathapasenan (1991) in case of mung bean and Guroo and Patel (1993) in mustard plant also reported increased nitrogen content in leaf and stem as compared to control. Lone *et al.*, (2001) also witnessed improved nitrogen content in *Brassica juncea* cultivars subjected to non-irrigated conditions due to spray of CCC at 400ppm. However, observation made by Saeed *et al.*, (1972) in Safflower (*Carthamus tinctorius* L.) goes in agreement with present finding. In safflower nitrogen content was reduced by all treatments (100 to 400ppm) of CCC in tops and roots. Patil (2011) in *Simaruba glauca* reported that the nitrogen content of leaf tissue increased in response 20 ppm CCC while nitrogen content decreases in response to 5 ppm CCC. The nitrogen content in root tissue decreases in response to all treatments. This finding supports the present findings.

Khuankaew *et al.*, (2009) studied the effect of ethephon application by soil drenching method on *Curcuma alismatifolia* Gagnep. at 0, 100, 300 and 500ppm concentration twice at shoot emergence/4 weeks after planting (4WAP) and at 6 WAP of curcuma plant. They reported reduced accumulation of nitrogen content in
above ground plant organs like leaves, flower, flower stalks and new shoots and underground plant parts like rhizome, storage roots and roots. This finding goes in tune with the present finding in *C. forskohlii* as lower dose of ethephon caused decrease in nitrogen content in stem and root part but not in leaf part where as high dose of ethephon enhanced nitrogen content in all plant parts of Coleus. Total nitrogen was enhanced in leaf tissue of sugarcane cultivar Co-671 due to pre-treatment of DW and ethephon (Patil, 1995). This is also found true in present studies.

As discussed earlier Nitrogen is the important constituent of proteins and nucleic acid as well as many metabolic intermediates. It also acts as an important component of many vitamins and some of growth regulators as indicated by several researchers. In the present study an increased level of nitrogen content in response to triacontanol at high dose as foliar spray treatment appear to be advantageous in terms of enhanced nitrogen content of plants which might be useful for the improvement of many metabolic reactions and stimulation of IAA and cytokinin like growth hormones leading to improved growth and productivity. Also altered nitrogen content in plant tissues, may be enhanced or reduced due to PGRs is an indicative of modified carbon and nitrogen metabolism.

2. Nitrate content \((\text{NO}_3^-)\)

The effect of foliar spray of Plant growth regulators Triacontanol, BRs, CCC and ethephon on nitrate content from leaf and root part of *C. forskohlii* is shown in Table no. 23 and Fig. 75. It can be revealed from the results that in root tissue of *C. forskohlii* the amount of nitrate content is reduced due to both concentrations of triacontanol, BRs, CCC and ethephon however in case of leaf tissue it is increased due to both concentrations of all PGRs except in high concentration of ethephon there is decrease in leaf nitrate content as compared to control. Highest nitrate content is observed in leaves treated with CCC low dosage and in root of control treatment whereas lowest nitrate accumulation is seen in leaves treated with Ethephon high dosage among all PGR treatments.

Nitrate is the most abundant readily available nitrogen source for plant absorption (Beevers and Hagemen, 1969). According to Crawford *et al.*, (2000) diffusion of nitrate from soil solution takes place at apoplast. Nitrogen in the form of nitrate from soil solution is taken up by the epidermal and cortical cells of roots. The uptake or absorption of nitrate is an active process across the plasma membrane.
Mechanism of high affinity and low affinity is involved in the import of nitrate which is controlled by multiple gene family encoded carriers. Nitrate uptake (NO$_3^-$) by root cells of plant is the first key step in nitrogen metabolism. Once its inside the plant root cells, nitrate can be transported out of root cells either by extrusion in the external medium or by unloading in the xylem vessel to reach the above ground organs (Forde and Clarkson, 1999). Once nitrate enters inside the root cells or leaf cells, the reduction of nitrate is carried out by Nitrate reductase depending upon the species and nitrogen condition. The reduction of nitrate results in nitrite and then eventually in ammonium which takes place in cytoplasm and plastids. According to de Leij et al., (1998) possible fate of NO$_3^-$ in roots and leaves may be its transport into vacuole where it acts as a general osmoticum and serves as a reservoir of nitrate to keep up the growth process in limited availability of external nitrogen supply. Thus, nitrate also acts as a vacuolar osmoticum. According to Stitt (1999), nitrate content alters metabolism and provides energy for nitrate and nitrite reduction and also induces the expression of many genes responsible for nitrite reduction. According to Coruzzi and Zhou (2001) nitrate assimilation can be integrated into overall nitrogen and carbon metabolism because many of genes associated with nitrate / nitrite reduction are also regulated by carbon and amino acid signals. These responses subsequently cause differential organ growth such as with high nitrate levels, root growth inhibited and shoot growth stimulated with delayed onset of flowering (Stitt, 1999). Nitrate content also influences root architecture such as with high levels of nitrate in soil causes systemic inhibition of lateral root growth whereas localized zones of high nitrate induces root branching (Drew and Saker, 1975; Zhang et al., 1999; Vidal et al., 2010).

As opined by Smith et al., (2000) nitrate uptake is regulated by nitrate content itself, a specific property which is not shared with other ion transport systems like phosphate or sulphate ions. It also depends on availability of nitrate in soil, pH of soil solution, internal ionic status of root cell, age of the plant and species to species variation. In higher plants, nitrate uptake is altered due to sequential responses as a result of environmental factors like light intensity, temperature, stress and during ontogeny. Supply of carbohydrate in the phloem sap also regulates nitrate uptake. Observations made by Muller and Touraine (1992) states that some amino acids also interfere or modify nitrate uptake. According to them, amino acids like arginine, alanine, asparagine and glutamine strongly inhibited nitrate uptake while glutamate,
methionine and aspartate weakly hampered nitrate uptake whereas amino acids like histidine, valine, serine, phenylalanine, leucine slightly stimulated or affectless in uptake of nitrate. This suggests that amino acids or peptides circulating in phloem sap control the rate of nitrate uptake by root cells. Intracellular $P^H$ also regulates nitrate uptake. As mentioned by Ismande and Touraine (1994) stimulation or inhibition of nitrate uptake is also regulated by nitrate reduction process in shoot part of plant as stimulation or inhibition of nitrate reduction induces or depresses nitrate uptake in roots. Even the physiological state of leaf at the time of experimental also decides the level of nitrate content. Rate of assimilation of nitrate and or nitrite in the leaves also affects the level of nitrate content in leaf which in turn depends upon reducing ability of leaf that varies from species to species. Thus, level of nitrate in the plant tissue is governed by various endogenous and exogenous factors like light intensity, photoperiod, soil water availability, stress etc. (Steingrover et al., 1986; Huffaker et al., 1970; Yamada, 1984; Nikam, 2007)). Apart from being a nitrogen source for protein synthesis, nitrate itself might also acts as a growth regulator influencing plant growth and metabolism (Trewavas, 1985). However, there are also several evidences which have proved role of various endogenous and exogenous PGRs in governing nitrate uptake, assimilation and its level in various plants or plant parts (Knowles and Ries, 1981; Reinink and Biom-Zandastra, 1989; Bashist 1988 and 1990; Naeem et al., 2011 and 2012; Lone et al., 2010; Patil (2011); Jadhav (2015)).

Knowles and Ries, (1981) reported that there was no change in the nitrate uptake or endogenous level of nitrate content in corn and rice seedlings treated with triacontanol. Vijayraghavan and Balkrishnan (1985) recorded enhanced level of nitrate content in leaves of Upasi-9 and Upasi-10 varieties of tea plant. Czapla et al., (2007) also observed similar increase in nitrate content in leaves of spring triticale due to application of triacontanol but they observed reduction in nitrate content in culms as compared to control. This goes in agreement with the present findings as triacontanol stimulated nitrate content in leaves of $C. forskohlii$ also. However, Kumaravelu et al., (2000) has reported reduced nitrate content in green gram in response to triacontanol application at 0.5 and 1 mg dm$^{-3}$ concentration in contrast to present findings. The knowledge about effect of triacontanol on root nitrate content is scarce. However decreased nitrate content in roots of $C. forskohlii$ due to triacontanol is an indicative of its good quality for consumption (Prusakova et al., 1999).
Khripach et al., (1995 and 1996) studied the effect of 28-HBR and 24-EBR application on potato plants at a dose of 10-20 mg/ha and recorded 20% rise in productivity of potato along with diminished nitrate content in tuber but increased starch and vitamin C as compared to control. Hayat et al., (2001) also witnessed similar trend in Brassica juncea plants treated with HBL under the influence of salt stress. They reported that nitrate content was reduced due to stress in leaf and root of Brassica juncea but BRs application improved nitrate content in both parts however roots of Brassica juncea possessed more nitrate than leaves in salt stress which was reversed by application of HBL. HBL improved nitrate content in leaves of unstressed plant of Brassica juncea than root part. Similar trend is observed in C. forskohlii in present study. According to Prusakova et al., (1999) an important feature of BRs is its ability to increase yield of crops along with increased quality of crops but diminished nitrate content and other heavy metal elements. Same kind of observations are made in present study w. r. t. BRs. Thus, BRs proved beneficial in coleus roots by increasing its quality by reducing nitrate content in root but increasing productivity of coleus by high nitrate content in leaf which is a site of N and C metabolism.

Reinkink and Biom-Zandastra (1989) found decreased nitrate content in lettuce (Lactuca sativa L.) due to CCC whereas Humpheries (1963) reported increased nitrate content in leaves of tobacco subjected to CCC treatment. Bashist (1990) also recorded increased uptake of nitrate in Sesamum seedlings. Lone et al., (2010) also reported improved nitrate content in mustard plant subjected to combined application of nitrogen and CCC. Patil (2011) reported differing observation in Simaruba glauca. She reported increased accumulation of nitrate in both leaf and root tissue of Simaruba glauca due to 5 ppm CCC foliar spray but it is decreased due to 20 ppm CCC in leaf tissue. These findings are in tune with the present findings. In C. forskohlii also nitrate content is increased in leaf but decreased in root due to CCC as compared to control. So, it can be concluded that the enhanced level of nitrate content in leaf tissue which is a site of N and C metabolism, as an effect of CCC application in Coleus forskohlii might be an indicative of improved photosynthesis and productivity of plant. Reduced nitrate content in root due to CCC as compared to control also proves that CCC might be beneficial for qualitative growth of Coleus roots. In the present investigation, the amount of nitrate is decreased in root tissue of C. forskohlii due to both concentrations of ethephon while in leaf tissue it is increased.
due to only low concentration of ethephon as compared to control. Palmer (1985) studies the influence of ethephon on nitrogen metabolism in *Solanum tuberosum* L. They reported that ethephon treatment stimulated the ethylene production by roots, stem and leaves. They recorded that nitrate accumulation in stem tissues was not affected by ethephon treatment but was increased in roots at 24 and 48 h. however they found leaf NO\textsuperscript{3} content declined with time in ethephon-treated plants and after 24 h significantly less NO\textsuperscript{3} accumulated was found in ethephon treated leaves. This finding is in tune with the present finding because in *C. forskohlii* also there is decrease in leaf nitrate content due to high dose of ethephon while root nitrate content is decreased which contrasts with Palmer (1985). There are lacunae in our knowledge regarding the influence of ethephon treatment on nitrate accumulation in plants though several evidences support that endogenous ethylene biosynthesis, signalling and functioning is regulated by nitrogen availability or scarcity in plant system (Iqbal et al., 2015; Khan et al., 2015; Romera et al., 2015). These evidences prove that ethylene has to perform a key role in nitrogen metabolism and in overall plant productivity. These results of present investigation are explained in terms of ethephon stimulated production of ethylene, altered protein synthesis, NRA and nitrogen content and increase in cellular metabolism and permeability in response to ethephon.

Thus, it can be concluded that decreased nitrate content in roots and increased nitrate content in leaves of *C. forskohlii* due to triacontanol, BRs, CCC and ethephon is an indicative of its good quality and suitability for consumption (Prusakova et al., 1999) as well as of increased productivity because nitrate assimilation can be integrated into overall nitrogen and carbon metabolism (Coruzzi and Zhou (2001).

**3. Enzyme Nitrate Reductase (NR: E C 1.6.6.1-3)**

The influence of foliar application of both concentrations of triacontanol, BRs, CCC and ethephon on the activity of enzyme nitrate reductase (NR) from leaf and root tissue *C. forskohlii* is presented in Table No. 24 and Fig. 76. It can be clearly noticed from the results. that nitrate reductase (NR) activity is enhanced in both leaf and root tissue of *C. forskohlii* due to both concentrations of triacontanol, CCC, BRs except in leaf tissue treated with low concentration of BRs, it is reduced as compared to control. Whereas among all PGR treatments NR activity is reduced significantly in both leaf and root tissue due to both concentrations of ethephon. Among all the treatments, highest NR activity is found in leaves due to BRs high concentration while
in root tissue due to triacontanol low dosage. The activity of enzyme nitrate reductase is seen more dominantly in leaf tissue than root tissue. Similar trend can be noticed in all PGRs and control treatment.

Nitrate reductase (NR) is a complex metalloflavoprotein, catalysing the first, rate limiting step of nitrate assimilation that is a reduction of nitrate (NO\(_3^-\)) to nitrite (NO\(_2^-\)). Nitrate reductase enzyme activity (NRA) is a control point in the process of nitrate assimilation and amino acid production (Beevers and Hagemen, 1969; Srivastava, 1980; Salisbury and Ross, 1992; Lea, 1997). NR is a complex, oligomeric, metalloenzyme having showing variation in molecular masses isolated from various sources. It ranges from 197 to 460 kD molecular weight and consists of two subunits flavin and flavoprotein with Molybdenum. Nitrate (NO\(_3^-\)) absorbed by roots is reduced to nitrite (NO\(_2^-\)) by enzyme nitrate reductase and then nitrite is reduced by nitrite reductase (NiR) to ammonium ion. Ammonium is thereafter integrated into amino acids by the glutamine synthetase- glutamine 2- oxoglutarate transaminase (GS-GOGAT) enzyme system providing plant autotrophy for nitrogen. NR enzyme requires NADPH and NADH as an electron donor and proton is consumed in the reaction. NAD(P)H donates two electrons to the FAD and serves as reductant. These electrons pass to the Mo-MPT (Molybdenum-molybdoprotein). NR enzyme has two active sites, one at the 'C' terminus for accepting electrons from NADPH/NADH and the other at the 'N' terminus for reducing nitrate to nitrite. Thus, in leaves, NR is closely associated with photosynthesis as reductants NADPH and or NADH for nitrate reduction are obtained from the oxidation of Calvin cycle intermediates (Schrader et al., 1968; Beevers and Hagemen, 1969). So, the activity of NR can be correlated with photosynthesis and thus with total dry matter production as opined by Antony, (1995) after his studies in soybean. NR enzyme is present in both roots and shoots in many species. In roots, the epidermal cells and cortical cells of the root surface shows accumulation of NR activity at low nitrate level while at high nitrate concentration, its activity is significantly noticed in cortex and vascular tissue (Crawford et al., 2000). In roots, there exists two distinct types of NR enzyme, one present in the cytosol (cNR) and second situated on plasma membrane and facing the apoplast (PM-NR) (Stohr and Mack, 2001). Yamaski et al., (1999) purified cNR from maize while activity of PM-NR is noticed only in root tissue where activity of PM-NR goes above the activity of cNR particularly during night (Stohr and Mack, 2001).
According to Srivastava, (1975) the distribution of nitrate reductase is varied in different plants and plant parts depending upon the species and age of plant. The reduction and assimilation of nitrate depends upon the relative level of NR in above and below ground plant parts and thus influenced by various factors such as species, age of plant. In wheat and maize, leaf is found to be major site of nitrate reduction as maximum of nitrate assimilation takes place in leaf (Beevers and Hagemen, 1969; Reilly, 1976) while in pea, root tissue is the major site for nitrate reduction and assimilation (Wallace and Pate, 1965). Kapoor and Lii, (1982) and Marwaha, (1998) found that in tuberous crop potato, leaves are the major sites of nitrate reduction as compared to roots and tubers. Nikam (2007) also found similar trend in Chlorophytum borivillianum. She also noticed that leaf tissue acts as a main site for nitrate reduction for most of its earlier growth span while its activity was found more in tuberous roots in later stages of growth as compared to leaf tissue. In the present investigation, in C. forskohlii also leaves exhibit higher activity of NR as compared to root tissue in response to all PGR treatments and control.

The catalytic efficiency of NR depends upon various factors 1). availability of substrate nitrate in the cytoplasm and steady-state concentration of NAD(P)H 2). the level of functional NR (amount of NR protein and availability of cofactors and metal ions- FAD, heme, Fe, Mo-MPT and Mo) and 3). the activity level of functional NR. All these factors are regulated directly or indirectly by metabolic sensors and signal transducers (Campbell, 1999). According to Crawford, (1995) and Scheible et al., (1997), the free glutamine level and its ratio to free glutamate and also nitrate level are presumably the key metabolites regulating the level of nitrate reducing capacity in plants. As low level of glutamine and availability of nitrate enhance NR level and nitrate reducing capacity while high glutamine level ceases nitrate reduction and decrease activity level of NR. According to Solomonson and Barber (1990), this enzyme is regulated by protein synthesis and degradation cycles (control by redox and allosteric modulation (Kaiser & Brendle-Behnisch 1991), and phosphorylation and dephosphorylation processes (Huber et al. 1992). Thus, as quoted by Kaiser et al., (2002) protein phosphorylation and dephosphorylation regulates NR and its control of activity and protein synthesis and denaturation are coupled. The information about nitrate reductase in roots is inadequate. Apart from availability and content of nitrate, there are so many factors which regulate NRA such as inorganic salts and ions,
RESULTS AND DISCUSSION

antibiotics and metabolic inhibitors, herbicides, fungicides, age and diurnal rhythms, temperature, stresses, atmospheric pollutants etc. (Srivastava, 1980; Bose and Srivastava, 2001). Since NR enzyme is one focal point for integration of control of carbon and nitrogen metabolism (Campbell, 1999) and nitrate is the most important source of nitrogen in many crops, understanding the role of NR in higher plants in response to PGRs influence is very important through economic point of view. Several attempts have been made to evaluate the activity of NR in response to plant growth regulators. There are plenty reports of positive influence of triacontanol on NR activity in both leaves and roots of medicinal plants.


Increased activity of NR in present investigation due to triacontanol may be attributed to overall improved plant metabolism.

Mai et al., (1989) and Sivakumar et al., (2002) recorded increased activity of NR due to BRs in rice and pearl millet leaves respectively. Anuradha and Rao (2003) reported improved NR activity in rice plants subjected to salt stress due to BRs
RESULTS AND DISCUSSION

application proving ameliorating effect of BRs. Efficacy of BRs application in *Brassica juncea* plant against Cd-stress was witnessed by Hayat *et al.*, (2007). They reported that in Brassica plants sprayed with 0.01 µM of HBL helped plant to overcome adverse effects of Cd stress by improving activity of NR and nitrate content level which was found to be reduced due to stress. Alam *et al.*, (2012) recorded significantly increased NR activity in leaves due to HBR at $10^{-7}$M concentration in Rosea and Alba varieties of *Catharanthus roseus* when applied as a foliar spray about 36% more than control. Yadav *et al.*, (2012) did comparative analysis of 24-EBR and 28-HBR on NR activity and other biochemical parameters in tomato Cv. K-21plant and reported increased NR activity due to BRs more significantly at $10^{-8}$M concentration of 24-EBR. Hayat *et al.*, (2012) reported significantly lower activity of NR in Cd-stressed leaves of tomato which was reported to be increased due to foliar application of HBR and EBR at $10^{-8}$M concentration under both stressed and normal condition in tomato.

Sairam (1994) studied effect of HBR on wheat plants under normal and water-stressed condition. They reported that HBR spray improved NR activity in wheat leaves under both conditions and alleviated the negative effect of stress on nitrate reduction.

Enhanced activity of NR enzyme due to BRs may be because of increased concentration of nitrate due to activity of BRs at membrane level (Mai *et al.*, 1989; Hayat *et al.*, 2012). According to Khripach *et al.*, (1999) this could be an additional effect of BRs on expression of specific genes. Additionally, BRs are also involved in the processes like transcription and translation which might have influenced activity of NR positively (Khripach *et al.*, 2003; Hayat *et al.*, 2012). Thus, it is possible that BRs besides influencing various biochemical activities also improves water and nutrient (nitrate) uptake by increasing NR activity resulting in elevated protein synthesis, growth and yield.

application @ 500ppm and 1000 ppm in leaves of Patchouli (*Pogostemon cablin* Benth.) plant at later stages of growth @ 120 DAP. These findings go in agreement with the present findings. In contrast to this the effect of CCC in water-stressed plants of *Vetiveria zizanoides* was studied by Khan *et al.*, (2008). They reported that activity of NR was reduced due to drought stress however CCC foliar application increased NR activity in stressed plants whereas in unstressed normal vetiver grass leaves it was found decreased due to CCC.

Increased activity of NR in both leaves and root of *C. forskohlii* in present study due to CCC will surely increase the rate of nitrate assimilation and thus will improve both C and N metabolism to attain maximum growth and yield.

Singh and Misra (2001) reported increased NR activity in *Mentha spicata* Var. MSS-5 in response to ethrel application at high concentration(1000ppm) to plantlets of Mentha cultured in nutrient solution. Khan *et al.*, (2008) analysed impact of ethephon application on *Vetiveria zizanoides* plants subjected to both normal and drought stressed condition. They recorded that activity of NR was decreased due to stress but elevated due to application of ethephon to stressed plants where as in unstressed plants NR activity was reduced due to ethephon. Similar observations are made in present investigation in both leaf and root of Coleus. Synergistic effect of auxin and ethylene on physiology of *Jatropa curcas* L. plant was assessed by Joshi *et al.*, (2011). They reported that ethrel at 50, 100 and 150ppm along with IAA and NAA caused positive effect on nitrate reductase activity as compared to control and when applied alone ethrel treated plants of jatropha showed higher NR activity @ 150 ppm. Sharma and Sardana (2012) also recorded enhanced activity of NR in groundnut by ethrel treatment. They also reported that different combinations of IAA, mepiquat chloride and ethrel positively influenced NR activity along with its productivity.

Decreased activity of NR due to ethephon in leaves and roots of Coleus in present study might be attributed reduced uptake and translocation of nitrate or may be due to alterations in metabolic processes triggered due to PGRs. These results are explained in terms of ethephon stimulated protein synthesis and increase in cellular metabolism and permeability.

Thus, it can be concluded that increased activity of NR in leaves and roots of Coleus in present investigation is an indicator of enhanced rate of nitrate assimilation
RESULTS AND DISCUSSION

and thus photosynthesis triggered by PGRs which might boost plant’s growth and productivity and efficiency of nitrogen uptake and utilization.

4. Soluble protein content

The variation in the level of soluble protein content in leaf and root tissue of *C. forskohlii* in response to foliar application of low and high concentration of Triacontanol, BRs, CCC and ethephon is presented in Table No. 25 and Fig. 77.

Proteins are polypeptides made up of amino acids. Proteins are most important macromolecules which actually carry out all the cell processes.

It can be clearly seen from the figure that soluble protein content is enhanced in root tissue due to both concentrations of all PGRs as compared to control where as in leaf tissue exactly opposite trend is noticed. Amongst all important biomolecules proteins occupy most crucial position as without which existence of life is not possible. Enzymes or biological catalysts forms the major part of soluble proteins. Thus, being enzymes proteins catalyse all biochemical reaction and play a critical role in all metabolic processes in plant cell. All biological membranes are made up of proteins. Proteins also provide mechanical support in both within the cell and outside the cell. In plant growth regulation, hormone signalling, proteins are actually hormone receptors, growth factors and also gene activators.

Proteins differ according to type, number and sequence of amino acids so they also differ in molecular structures and physiochemical properties. Proteins are classified as simple proteins, conjugated proteins and derived proteins. Simple proteins like albumin are soluble in water and in dilute salt solution and which can be precipitated by saturation with ammonium sulphate solutions and then coagulated by heat. As proteins perform a significantly vital role in wellbeing of any biological system so analysis of proteins attains utmost importance in biochemical and physiological studies. The analysis of protein composition in plants provide knowledge about how these molecules interact and cooperate to produce and maintain plant growth and development under any environmental condition.

The cell responds to internal and external changes by regulating and modifying the level and activity of its proteins so changes in protein content and its type gives idea about the qualitative and quantitative changes taking place in plant system. In plants, protein synthesis takes place in three different compartments such as cytoplasm, plastids and mitochondria. Each cell organelle also has its own protein
RESULTS AND DISCUSSION

synthesis machinery. The process of protein synthesis requires large amount of energy to be spent by cell and this is highly regulated processes depending upon up and down regulation of gene expression. Thus, as opined by Spremulli, (2000) to maintain proper plant metabolism, regulation of protein synthesis and degradation in response to internal and external signals by adjusting the amount of specific protein present is essential to cop up with cellular environment. As enzymes forms the major portion of soluble proteins in plant system, responsible for various biochemical and physiological functions these are always in a state of transformation which involves synthesis of new essential proteins and degradation of unwanted decomposed proteins. Various factors bring about changes in protein level such as temp, nutrient ion status, various stresses, endogenous or exogenous level of plant growth regulators, age of plant and physiological status of cell and so on. There are numerous evidences regarding alterations in the level and type of proteins in response to influence of various PGRs. Plant growth regulators have been found to influence the protein content significantly in many crop plants. Positive effect of triacontanol on leaf protein content in various plants is recorded by various workers such as Knowles and Ries (1981), Ries and Houtz, (1983), Ries (1991) in rice and corn, Kumarvelu et al., (2000) in green gram, Muthuchelian et al., (2003) in Erythrina variegata, Naeem et al., (2009) in Hycinth bean, Naeem et al., (2010) in coffee senna, Naeem et al., (2011) in Mentha arvensis, Hashmi et al., (2011) in Ocimum basilicum,Khan et al., (2014) in lemon grass which are contrary to present investigation as in Coleus forskohlii leaf soluble protein content is decreased due to triacontanol but increased in root tissue.

Singh et al., (2008) and Singh et al., (2012) recorded enhanced level of soluble protein in both leaf and rhizome tissue of Zingiber officinale Rosc. Ginger plant and turmeric (Curcuma longa) plant due to foliar spray of triacontanol at 10$^{-6}$M concentration. This finding supports increase in soluble protein content of root of Coleus in present study. In viviparous hypocotyls of Rhizophora apiculata, a mangrove, Moorthy and Kathiresan, (1993) observed increased soluble proteins in both leaves and roots in response to triacontanol.

Bajguz (2000) found highest growth of the protein content and nucleic acids in case of green alga Chlorella vulgaris Beijerinck exposed to BRs at concentration of 10$^{8}$M in 36th hour of its cultivation. Swamy and Rao (2008) reported that in case of
RESULTS AND DISCUSSION

*C. forskohlii* subjected to foliar application of 24-EBR and 28-HBR there was enhancement in contents of proteins and nucleic acids with increased tuber yield. Bajguz and Asami (2005) observed increase in the content of protein by 24-EBR at $10^{-9}$M concentration in *Wolffia arrhiza*. In case of *Arachis hypogaea* increased level of nucleic acids and soluble proteins in leaves and nuts due to application of 24-EBR Vardhini and Rao, (1998). Swamy and Rao, (2008) and Ayad *et al.*, (2009) found increased soluble protein content in geranium (*Pelargonium graveolens* L.) plant due to 28-HBR application. Nakajima *et al.*, (1996) also noticed increased soluble proteins in Chinese cabbage protoplast by addition of 24-EBR in culture media. Vardhini *et al.*, (2012) in storage roots of radish also observed elevated level of soluble proteins due to 24-EBR and 28-HBR application. Vardhini and Rao (1998) and Vardhini *et al.*, (2008) also reported increased soluble proteins in leaves and fruits of groundnut and tomato resp. Sirhindi *et al.*, (2009) reported increased total protein content in seedlings of *Brassica juncea* due to 24-EBR and 28-HBR. Numerous studies have shown that BRs has increased protein synthesis in leaves and roots of plants either under stressed or unstressed condition (Anuradha and Rao, 2001; Sirhindi, 2009). These findings go in agreement with the findings of present investigation. In *C. forskohlii* also root soluble protein contents are increased due to both doses of BRs as compared to control. As mentioned by Kalinich *et al.*, (1985) BRs stimulated increase in soluble proteins might be attributed to enhance activity of RNA and DNA polymerase enzyme which are engaged in a physiological response to BRs hormone. According to Sairam (1994) BR induced increase in soluble (enzyme) proteins results in increased rate of photosynthesis and ultimately yield. As opined by Gregory (1981) enhanced level of soluble proteins in response to BRs might be because of increased protein synthesis and specific metabolic changes. Similar hypothesis is proposed by Bajguz and Hayat (2009) that BR regulated plant response to environmental conditions is a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions causing induction of protein synthesis and production of various defensive chemical compounds. In the present investigation, increased protein content in roots might be presumably because of increased protein synthesis and sequential metabolic enzymatic reactions induced due to BRs. Increased soluble proteins in root as compared to leaves induced by BRs in present study might be indicative of increased transport of protein towards roots being
RESULTS AND DISCUSSION

tuberous root as a major site of dry matter accumulation in Coleus or might be because of altered membrane permeability or increased rate of amino acid synthesis and transportation from leaf to root. Thus, irrespective of process involved in protein synthesis, increased protein content due to BRs is beneficial for coleus productivity. In the present investigation both concentrations of CCC induced reduction in soluble protein content in leaf tissue while enhanced level of protein content is seen in root tissue by CCC. Similar observation was made by Khan et al., (2008) in Vetiveria zizanoides subjected to drought stress and foliar application of CCC. They reported that CCC caused decrease in protein content of leaves under both stressed and unstressed condition. However, Bode and Wild (1984) in wheat var. Kolibri reported increased level of soluble proteins in CCC treated leaves. Similar increase in leaf soluble protein is reported by Kar et al., (1989) in Safflower, Shah and Prathapasenan (1991) in mungbean, Giridhar and Giri (1997) in ground nut in response to CCC application. These evidences are antagonistic to present findings. However enhanced protein content in root tissue of C. forskohlii due to CCC may be beneficial for increasing root yield and productivity. Khan et al., (2008) reported reduced protein content in leaves of Vetiveria zizanoides plant in response to ethrel application under both drought stressed and unstressed conditions. This goes in tune with the present finding.

Thus, it can be concluded that increased content of soluble protein in root tissue due to foliar application of all PGRs as compared to control is an indicative of positively altered metabolism and membrane permeability w. r. t increased nutrition induced due to PGRs. It is also an indicator of improved nitrogen metabolism. It might lead to increased production as proteins are the most important biomolecules essential for enhanced growth.

5. Free amino acids

The changes occurred in free amino acids content in leaf and root tissue of C. forskohlii in response to low and high concentration of triacontanol, BRs, CCC and ethephon are depicted in Table No. 26 and Fig. 78. It is clearly evident that the level of free amino acids is elevated in both leaf and root tissue of coleus due to both concentrations of triacontanol, BRs and CCC however in case of ethephon treated plants, free amino acids are increased only leaf but not in root tissue as compared to control. Among all the treatments highest content of free amino acid is found in root
tissue treated with low dose of triacontanol and BRs while in leaf tissue treated with both doses of ethephon. Amino acids being building blocks of proteins becomes one of the most essential organic compounds in all living organisms. In most of non-leguminous crops, amino acid synthesis and distribution takes place in leaf tissue for which energy and carbon skeleton is provided by photosynthesis to assimilate nitrogen in the form of nitrate to nitrite and finally into ammonium which is further converted into primary amino acid products (glutamine and glutamate). The amino groups of these products are further allocated through transamination to form the host of amino acids essential for the process of protein synthesis and all the other biochemical reactions. Amino acid is the most preferable form of organic nitrogen for transportation in many plants which serves as a hub for many processes including nitrogen metabolism, protein synthesis and as a precursor for many important cellular components (Lalonde et al., 2004; Nikam, 2007). According to Lalonde et al., (2004) long distance transport of amino acids occurs in phloem as well as xylem, by generating a quasi-circulatory system for organic nitrogen transport. Broad selectivity carriers may also be used for taking up broad spectrum of different amino acids. Amino acids contribute to the base for different enzymatic and non-enzymatic proteins. Plants also contain free amino acids. Amino acids are classified as essential, imino acids and non-protein amino acids (Sonar, 2013). As amino acids play vital role in all processes of cellular metabolism and involved in multiple metabolic pathways it is important to study the effect of various exogenous and endogenous factors like pH, stresses, PGRs, temperature etc. on amino acid synthesis, metabolism or distribution inside the plant system. Several attempts have been made by many workers to evaluate the effect of PGRs on the content of free amino acid in many plants. In present investigation, in C. forskohlii, the level of free amino acid content is increased in both leaf and root tissue by triacontanol. Many researchers have reported positive effect of triacontanol on free amino acid content (Naeem et al., (2011). Knowles and Ries (1981), Ries and Wert, (1982), Ries, (1985), Kim et al., (1989) have recorded increment in amino acids in leaves of rice (Oryza sativa) and corn (Zea mays) in response to triacontanol. Muthuchelian et al., (1990) and Thakur and Thakur, (1992) also noticed enhanced free amino acids due to triacontanol in leaves of Pennisetum polystachyon and Acacia leaves respectively. Muthuchelian et al., (1995) recorded enhanced free amino acids in Erythrina variegata plants under the influence of
RESULTS AND DISCUSSION

triacontanol and flooded conditions. Kadam et al., (1998) and Kumarvelu et al., (2000) noticed increased free amino acids in leaves of spinach and green gram at 0.5 mg/l and 5ppm concentration of triacontanol spray respectively. All these evidences support present readings of triacontanol. As reviewed by Khripach et al., (2000) BRs significantly influence protein spectrum and amino acid composition of proteins in plants under normal and stressed conditions. El-Bassiony et al., (2012) found ameliorating effect of BRs on growth and productivity of snap bean grown under high temperature stress. They reported increased total free amino acid in the leaves due to BRs spray application at 25 ppm against control. This evidence goes in agreement with the present investigation while Abd El-Wahed et al., (2004) reported negative effect of BRs on amino acids. They studied effect of spraying of stigmasterol, a type of BRs on chamomile (Chamomilia recutita L.) plant at 25, 50, 75 and 100mg/l concentration. They reported that stigmasterol significantly decreased total free amino acids against control at 100mg/l dose. According to Mandava, (1988), BRs influence enzymatic activities of membrane potential, DNA, RNA and protein synthesis which may indirectly affect amino acid content. Possibility of similar situation cannot be denied in the present investigation.

Jain and Guruprasad (1989) recorded an enhancement the level of free amino acid in radish seedlings treated with CCC more specifically in amino acid phenylalanine. Naylor and Stephen (1993) reported that early application of CCC increased the proportions of various amino acids like histidine, aspartic acid and alanine while amino acid like leucine content was found to be decreased in their field studies with triticale. However, they reported that late application of CCC in triticale had no change on histidine amino acid but enhanced level of aspartic acid, glutamic acid and leucine. Choudhary and Gupta (1996) also found increased amount of free amino acid in Catharanthus roseus in response to CCC treatment. All these evidences support present findings of CCC.

Joshi et al., (2011) reported enhanced level of amino acid in Jatropha curcas plants subjected to effect of auxin and ethrel in sequential combination or alone. They also reported increased activity of NR and content of proline are beneficial to Jatropha curcas.

In the present investigation, overall increment in free amino acid content in leaf and root part may be beneficial to C. forskohlii and suggests overall positive
RESULTS AND DISCUSSION

effect of PGRs on plant metabolism and induction of various sequential biochemical changes stimulated by PGRs at cellular level leading to altered nucleic acid, protein synthesis and N metabolism.

H. Phosphorus Metabolism

Influence of foliar spray of PGRs on phosphorus metabolism

1. Phosphorus Content

   The effect of low and high concentration of Plant growth regulators, Triacontanol, Brassinosteroids, Chlorocholine chloride/CCC and ethephon on the Phosphorus content from leaf, stem and root tissue of *Coleus forskohlii* can be observed from Table No. 27 and Fig. 79.

   Phosphorus is an important plant macronutrient being essential part of nucleic acids which makes it highly indispensable element in all living organisms. It is also an essential constituent of phospholipids, sugar phosphates, NADP etc. It is also a key component of biological energy currency ATP. Phosphorus plays a key role in various metabolic activities and plants cannot grow without phosphorus.

   It is evident from the figure that the Phosphorus content is increased in leaf, stem and root tissue of *Coleus forskohlii* plants treated with both low and high concentration of triacontanol/ Vipul as foliar spray as compared to control plants. Though both doses of triacontanol increased the P content in all plant parts, its concentration is found to be more in leaf and stem and low concentration of triacontanol is found to be more effective in improving P content in all plant parts as compared to control and all the other PGRs tried.

   It can be revealed that brassinosteroids concentration at 1ppm and 5ppm have increased the content of Phosphorus in leaf, stem and root as compared to control. Highest amount of phosphorus is visible in stem due to both concentrations of BRs.

   In case of CCC, the level of phosphorus is reduced in all plant parts of *Coleus forskohlii* due to foliar sprays of CCC at both concentrations but the amount of phosphorus is found to be more in leaf than stem than root tissue.

   The level of phosphorus is increased in all plant parts leaf, stem and root of *Coleus forskohlii* along with increase in concentration of ethephon foliar spray. High concentration of ethephon caused very sharp increase in P content as compared to low concentration.
Phosphorus is involved in regulation of various metabolic pathways, as phosphorylation and dephosphorylation of enzyme proteins play a key role in regulation of various life processes in plant cell. Munson (1998) revealed that the level of phosphorus required for the optimum plant growth is 0.2% to 0.75% of dry weight. According to Schachtman et al., (1998) after nitrogen, phosphorus is the second most limiting mineral element for plant growth. According to Kochian (2000), phosphorus nutrition is unique as the phosphorus availability to plants is major constraint to growth of plants. Though the amount of phosphorus in soil may be high, most of it is present in unavailable form or forms that are available outside the rhizosphere of plant.

Phosphorus in the soil is present mostly in different fractions, such as organic and mineral or inorganic phosphorus. According to Richardson (1994) about 20 to 80% of phosphorus in soil occurs in the organic form, of which phytic acid (inositol hexa phosphate) is major component while remaining fraction of phosphorus is found in inorganic form containing 170 mineral forms of phosphorus (Holford, 1997) such as Hydroxyapatite, flurapatite, Di or tri calcium phosphate etc. so only phosphorus fraction present in the soil solution is readily available for the uptake plant roots while 80% of phosphorus became immobile or unavailable for plant uptake due to absorption, precipitation or conversion to organic form (Holford, 1997). According to Lynch (1995) root systems with higher ratios of surface area to volume will explore larger volume of soil. According to Bieleski (1973) plants can readily absorb inorganic phosphorus (Pi) which is found in very low concentration in soil solution (not more than 10µM in soil solution). So, there is imbalance between the requirement of phosphorus by plants and its concentration in soil. So as stated by Schachtman et al., (1998) the plants must have developed specialized transporters at root-soil interface for absorption of Pi from soil solution and also mechanisms for Pi transport across membranes for intra and intercellular compartments where the concentration of Pi is 1000-fold more than the external solution. The kinetic and molecular data reveals that higher plants have multiple transporters for transport of Pi across cellular membranes. The molecular data available supports the presence of at least 4 genes that encode Pi transporters and kinetic data supports the presence of two types of transporters with different affinities for Pi (Schachtman et al., 1998).
RESULTS AND DISCUSSION

In higher plants, in vacuolated cells two major phosphate pools occur, metabolic and non-metabolic pool. In metabolic pool which is represented cytoplasm including chloroplast, phosphate esters are present in higher proportion where as in the non-metabolic pool or storage form including vacuole, Pi is the dominant fraction. According to Munson, (1998) these pools can be classified according to their location in physical compartments such as cytoplasm, vacuole, apoplast and nucleus. Phosphorus is taken into plant through active transport system (Rausch et al., 2004) and the form of Pi in these compartments will be pH dependent. Like at pH 7.2, Pi in the cytoplasm will have equally partitioned ionic forms H2PO4\(^-\) and HPO4\(^{2-}\) while in vacuoles or apoplasts where there is acidic pH H2PO4\(^-\) will be dominant form of Pi. They can be also classified according to chemical form of ‘P’ such as Pi, P-esters, P-lipids and nucleic acids. The concentration of total ‘P’ in each such chemical form is altered according to type of tissue, its age and in response to ‘P’ nutrition except in DNA. Pi plays a key role in metabolic pool by acting either as a substrate or as an end product and also as regulators of enzyme activities. So, compartmentalization of Pi is more essential for the regulation of various metabolic pathways in cytoplasm and chloroplast (Marschner, 1986). Thus, many enzymatic activities require stable cytoplasmic Pi concentration which is attained by a combination of membrane transport and exchange between various intracellular metabolic pools of Pi (Schachtman et al., 1998).

According to Ratcliffe (1994), NMR studies contributed majorly to our knowledge of Pi pools within the plant. NMR studies confirmed the presence of a small, rapidly turning over pool of Pi (about 1-5% of total Pi) located in the cytoplasm and larger storage pool located in the vacuole.

Recent studies done by Mimura et al., (1996) provide an idea about ‘P’ translocation in whole plant. They revealed that in P–sufficient plants Pi absorbed by the roots is transported into xylem and then to the younger leaves. There is also significant retranslocation of Pi into the phloem from older leaves to the younger growing tissues and from shoots to the roots. The concentration of Pi in the xylem tissue ranges from 1mM in scarcity of Pi while increase up to 7mM in plants grown in Pi sufficient conditions. According to Schachtman et al., (1998) in scarcity of ‘P’, plants tend to grow more roots to increase the rate of ‘P’ uptake by roots from soil, re-translocate Pi from older leaves and obtain the Pi from vacuolar stores. On the other
hand, under the condition of adequate supply of Pi and at high rate of Pi uptake, number of processes act to prevent the accumulation of Pi concentration at toxic level. This can be achieved by conversion of Pi into organic storage forms (e.g. phytic acid) or by reducing the rate of Pi uptake from outside soil solution (Lee et al., 1990) and Pi may be lost by efflux process. Any of this or all the processes may have applied by plants for the maintenance of intracellular Pi homeostasis.

Krishnan and Chandrasekaran (1989) in fenugreek and Sharma et al., (2006) in pea var. Arkel plant also found increased level of Phosphorus under the influence of triacontanol. Observations made by Naeem et al., (2009) in plant Hycinth bean (Lablab purpureus L.) and Naeem et al., (2011) in Mentha arvensis L. also supports stimulation of phosphorus content in leaf tissue due to foliar spray of triacontanol at $10^{-6}$M concentration. Alam et al., (2012) in Catharanthus roseus also found enhanced level of phosphorus in leaf due to foliar application of triacontanol at $10^{-7}$M concentration as compared to control plants. Khandaker et al., (2013) observed similar type of increase in phosphorus content in leaves of Bougainvillea plant. They found that triacontanol at 2.5mg/L concentration resulted in accumulation of 37% of phosphorus as compared to control.


Sumeria (2003) and Rani et al., (2013) reported increased uptake and accumulation of phosphorus in straw part of mustard (Brassica juncea (L.)) and Okra (Abelmoschus esculentus L. Moench)) CV. Arka Anamik due to foliar application of triacontanol at 0.05% and 4000ppm respectively as compared to control. Singh et al., (2012) reported high level of phosphorus content in rhizome and leaf tissue of medicinally important plant ginger (Zingiber officinale Ros.) due to foliar spray of triacontanol at $10^{-6}$M concentration which also goes in conformity with our results.

In case of Coleus forskohlii a significant increase in P content of all plant tissues like leaf, stem and root is evident under the influence of triacontanol as compared to control. In view of indispensable role of P content in overall growth and development processes of plant, an increase in P level by both concentrations of triacontanol will certainly boost the metabolic and developmental processes in Coleus
A marked increase in P content in leaf and stem tissue than root of *Coleus forskohlii* due to triacontanol indicate its efficiency in positively modifying the P uptake and translocation. Low concentration of triacontanol is found to be more effective in improving P content in all plant parts as compared to control and all the other PGRs tried.


El-Moursi *et al.*, (2012) studied effect of stigmasterol which is also one of BRs and also found increased phosphorus in *Moringa oleifera*. Results found by Alam *et al.*, (2012) in leaves of *Catharanthus roseus* L., Vardhini *et al.*, (2012) in radish roots and by Bera and Kalipada (2013) in stalk and grains of lentil (*Lens culinaris* Medik) also goes in tune with our results.

In case of *Coleus forskohlii* a significant increase in P content of all plant tissues like leaf, stem and root is evident under the influence of both the concentrations of brassinosteroids as compared to control. Phosphorus plays a vital role in all the metabolic processes in plant life. An increase in P level by both concentrations of BRs will certainly help in the metabolic and developmental processes in *Coleus forskohlii*. The increase in P content is very sharp in leaf and stem as compared to root is indicative of improved uptake and translocation of P. This increment of P content in all plant parts due to high concentration of BRs is more significant as compared to control and all the other PGRs tried in case of *Coleus forskohlii*.

El-Fouly *et al.*, (1970b) and Saeed *et al.*, (1972) reported similar trend of reduction in phosphorus content in all plant parts under the influence of CCC in case
RESULTS AND DISCUSSION

of cotton and safflower (*Carthamus tinctorius* L.) respectively. According to El-Fouly *et al.*, (1970) in cotton plant phosphorus content was found more in stem than root which is also found true in our study. Husien and Abdul (1985) and Patil (1995) observed similar reduction in phosphorus level due to CCC application in okra and sugarcane plants respectively. Andrievic (1988) observed similar decrease in phosphorus content in poppy plants due to CCC spraying at 300ppm and 600ppm during flowering but increased phosphorus content at 600ppm during stem elongation and at 300ppm sprayed during immature capsule period. Thus, he observed alterations in phosphorus content in poppy plant as per the dose of CCC and stages of plant growth. Under drought stress Zeid and El-Semary (2001) observed decreased phosphorus level due to CCC treatment in two different drought tolerant varieties of maize.


Though Gohlke and Tolbert (1962) found inhibition of translocation of radioactive phosphorus $^{32}$P in barley seedlings due to CCC when added to nutrient solution, Adepipe (1975) found no significant effect on leaf fed radioactive phosphorus $[^{32}\text{P}]$ translocation to root and shoot of pea plants under the influence of foliar and root application of CCC even at high concentration. Karivaratharaju *et al.*, (1975) found that foliar application of CCC enhanced the uptake of phosphorus $[^{32}\text{P}]$ content in leaves and stem of bean (*Dolichos lablab* L.) seedlings up to 100ppm concentration of CCC but increasing concentration of CCC had inhibitory effect.

In the present investigation we found declined level of P in all plant parts of *Coleus forskohlii* under the influence of both concentration of CCC as compared to control and all the other PGRs tried. This indicates altered P nutrition by CCC application. This sharp decline in P content in all plant parts would surely affect the phosphorus metabolism in case of *Coleus forskohlii*. 
Karivaratharaju et al., (1975) studied the effect of ethrel or ethephon on uptake and distribution of radioactive phosphorus $[^{32}\text{P}]$ in bean (*Dolichos lablab* L.) seedlings, they found enhanced phosphorus $[^{32}\text{P}]$ content in leaves and stem but increasing concentration of ethrel (above 100ppm) had inhibitory effect.

Waidyanatha (2003) investigated the effect of ethrel on yield and content of inflorescence sap of coconut (*Cocos nucifera*) and forty-year-old palm. They found increased content of phosphorus with the sap at 2.5% ethrel concentration as compared to control along with increased sap yield. Khuankaew et al., (2009) in *Curcuma alismatifolia* Gagnep. found that ethephon application at 300ppm increase the accumulation of phosphorus content in underground plant organs.

Our findings showed increased level of P in all plant parts of *Coleus forskohlii* due to both concentrations of ethephon. This increase in phosphorus is an indicative of altered P nutrition in *Coleus forskohlii* because of ethephon. This increase in P content may be due to the fact that ethephon releases ethylene which increases respiration rate which results in decrease in carbon accumulation and increase in break down and loss of energy (ATP). It causes decrease in ATP content which finally affects the important life processes such as photosynthesis and amino acid metabolism which collectively inhibits plant growth which is found true in our investigation w. r. t. ethephon high concentration.

It can be concluded that increased content of P in leaf and root in coleus in response to triacontanol, BRs and ethephon may be beneficial to plant growth and productivity which is an indicator of increased nutrient uptake, membrane permeability and enhanced rate of metabolism.

2. Enzyme ATPase activity

The effect of foliar spray of low and high concentrations of Plant growth regulators Triacontanol/Vipul, Brassinosteroids, Chlorocholine chloride/CCC and ethephon on the activity of enzyme ATPase from leaf and root tissue of *Coleus forskohlii* Willd. can be observed from Fig. 80 and Table No. 28. It is clear from the figure that the activity of enzyme ATPase is remarkably increased in both leaf and root tissue of *Coleus forskohlii* Willd. in response to both concentrations of all the plant growth regulators applied as compared to control.

Enzyme ATPase is called as ‘master enzyme’ of plant cells and also as ‘eco enzyme’ because of its varied adjustability under varied environmental conditions.
RESULTS AND DISCUSSION

(Rockel et al., 1998, Xia et al., 2000 and Yu et al., 2001). This enzyme is present in almost all plant cells. This is widely distributed within the eukaryotic endomembrane systems including ER, the Golgi apparatus, membrane vesicles, the tonoplast and the plasma membrane (Rockel et al., 1998), LIU Guan – Shan et al., 2004). Logan (2006) reported ATPase from vacuolar and other endomembranes from plants such as thylakoid membrane of chloroplast, inner mitochondrial membrane and also in plasma membrane of bacteria and blue green algae. ATPase is found to be involved in the hydrolysis of biological currency ATP. ATPases are mainly of two types namely P-ATPase (E. C. 3.6.1.35) and V-ATPase (E. C. 3.6.1.34) based on its function and locality. P-ATPase works at plasma membrane and V-ATPase at tonoplast or vacuolar membrane (Serrano, 1989), Taiz (1992).

According to observations made by Klink and Lottege (1990) and LIU Guan – Shan et al., 2004) V-ATPase is highly abundant at plant tonoplast making up to 6.5%-35% of total tonoplast protein in different plant species. The V-ATPase provides the driving force for the secondary active transport of ions and metabolites. The V-ATPase is a multi-subunit protein complex made up of up to 10 subunits, of which Subunits A, B and C exist in all species and catalyses ATP hydrolysis, regulating activity of enzyme and transporting proton from cytoplasm to vacuole respectively. LIU Guan – Shan et al., 2004) stated that V-ATPase holoenzyme consists of two domains V1 which is membrane peripheral domain hydrolyzing ATP and V0 membrane integral domain transporting proton. Subunits A and B are located in V1 domain and subunit C in V0 sector. Subunit A is mostly conserved having molecular mass ranging from 67 to 73kD in different plant species. In maize (Zea mays L.) it is reported that V-ATPase subunit A is composed of 561 amino acids with molecular mass 69 kD (Gene Bank accession P49087). LIU Guan – Shan et al., 2004) for the first time determined that in maize, phosphorylation play important role in V-ATPase activity regulation. They also reported that Ser525 is the potential phosphorylation site on subunit A of V-ATPase in maize roots. Phosphorylation and dephosphorylation plays important regulatory role in activity of both V-ATPase and P type H+-ATPases.

It has been approved that a ‘C’-terminal penultimate threonine residue can be phosphorylated by application of P type H+ -ATPases isolated from spinach (Spinacia oleracea) leaf tissue and Nicotiana plumbaginifolia. P type H+ -ATPase isoforms
PMA2 expressed in *Saccharomyces cerevisiae* and purified (Olsson *et al.*, 1998; Maudoux *et al.*, 2000). The tonoplast H\(^+\)-ATPases also known as V-type H\(^+\)-ATPase in plant cells does not form phosphorylated intermediate and also not inhibited by vanadate. It belongs to the family V-ATPases, operating as proton pumps at endomembranes of eukaryotic cells (Nelson, 1992). The V- H\(^+\)- ATPase is inhibited by the antibiotic bafilomycin (Bowman *et al.*, 1988) and by NO\(^-\). It is stimulated by Cl\(^-\) and not by monovalent cations. The P\(^H\) optimum for this enzyme is 7.9 (Nelson and Taiz, 1989).

The P types H\(^+\)- ATPases are nothing but plasma membrane H\(^+\)- ATPases called so because these enzymes undergoes phosphorylation during its catalytic cycle. P-type H\(^+\)- ATPase constitutes a family of proton pumps driven by hydrolysis of ATP and are found exclusively in plasma membrane of plants and fungi. The primary role of this enzyme is to provide an energy source for transport of nutrients into the cell. Mg\(^{2+}\) is absolutely required by the plasma membrane H\(^+\)-ATPase for ATP hydrolysis (Vara and Serrano 1982). Michelet and Boutry (1995) reported that proton pump ATPase (H\(^+\)- ATPase) of the plant plasma membrane acts as of primary transporter by pumping protons out of the cell thereby creating P\(^H\) and electrical potential difference across the plasma membrane. Transport of many solutes into and out of the cell requires secondary transporters whose function is directly dependent on the proton-motive force created by H\(^+\)- ATPase. This mechanism involves building of P\(^H\) gradient across membranes. The energy bound in this electrochemical gradient considered to be the driving force for solute carriers and channels that are essential for nutrient uptake and maintenance of cell turgor.

The H\(^+\)- ATPase has a P\(^H\) optimum of 6.6, well below the physiological P\(^H\) of the plant cell cytoplasm which is usually 7.2 – 7.5. Thus, whenever protons start accumulating in the cytoplasm the activity of H\(^+\)- ATPase increases resulting in the expulsion of excess H\(^+\) from the cell.

Cytoplasm alkalinization which may result from increased H\(^+\)- ATPase pumping activity can trigger important cellular events in response to hormonal and developmental signals (Kurkdjian and Guern, 1989; Blatt and Armstrong, 1993). Consequently, the H\(^+\)- ATPase might act as an intermediate in certain signal transduction pathways rather than simply being the final target. The enzymatic cycle
and kinetics of the plant P type H\(^+\)-ATPase are reviewed by Serrano (1989) and Briskin and Hanson (1992).

According to Wach et al., (1992) the H\(^+\)-ATPase is a protein with a molecular mass of about 100 kD and is composed of a single polypeptide. It transports one proton per molecule of ATP hydrolysed and P\(^\text{H}\) optimum 6.6 and Km of MgATP of 0.3 to 1.4 mM. Its specific activity in purified plasma membrane is usually of the order of 1 to 2 \(\mu\) mol Pi min \(^{-1}\)mg\(^{-1}\) protein. Its activity is inhibited by vanadate, dichlorohexyl caebodiimide, diethyl styldestrol and erythrosin B, but not by NaNO\(_3\) or oligomycin which are inhibitors of the mitochondrial and chloroplastic ATPases. It’s also not inhibited by nitrate (an inhibitor of the vacuolar membrane ATPase) or molybdate (an inhibitor of non-specific phosphatases). Carafoli et al., (1980) proposed that ATPase is activated by Ca\(^{2+}\) binding protein calmodulin both *in vitro* and *in vivo*. ATPase is more sensitive to anions than cations (Walker and Leigh, 1981).

De Witt et al., (1991) gave proof for presence of plasma membrane proton pump i.e. ATPase pump in phloem cells of higher plants. They stated that metabolic energy is required for loading of sucrose into phloem and translocation of sugars throughout the plant and this energy is provided by the proton electrochemical gradient generated by plasma membrane proton pump (H\(^+\) ATPase). According to them the plasma membrane H\(^+\) ATPase is encoded by a multigene family in *A. thaliana*. They assessed the expression of the AHA3/GUS fusion by using histochemical localization or stain. They found the expression of the AHA3/GUS fusion predominantly in phloem cells of leaves, stems, roots and flowers along with pollens and regions of ovule tissues which are the sites for high levels of solute transport and thus requires high activity of H\(^+\) ATPase.

According to Sussman, (1994) molecular genetic studies put forth that there are multiple genes coding for H\(^+\) ATPases. Ewing and Bonnette, (1994) proved presence of a gene family with at least 7 genes in tomato and 10 genes in *A. thaliana* by Harper et al., (1994) which perform the fine regulation of H\(^+\) ATPase in diverse cells and tissues as supported by Michelet and Boutry, (1995)).

The plasma membrane of higher plants contains H\(^+\) ATPase as its major ion pump. It is involved in multiple physiological functions. Being ‘master enzyme’ it plays an important role in the nutrition and growth of the plant (Sussman and Surowy, 1994).
RESULTS AND DISCUSSION

1987). It also plays an important role in energy relations of cell and transport process and as it is also involved in triggering important cellular events in response to hormonal and developmental signals and might be an important intermediate in certain signal transduction pathways. According to Palmgren, (1991) the activity of the plasma membrane H⁺-ATPase is influenced after exposure of plant tissues to plant growth factors such as plant hormones, light and pathogens but very little is known about its regulation. Attempts have been made to study the effect of various plant hormones on ATPase activity. In our case also it is found true that plant growth regulators affected the ATPase activity positively in both leaf and root tissues of *C. forskohlii*. Exogenous application of triacontanol to barley roots caused rapid stimulation of membrane associated Ca²⁺/Mg²⁺ dependent ATPase activity in a calmodulin-dependent manner (Lesniak *et al.*, 1986) both *In-vivo* and *In-vitro*. Lesniak *et al.*, (1989) found stimulated activity of enzyme ATPase in barley (*Hordeum vulgare*) root vesicles plasma membranes in response to triacontanol and calmodulin. They also supported contention that triacontanol affects the membrane structure and function. Triacontanol induces the activation of a number of membrane bound enzymes (Ries and Houtz, 1983; Savithiry *et al.*, 1992). The stimulation of these enzymes leads to dephosphorylation of ‘L’ (+) forms of AMP, ADP and ATP which in turn results in formation of 9-β-L (+) adenosine which brings cascade of events leading to rapid physiological responses (Ries *et al.*, 1990; Ries, 1991). Similar kind of increase in ATPase activity was observed by Henry *et al.*, (1981) in Mung Bean plant leaf, upper internode and root tissue exposed to light at BRs concentration of 10⁻⁶, 10⁻⁷, 10⁻⁸ and 10⁻⁹M. Kalinich *et al.*, (1985) reported increased activity of enzyme ATPase in bean epicotyls in response to BRs application. Xu *et al.*, (1995) also observed same in wheat roots. Deeva *et al.*, (1996) studied the effect of 24 epibrassinolide on ATPase activity of plasma membrane of diploid and tetraploid buck wheat genotype seedlings, and reported about 35% increase in tetraploid wheat seedlings. Khripach *et al.*, (2003) proposed that BRs favors plant basic processes resulting in growth promotions as a result of induced activity of ATPase-pump in vacuoles because of metal detoxification or acid growth promotion.

Schumacher *et al.*, (1999) have shown that tonoplast H⁺-ATPase (V-ATPase) played on important role in elongation of hypocotyls which is promoted by BRs. Khripach *et al.*, (1999) made an attempt to evaluate the effect of EBI (BRs) on the
activity of H\(^+\)-ATPase in plasma membrane preparations obtained from maize roots. They reported that all the three different treatments with BRs (seed soaking, root exposure to BRs solution and spraying of upper part of maize seedlings) In Vivo increased the H\(^+\)-ATPase activity.

It can be concluded that increased activity of ATPase in leaf and root tissue in response to all PGRs can be beneficial to plant and reflects increased rate of metabolic activities, photosynthesis and respiration. ATPase enzyme breaks down ATP and provide energy for activation of several enzymes associated with plant growth and metabolism. This will surely boost plant growth both qualitatively and quantitatively.

3. Enzyme Acid phosphatase

The effect of low and high concentration of plant growth regulators, Triacontanol/Vipul, Brassinosteroids, Chlorocholine chloride/CCC and ethephon on the activity of enzyme acid phosphatase from leaf and root tissue of *Coleus forskohlii* can be observed from Fig. 81 and Table No. 29. Phosphorus as an integral part of vital plant processes and plays a significant role in plant growth and development so in view of this its assimilation, storage and metabolism are of immense importance. Phosphatase is a group of enzymes found ubiquitous in all types of tissues of all plant organs and plays an important role in efficient utilization of phosphorus by hydrolyzing several organic phosphatemonoesters, liberating available Pi, for plant assimilation (Machado and Furlani, 2004)

It is evident that in leaf tissue, the activity of enzyme acid phosphatase is decreased due to both concentrations of triacontanol but in root tissue low concentration of triacontanol is found to be effective in increasing the acid phosphatase activity as compared to control.

It is clear from results that in both leaf and root tissue of *C. forskohlii* the activity of enzyme acid phosphatase is decreased due to low concentration of BRs but increased due to high concentration of BRs.

In case of CCC sprayed plants, in root tissue, both concentrations of CCC caused increase in enzyme acid phosphatase activity while in leaf tissue CCC decreased the enzyme acid phosphatase activity while both concentrations of ethephon caused increase in enzyme acid phosphatase activity in both leaf and root tissue of *C. forskohlii*. Thus, the activity of enzyme acid phosphatase is found to be more remarkable in root tissue than leaf tissue due to all treatments.
Phosphatase is a widely distributed class of enzymes in all cells of all plant organs and has both intra and extracellular activity. These enzymes are grouped into two types on the basis of hydrolysis of organic phosphates carried out at below or above $P^H$-7 (Barrett-Lennad et al., 1982) as acid phosphatase (ACP) and alkaline phosphatase (ALP) resp. Acid phosphatase hydrolyses organic phosphates in acidic $P^H$ range. Acid phosphatase is widely distributed in higher plants (Parida and Das, 2004).

In view of Miernyk (1992) extracellular ACP exists abundant in roots, plant cell cultures and located within the cell wall or secreted by the root/suspension cells into surrounding root zone or culture media. Lee, (1988) and Lefebvre et al., (1990) stated that extracellular ACP of roots is present chiefly in apical meristem and exterior surface cells of roots and causes hydrolysis and mobilization of Pi from organic phosphates from the soil for plant absorption. According to Duff et al., (1994) being most acidified region, root surfaces are majorly suitable for extracellular ACP. It is the ability of plant to make soil P available for plant absorption. At low P availability, plants secrete root ACP; but plant species differ in secretion ability and enzyme activity (Fukuda et al., 2001). While intracellular plant ACP is also found ubiquitously in plants as in leaves, stems, fruits, and storage tubers as reported by De Leo and Sacher, (1970a and 1970b), Lorenckubis, (1989), Kanellis et al., (1989), Kamenan and Diopoh, (1983), Polya and Wettenhall, (1992) and Nikam, (2007). This intracellular ACP is present in cytosol, vacuoles and plastids and it also helps in release of Pi from organic compounds during seed germination and translocation of Pi from senescent tissue to other plant parts (Lee, (1988) and Duff et al., (1994)). According to Tabaldi et al., (2007) and Mishra and Dubey, (2008) this enzyme play central role in supplement, metabolism of inorganic phosphates for the maintaining cellular metabolism. So, this enzyme activity is a physiological characteristic related to plant efficiency in relation to P acquisition and utilization, and is genetically variable (Machado et al., 2004).

According to Duff et al., (1994) plant ACP shows considerable heterogeneity regarding native molecular mass, subunit structure but majority of ACP exist as monomeric or dimeric glycoproteins with subunit molecular masses of approx. 50-60 KDa. They also stated that plant intracellular ACP shows clear and non-absolute substrate specificity such as phytases (most of seed phytases are active in between $P^H$
phosphoglycolate phosphatase (found as chloroplast localized ACP, active at pH 6.3), 3-PGA phosphatase, PEP and phosphoprotein phosphatase.

According to Besford, (1980) in ‘P’ deficiency, ACP activity in leaf tissues increases to remobilize Pi from vacuoles, plastids to other active plant parts. Mc Lachlan et al., (1987) also found in wheat shoots that ACP activity from leaves was associated with severity of P deficiency symptoms in plants. Acid phosphatase activity may be related to phosphorus use efficiency in shoot tissue because it causes mobilization of Pi from metabolically poor sites such as old senescent leaves and vacuoles to other metabolically active plant parts (Schachtman et al., 1998). So, ACP activity could be considered as a diagnostic criterion for P deficiency and ACP in leaf tissue may be related to phosphorus use efficiency (Duff et al., 1994).

Thus, it is difficult to understand whether stimulated activity of ACP either from root or shoot tissue is a mechanism of plant’s phosphorus use efficiency (hydrolysis of Pi from soil to soluble forms) or merely an indication of phosphorus deficiency (remobilization of Pi from storage to active site).

Lesniak and Ries (1983) studied the influence of foliar spray of triacontanol on corn seedlings (Zea mays L.) and noticed decrease in ACP activity on per mg protein basis in shoot tissue while Kamble and Chavan (2011) noticed slight increase in ACP activity in roots of Vetiver grass (Chrysopogon zizanoides (L.) Roberty) which goes in tune with our observations in C. forskohlii.

Thakur and Thakur (1993) reported increased level of ACP activity in tomato cultivars exposed to moisture deficit which was found reduced when those cultivars were treated with traicontanol and mixtalol which indicates that triacontanol reduces activity of ACP which was found true in C. forskohlii.

In C. forskohlii plants it can be seen that there is increased level of P content in all plant parts and also increased ATPase activity in both leaf and root tissue in response to triacontanol, it can be correlated with low activity of ACP in response to triacontanol but exact reason behind the low activity of ACP due to triacontanol cannot be explained. Whether it is because of availability of higher P content or higher activity of enzyme ATPase or higher P uptake. Again, according to Duff et al., 1994 acid phosphatase expression is regulated by a variety of developmental and environmental factors. Some of the authors also observed inconsistent results for other plant species such as inverse relationships between ACP activity and root P contents.
have been found for sorghum by Furlani et al., (1984), for white clover by Dracup et al., (1984), and common beans (Helal, 1990); between leaf and root phosphatase activity and P deficiency in wheat plants (Mc Lachlan & De Marco, (1982) and Machado et al., 2004).

Kathju and Tewari (1970) found decreased activity of ACP enzyme in cotyledons, plumule, radical and seedling axis in seedlings of guar (Cyamopsis tetragonoloba) subjected to seed soaking treatment at 2000ppm solution of CCC.

Muthukrishnan et al., (1974) studied the effect of foliar spray of ethephon on tomato and sweet potato shoot tissue and observed increased activity of enzyme acid phosphatase along with increase in ethephon concentration or dosage (100ppm to 1000ppm) which goes in agreement with our findings.

Review of evidences suggests that increased activity of ACP enzyme due to different concentrations of ethephon might be due to de novo synthesis of enzyme or due to an activation of ACP. As per the view expressed by Muthukrishnan et al., (1974) this increase in activity of enzyme acid phosphatase might be due to increased rate of respiration triggered by ethylene released by ethephon treatment. This increase demands more breakdown of ATP or translocation of phosphates to metabolically active sites which causes inhibition of growth of plant. This is also found true in C. forskohlii.

Thus, it can be concluded that increased activity of enzyme ACP in both leaf and root tissue induced due to PGRs at different concentration will be beneficial to plant in increasing P acquisition and utilization and will surely enhance P metabolism and finally growth and productivity.

4. Alkaline Phosphatase (ALP) (E.C 3.1.3.1)

Effect of various plant growth regulators on alkaline phosphatase activity in both leaf and root tissue of C. forskohlii can be seen in Fig. 82 and Table No. 30.

Alkaline phosphatase belongs to group of phosphatase enzyme which catalyses hydrolysis of organic phosphates in a range of pH 7. (Barrette-Lennard et al., 1982). Alkaline phosphatases in plants play a crucial role in the provision and metabolism of inorganic phosphate for the maintenance of cellular metabolism (Tabaldi et al., 2007; Mishra and Dubey, 2008)

It is evident from the Fig. 82 that due to low concentration of triacontanol there is decrease and slight change in the activity of enzyme alkaline phosphatase in
both leaf and root tissues while due to high concentration of triacontanol there is increase in enzyme activity as compared to control in both the tissues of *C. forskohlii*.

Due to low concentration of BRs the activity of enzyme alkaline phosphatase is increased in leaf and decreased in root but opposite trend is seen due to high concentration of BRs as compared to control. The effect of CCC on alkaline phosphatase enzyme activity from leaf and root tissue of *C. forskohlii* can be seen that due to both concentrations of CCC there is increase in enzyme activity in both leaf and root tissue but increase is remarkable in leaf tissue.

Lower dose of ethephon caused increase in activity of enzyme alkaline phosphatase while higher dose of ethephon caused decrease in enzyme activity in both leaf and root tissues of *C. forskohlii* as compared to control.

The review of literature on phosphatases in plants clearly reveals that in comparison with acid phosphatases there is more scarcity in our knowledge regarding alkaline phosphatase (ALP). Thus, our knowledge about this enzyme system is very limited or its role is obscure. According to Stadtman (1961) ALP (E.C 3.1.3.1) functions at higher pH level (>8) on various phosphate esters. It is reported by Duff *et al.*, (1994) that plant alkaline phosphatase shows absolute substrate specificity and examples of such ALP are fructose 1-6 bisphosphatase and sucrose phosphatases which are involved in sucrose metabolism. ALPs are reported to occur in low quantities. Higher dilution causes unstable activities while pure state of this enzyme subjected to surface denaturation. It is difficult to isolate but Cohen (1989) studied its properties, tendency of structure, regulation and action in multiple forms. In case of plants the exact role of ALP is not fairly understood. As mentioned by Vemuri *et al.*, (2010), one of the role of ALP might be providing inorganic phosphate for metabolic excretory and secretory functions in plants. Ross and Ely (1951) put forth that ALP found in fixed plant tissues of onion and corn nuclei hydrolyses metaphosphates to orthophosphate. They also compared it with the ALP activity in rat tissues and proposed that ALP enzyme from both plant and animal cell requires magnesium ions for hydrolysis and fluoride ions acts as its inhibitors. Coleman and Gettins (1983) reported ALP as a Zn (II) metalloenzyme which causes hydrolysis of phosphate monoesters and formation of phosphoseryl as an intermediate.

Kieleczawa *et al.*, (1992) performed isolation and characterization of ALP from pea thylakoids. They estimated the molecular mass of ALP from pea thylakoid
as 51.5 KD and active at pH 8.0. They reported that it required Mg$^{2+}$ but wasn’t dependent on this ion and showed sensitivity to fluoride. Ganjewla et al., (2010) revealed that ALP also participates in the breaking down and mobilization of starch and sucrose for biosynthesis of essential oil in lemongrass (*Cymbopogon flexuosus* Steud.) Wats.

Lesniak and Ries (1983) noticed that activity of enzyme alkaline phosphatase in corn seedling tissue decreased slightly on per mg protein basis under the influence of triacontanol treatment.

There are very less or no reports regarding the effect of triacontanol, brassinosteroides, CCC and ethephon on activity of enzyme ALP. It is really difficult to predict the role of these PGRs in alteration of the activity of enzyme ALP and thus in P metabolism in case of *C. forskohlii*.

Altered activity of ALPase due to PGRs application might be attributed to chemical and compositional changes triggered by PGRs application. Thus, it can be concluded that increased activity of ALPase due to PGRs will be beneficial to increased P metabolism.

5. Inorganic pyrophosphatase

The effect of low and high concentration of plant growth regulators like triacontanol/vipul, brassinosteroids, CCC and ethephon on the activity of enzyme inorganic pyrophosphatase from leaf and root tissue of *C. forskohlii* can be seen from Fig. 83 and Table No. 31.

It can be clearly seen from all the results that the activity of enzyme inorganic pyrophosphatase is remarkably decreased in both leaf and root tissue subjected to both concentrations of all the PGRs as compared to control. Also, the inorganic pyrophosphatase activity is found more in leaf tissue than root tissue in all the treatments including control.

Inorganic pyrophosphatase enzyme (PPase) (E.C.3.6.1.1) is present ubiquitously in nature that catalyzes the conversion of inorganic pyrophosphate (PPI) into two orthophosphates (Pi) in the presence of water and divalent metal cations (PPI + H$_2$O $\rightarrow$2Pi). This enzyme is essential for plant life (Sonnewald (1992), Sivula et al., (1999) by providing a thermodynamic pull for biosynthetic reactions (Kornberg, 1957). Pyrophosphate is produced as a byproduct of ATP hydrolysis and it is generated during many cellular processes specially during the synthesis of proteins.
DNA, RNA, starch and cellulose. (Grzechowiak et al., 2013). It is necessary to remove inorganic pyrophosphate produced as a byproduct during biopolymer synthesis to maintain the direction of the reaction and thus to drive anabolism (Kornberg, 1957).

Soluble PPases from various organisms have been studied and divided into two families, Family I and II. Family I include most of the currently known eukaryotic and prokaryotic PPases, representing PPases from *Saccharomyces cerevisiae* (Heilkinheimo et al., 1996 and 2001) and *E. coli* (Harutyunyan et al., 1997; Samygina et al., 2007) and *A. thaliana* (Grzechowiak et al., 2013).

Family II includes only prokaryotic representatives such as PPases from *Bacillus subtilis* and putative PPases from four other bacterial strains (Shintani et al., 1998, Sivula et al., 1999 and Rantanen et al., 2007). Both enzyme PPase families have same function but have completely different three-dimensional structure and different biochemical properties. The two families do not show any sequence similarity to each other (Shintani et al., (1998) and Sivula et al., (1999)). In addition to the soluble cytoplasmic PPases, plants and certain bacteria have much larger membrane bound PPase, which functions as a reversible proton pump but don't have similarity with both Family I and II of PPases (Serrano et al., 2004, Grzechowiak et al., 2013). Plant PPases acts as monomers with 25 KDa molecular mass (Grzechowiak et al., 2013).

According to Bucke (1970) an alkaline inorganic pyrophosphatase (E C 3.6.1.1) has been detected in a range of species and is associated partly with the chloroplast fraction. It has an absolute requirement for Mg $^{2+}$ ions and active at pH optimum of 8.3. Its activity is inhibited by orthophosphate, arsenate and EDTA but remains unaffected by -SH group inhibitors.

Maeshima (1991) also reported inorganic pyrophosphatase from vacuolar membrane which catalyzes both the hydrolysis of Ppi (inorganic pyrophosphate) and also carry out electrogenic translocation of protons into the vacuoles. They isolated and purified this vacuolar IPPase from mung bean vacuoles and compared this with other forms of inorganic IPPases. They also reported absolute requirement of Mg $^{2+}$ ions and Ca $^{2+}$ ions sensitivity for vacuolar IPPase enzyme activity but found Cd $^{2+}$, Co$^{2+}$, Cu$^{2+}$ as an inhibitors. Baltscheffsky et al., (1999) had reviewed about a tightly
membrane bound family of various H⁺ - proton pumping inorganic pyrophosphatase enzymes from various prokaryotes and eukaryotes.

Mukherjee and Pal (1982) surveyed around 51 plant species belonging to twenty-two families for the estimation of activity of enzyme IPPase from leaves and its distribution. They reported that different plant leaves show different level of alkaline IPPase activity but among the plant leaves they studied, the high level of activity was found in leaves of *Amaranthus blitum* and *Amaranthus gangeticus* of Amaranthaceae family and have optimum pH at 9.0. Soluble alkaline inorganic pyrophosphatases have been characterized in plant extracts and some of them have been purified (Mukherjee and Pal (1983), Kumar and Singh (1983)). Based on the subcellular fractionation studies of this enzyme carried out by Gross and Rees (1986) and Weiner et al. (1987), most of these alkaline IPPase activities corresponds to plastidic isoforms.

Kathju and Tewari (1970) found that activity of IPPase was inhibited by chlormequat or CCC in both cotyledons, plumule, radicle and seedling axis in guar (*Cyamopsis tetragonoloba*) in response to seed soaking treatments in a solution of chlormequat (2000ppm). The role of triacontanol, BRs and ethephon on the activity of enzyme IPPase is unclear.

Thus, it can be concluded that all the PGRs negatively affected the activity of enzyme IPPase indicating altered mode of P metabolism. This might be due to change in internal and external pH induced by PGRs by changing membrane permeability and alteration in biochemical activities triggered by PGRs.

**I. Mineral nutrition**

*Influence of foliar spray of PGRs on inorganic constituents*

**1. Potassium content**

The influence of foliar spray of triacontanol, brassinosteroids, CCC and ethephon on the Potassium content of leaf, root and stem of *Coleus forskohlii* is depicted in Table No. 32 and Fig. 84. It can be revealed from the results that level of potassium content is different for different concentrations of PGRs. Potassium content is seen increased in all plant parts due to both concentrations of triacontanol treatment as compared to control however highest Potassium content is found in stem than leaf than root and low dose of triacontanol is found more effective in 'K' content accumulation. In case of BRs treated plants, high dose of BRs shows no significant
variation in potassium content than control whereas low concentration of BRs shows higher potassium content in all plant parts as compared to control. Higher potassium accumulation in all plant parts is observed due to high dose CCC whereas low dose shows potassium content at par with control. In case of ethephon treatment higher potassium content is found in all plant parts due to both concentrations.

Potassium being an indispensable univalent cation mineral element for plant life. Potassium is among the most abundant cations in higher plants and is crucial for plant nutrition, growth, tropisms, enzyme homeostasis and osmoregulation (Epstein et al., 1963 and Schroeder et al., 1994). While K does not become a part of the chemical structure of plants, it plays many important regulatory roles in plant growth and development. According to Kochian (2000), potassium plays a role in innumerable cellular and whole plant functions such as an osmoticum for cellular growth and stomatal function, balancing the charges of diffusible and non-diffusible anions, activating more than 50 plant enzymes, and participating in numerous metabolic processes including photosynthesis, oxidative metabolisms and protein synthesis. According to Marschner, (1986) another key function of K$^+$ is the activation of membrane bound ATPases, which require Mg$^{2+}$ but are further stimulated by K$^+$. Actual soil concentrations of this mineral vary widely, ranging from 0.04 to 3% (Sparks and Huang, 1985). The concentrations of K$^+$ in soil solution [K$^+$] are low, in the range of only 0.1–6 mM depending upon the soil (Adams, 1971), still plants accumulate appreciable quantities of this element. Potassium is the dominant counter ion to light-induced H+ flux across the thylakoid membrane and the establishment of the trans membrane pH gradient necessary for the synthesis of ATP (photophosphorylation) in analogy to ATP synthesis in mitochondria (Laulchli and Pfluger (1978)). As suggested by Collins and Duke, (1981) high potassium concentrations in the sieve tubes also substantially contribute to the volume flow rates and thus enhance the photosynthate transport from source to sink. According to Mengel and Kirkby, (1982), potassium in the plant is very mobile. Its main transport direction is towards the meristematic tissues and potassium mainly acts as a charge carrier of high mobility that forms only weak complexes so that it is readily exchangeable (Wyn Jones et al., 1979). As opined by Marschner et al., (1996) the main reason behind the high cycling of potassium between shoot and roots is its involvement in photo assimilate transport from source to sink.
According to Jeschke and Wolf (1988) almost all the K required for plant root growth takes place by potassium re-translocation via the phloem. According to Kochian (2000), in the plant system exists an integrated and most regulative K transport system at different sites along the long distance pathway which allow the plant to direct the partitioning and circulation of K\(^+\) throughout the plant body. This integrated system of K\(^+\) transporters probably plays a central role in plant growth and development and also directs the allocation of mineral nutrients depending upon changes in availability and demand of nutrients. Although, K\(^+\) is not a basic component of protein, carbohydrates or fats, it plays an important role in their metabolism. Potassium stimulates net photosynthetic activity of a given leaf area and increases the translocation of photosynthates to the tuberous roots. It also plays an important role in metabolism of proteins, carbohydrates and fats, even though it is not a structural part of those molecules. Lalitha et al., (1997) reported an increase in lipid, crude protein, starch and ascorbic acid content in potato tubers in response to potassium application as noticed in some experiments. In tuberous crop like potato, potassium element is reported to play a role directly or indirectly in several physiological and biochemical processes and influences the tuber yield, growth and imparting of resistance against drought, frost stress and pests (Singh et al., 2001). In Cassava plant also, Nguyen Huu Hy et al., (1998) reported an increase in starch content with increasing applications of K. As opined by Herlihy and Carroll, (1969) Potassium has the greatest influence on potato tuber size and increases the proportion of large tubers. The optimum K content is about 22 to 31 mg g\(^{-1}\) of tubers on dry matter basis. Potassium has been identified as a necessary element for rapid translocation of nutrients at a later stage of tuberization and bulking. So, in present investigation also status of K content under the influence of PGRs is worth studying being coleus, a tuberous crop. Uptake of K\(^+\) from soils into roots is largely mediated by high-affinity K\(^+\) uptake (\(K_{\text{m}}\approx10–40 \ \text{μM}\)) (Newman et al., 1987; Maathuis and Sanders 1993). Supply of K is directly proportional to its translocation rate. Translocation of nitrates, phosphates, calcium, magnesium and amino acids is regulated by K\(^+\) supply. During plant growth and development, potassium activates at least 60 different enzymes (Evans and Sorger, 1966). Physical shape of the enzyme is changed by the presence of K in such a way that active site gets exposed for proceeding reaction.
As suggested by Qi and Spalding, (2004) uptake of potassium is inhibited by presence of high levels of monovalent cations such as Na$^+$ and NH$_4^+$. Potassium plays important role in osmoregulation and preferential uptake of potassium is noticed in many halophytes. Guard cells appear to have a high affinity for potassium as opening and closing of stomata needs K$^+$ concentration gradient (Fischer and Hsiao, 1968). According to Epstein, (1963) the K absorption by roots is biphasic. The uptake and accumulation of potassium varies during different phases of plant growth and according to the plant organ (Dastur and Bhatt (1965); Leidi et al., (2004); Nikam, (2007). As presumed by Epstein (1972), 1% potassium on a dry weight basis is adequate for plant growth while, Munson (1998) proposed as 1.15 to 5.50% potassium concentration on dry weight species as sufficient or normal level for the plant life.

In case of a tuber crop sugarbeet, Draycott (1972) recorded that average concentration of potassium in dried tops at harvest was about 3% whereas, the concentration in roots is 0.77%. In the leaves of Chlorophytum borivilianum the potassium content ranges from 0.88 to 2.86% during different growth stages (Nikam, 2007). In present studies in C. forskohlii K$^+$ content is about 1% in leaves, 0.74% in roots while 1% in stem part at the harvesting stage in control plants. The effect of plant growth regulators on potassium uptake and concentration in the plant is recorded by many workers.

Singletary and Foy (1980) reported that a foliar application of triacontanol did not significantly alter growth or K content in soybean. Ries et al., (1993) also reported that TRIA application stimulates K+, Ca 2+, and Mg 2+ accumulation in tomato, maize, and cucumber seedlings by eliciting a secondary messenger, L (+) adenosine. Naeem (2009) reported significant improvement in K content of leaves in hyacinth bean (Lablab purpureus L.) by foliar application of Triacontanol. The concentrations of these leaf-nutrients were found significantly higher at 106 M of TRIA over the control. In pea var. Arkel, triacontanol foliar spray increased the K uptake in both grain straw part in response to triacontanol (Sharma et al., (2006). Idrees et al., (2010) in coriander plant leaves found no enhancement in K content of leaf due to a foliar spray of 10-6 Triacontanol + 10-6 GA. Hashmi et al., (2011) reported higher K content due to triacontanol at 10$^{-6}$M in Basil leaves. Alam et al., (2012) reported higher potassium content in Catharanthus roseus leaves due to triacontanol and HBR.
RESULTS AND DISCUSSION

at concentration of $10^{-7}$M. Singh et al., (2012) in ginger (Zingiber officinale Rosc.) noticed enhanced potassium concentrations in both above and underground plant parts due to triacontanol at 10–6 M concentration. Rani et al., (2013) reported elevated uptake of K in okra (Abelmoschus esculentus L. Moench) plants with triacontanol @4000 ppm. In Bougainvillea glabra leaves increased content of K content is reported due to 0.5 and 1.0mg/L triacontanol treatments by Khandaker et al., (2013). The enhancement in leaf K contents due to triacontanol application is also reported by Chaudhary et al., (2006); Khan et al., (2009), Naeem et al., (2011), Hashmi et al., (2011) and Khan et al., (2014) on Capsicum annuum, tomato (Solanum lycopersicum L.), Mentha arvensis and Ocimum basilicum and lemon grass (Cymbopogon flexuosus (Steud.) Wats.) respectively. All these findings are in agreement with present findings. Perveen et al., (2014) studied influence of foliar spray of triacontanol on two wheat cultivars under saline condition. They reported that in both cultivars of wheat, K content is decreased due to salinity stress in both shoot and root tissue. However, application of triacontanol enhanced K content in both stressed and unstressed conditions. It is well established fact that triacontanol activates various membrane bound enzymes mainly phosphorylases and bring about a cascade of physiological events leading increase in growth.

Purbey and Sen (2007) recorded increased uptake of K content in both seeds and straw part of Fenugreek (Trigonella Foenum-Graecum L.) due to BRs Alam et al., (2012) reported higher potassium content in Catharanthus roseus leaves due to application of HBR at concentration of $10^{-7}$M. Ali et al., (2006) studied influence of 24-epiBL on two wheat cultivars grown in saline conditions and they reported that Foliar spray of brassinosteroids reduced leaf Na+, and enhanced leaf K+, leaf Ca2+, and K+/Na+ ratios. They concluded that brassinosteroids improved the Ca2+/Na+ and K+/Na+ ratios of the wheat cultivars by enhancing Ca2+ and K+ uptake, and reducing Na+ uptake, which may have contributed to enhance salt tolerance of both wheat cultivars. Vardhini et al., (2012) found that 24-epibrassinolide and 28-homobrassinolide stimulated the growth of radish roots along with increased levels potassium. Surender et al., (2013) in black gram found enhanced K content in leaves in response to the basal application of nitrogen 25 kg per hectare with foliar spray of urea 2% and 0.1ppm brassinolide treatment.
Higher potassium content enhances turgor maintenance and active growth of cells leading to better plant growth. Therefore BR, besides regulating various metabolic activities, also enhances water and nutrient uptake resulting in increased protein synthesis, growth and finally yield. In contrast to positive effect of BRs on nutrient uptake, the findings of Hayat et al. (2000) states that BRs did not have a significant effect on accumulation of K+ in mustard plants. There are several evidences in support of positive role of BRs in stress tolerance such as exogenous application of BRs induces abiotic stress tolerance in plants, e.g., exogenous application of BRs increased salinity tolerance in rice (Anuradha and Rao, 2001), tomato (Prakash et al., 1999), and in chickpea (Ali et al., 2007). Karlidag et al., (2011) studied effect of brassinosteroids 24-epibrassinolide (24-EBL) (0.0, 0.5, and 1 μM) by foliar application and recorded that 24-EBL (1 μM) application under saline condition significantly increased shoot and root dry matter of strawberry along with increased Macro-micro element content of leaf and root with increase in 24-EBL except for Na under salinity stress.

Wang et al., (2010) in potatoes (Solanum tuberosum L.) studied the effect of exogenous chloro choline chloride (CCC) foliar treatment significantly increased the contents of P, K, Ca, Mg, Fe, Mn, Zn and Cu in potato leaves. It is reported by Singh et al., (1987) that K level in the stems of soybean (Glycine max) increased on application of CCC grown under greenhouse conditions. Foliar application of chloro choline chloride at the rate of 300ppm at the flower initiation stage increased the uptake of N, P and K in soybean plants. Kutwal (1989) observed that pre-sowing soaking treatment of groundnut seeds with CCC and kinetin increased potassium content in all plant parts namely leaf, stem and root. According to Guroo and Patel (1993), K content in Brassica juncea was stimulated due to CCC application. CCC pretreatment decreases potassium content in leaves of sugarcane (Patil, 1995). Foliar application of CCC (400 ppm) stimulated the uptake of potassium in Brassica juncea (Lone, 2001). Zeid and EI-Semary (2001) reported application of CCC increased K content in two varieties of maize under drought stress. Khuankaew et al., (2009) tried to study the effect of ethephon foliar application at 100, 300 Ind 500 mg/L concentration on rhizomatous plant Curcuma alismatifolia Gagnep. They noticed reduced K content in aboveground plant part and increased K content in underground plant part. In aboveground plant parts K content decreased with increased ethephon.
concentration. An opposite trend was found in underground plant parts of Curcuma. In present study K content is increased in all plant part due to high concentration.

It can be concluded that in the present investigation the potassium content is in range of optimum concentration as indicated by Jayal and Kehar (1962) in Mulberry and Munson (1998). It is well documented that hormone application increases physiological and metabolic activities of the plant as a result of which, there might be more uptake of nutrients by plants from the soil (Nickell, 1982). Overall increased level of potassium in all plant parts in response to all PGRs will be surely helpful to plant in efficient nutrient uptake, increased photosynthesis and other several metabolic activities leading to increased growth of the plant and all the other yield attributes.

2. Calcium content

The effect of foliar treatment of PGRs, triacontanol, brassinosteroids, CCC and ethephon on the Potassium content of leaf, root and stem of *Coleus forskohlii* is depicted in Table No. 33 and Fig. 85. It can be revealed from the figure that in triacontanol and ethephon treated plants, the calcium Ca\(^{2+}\) content is increased in all plant parts namely leaf, root and stem due to both concentrations as compared to control. High concentration of BRs caused increase in Ca\(^{2+}\) content in all plant parts while at low concentration of BRs it is at par with the control. Calcium content is increased in leaf and stem but slightly decreased in root by the both concentrations of CCC as compared to control. The overall accumulation of calcium is found in the order as leaf>stem>root part in all treatments including control.

Calcium is a divalent cation which is a large secondary nutrient that is critical for crop development. It is needed in large amounts by all plants for the formation of cell walls and cell membranes, and it also plays a vital role in soil structure. Calcium uptake by the plant is passive and does not require energy input. Root hairs are main site of uptake of minerals from the soil bulk and Ca\(^{2+}\) directly enters into the root hair passively (Shear and Faust (1970). However, there is conflict regarding this as absorption of calcium is found more at normal temperature and also found inhibited by 2, 4 - nitrophenol as opined by Yang *et al.*, (2003). Calcium exists in plant tissue as free Ca\(^{2+}\) and or as Ca\(^{2+}\) adsorbed to in diffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It is also present in the form of Ca-oxalates, carbonates and phosphates (Mengel and Kirkby, 1982).
RESULTS AND DISCUSSION

Being most indispensable constituent of cell wall and membrane, calcium is required for cell elongation and cell division (Burstron, 1968). So as suggested by Mengel and Kirkby, (1982) Ca^{2+} plays an important role in nutrient ion uptake and in membrane permeability which is of extreme importance in any metabolic process. In general, Ca^{2+} stimulates membrane bound enzymes (Rensing and Cornelius, 1980), particularly ATPases at the plasma membrane of roots of certain plant species (Kuiper et al., 1974). Cytosolic calcium (Ca^{2+}) plays a pivotal role as a second messenger in plant signal transduction through a calcium binding protein named 'calmodulin' and calcium dependent protein kinases. According to Wyn Jones and Lunt, (1967) calcium increases the activity of only few enzymes such as α- amylase, phospholipases and ATPases. Rodenburg et al., (1994) reported that Ca2+ acts as a co-factor for Alpha amylase activity. The action of phytochrome, the photo morphogenetic pigment is also found to be partly mediated through calcium (Mustilli and Bowler, 1997). During plant growth, development and adaptation to environmental changes, calcium acts as a second messenger (White and Broadley, 2003). This role of calcium is expressed through significant increase in cytosolic Ca^{2+} level in response to hormonal or environmental signals which is mediated through the action of calcium activated protein kinases and calcium binding protein calmodulin. The plant signals like touch, wind, temperature shock, fungal elicitors, wounding, oxidative stress, anaerobiosis, ABA, osmotic stress and mineral nutrition are supposed to be transduced through calcium. The calcium content of plants varies between 0.1 and > 5.0% of dry weight depending on the growing conditions, plant species and plant organ (Marschner, 1986). As suggested by Tsujita et al., (1978) and Hocking et al., (1980) calcium is relatively immobile element and its accumulation in the senescent old leaves in contrast to young and mature leaves is well established. The sufficient or normal calcium level for the growth of the plant is found to be about 1-4% (Munson, 1998). Draycott (1972) recorded a value of 1% calcium in the tops and 0.24% calcium in the tuberous roots at the time of harvest in sugar beet. In the present investigation in Coleus forskohlii, calcium content found leaf is about 1.8%, in root about 0.9% and in stem 1.5% in control plants. There are few attempts made by some workers to see the effect of triacontanol on Ca content in plants.

As observed in tomato, maize, and cucumber seedlings treatment with TRIA increases L (+)-adenosine levels (Ries and Wert, 1988), and picomole concentrations
of (+)-adenosine can enhance Ca\(^{2+}\), Mg\(^{2+}\), and K\(^{+}\) concentrations (Ries et al., 1993). Naeem et al. (2009) reported significant improvement in K content of leaves in hyacinth bean (*Lablab purpureus* L.) by foliar application of Triacontanol. Khan et al., (2009) in tomato (*Solanum lycopersicum* L.) plant also recorded enhanced Ca content. Naeem et al., (2010) in Coffee senna (*Senna occidentalis*) noticed increased Ca content in response to triacontanol. According to Ries et al., (1993), exogenous application of TRIA as a spray, accelerates the influx of Ca\(^{2+}\) into the cytoplasm (which could bind to receptor proteins such as calmodulin as proposed by Evans et al., (1991), while increased uptake of K\(^{+}\) might be attributed to increased competition at the plasma membrane sites (Epstein, 1966) which regulates the growth processes in the face of certain external stimuli (Ries et al., 1993). These findings are in agreement with present findings. Perveen et al., (2014) studied influence of foliar spray of triacontanol on two wheat cultivars under saline condition. They reported that in both cultivars of wheat, Ca content is decreased due to salinity stress in both shoot and root tissue. However, application of triacontanol enhanced Ca content in both stressed and unstressed conditions.

Triacontanol is considered to play a role in water uptake, cell elongation, cell division, and permeability of membranes (Hangarter et al., 1978). Triacontanol stimulated mineral uptake and distribution in Coleus plant which will surely help plant in healthy mineral nutrition and certainly helpful for enhanced growth and yield of plant.

Ali et al., (2006) studied influence of 24-epiBL on two wheat cultivars grown in saline conditions and they reported that foliar spray of brassinosterioids decreased the leaf Na\(^{+}\), and enhanced leaf K\(^{+}\), Ca\(^{2+}\), and K\(^{+}\)/Na\(^{+}\) ratios. Vardhini et al., (2012) found that 24-epibrassinolide and 28-homobrassinolide stimulated the growth of radish roots along with increased levels minerals like calcium. In wheat *Triticum aestivum* L. subjected to saline stress, the effect of 24-epiBL was studied by Shahbaz and Ashraf (2007). They reported that application of 24-EBL enhanced plant biomass under both saline and non-saline conditions, but exerted a non-significant effect on leaf Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), and Cl\(^{-}\) contents or K\(^{+}\)/Na\(^{+}\) ratios while in roots a similar pattern was observed but only for root K\(^{+}\) and K\(^{+}\)/Na\(^{+}\) ratios.

According to Gabr et al., (1985) treatment of CCC leaded to increase in Ca content in tomato plant. The pre-sowing soaking treatment of groundnut with CCC
RESULTS AND DISCUSSION

reported to stimulate calcium content in leaf tissue as noticed by Kutwal, (1989). Patil (1995) observed pre-sowing soaking treatment with D.W., CCC and ethephon increases calcium status in leaf tissue of sugarcane. Application of CCC led to increase in Ca concentration under drought stress in two differentially drought tolerant varieties of maize (Zeid and EI -Semary, 2001). The effect of CCC on the translocation of Ca, Mg and K was studied by Tumal et al. (2007). The evidences regarding ethephon effect on Ca content is scarce.

Calcium acts as a second messenger during plant growth, development and adaptation to environmental changes, (White and Broadley, 2003). Calcium is important for membrane permeability and integrity. Enhanced calcium content will certainly play a key role in activating various enzymes of several metabolic process and will boost the overall growth and health of the Coleus plant.

3. Magnesium content

The influence of foliar application of plant growth regulators triacontanol, brassinosteroids, CCC and ethephon on the Calcium content of leaf, root and stem of Coleus forskohlii is depicted in Table No. 34 and Fig. 86. It can be revealed from the figure that in triacontanol treated plants, magnesium Mg\(^{2+}\) content is increased in leaf due to high concentration while decreased due to low dosage whereas in both root and stem part, Mg\(^{2+}\) is at par with the control due to both doses of triacontanol. In response to both concentrations of BRs, the content of Mg\(^{2+}\) is decreased in all plant parts as compared to control. Similar reduction in Mg\(^{2+}\) is also visible in all plant parts due to both concentrations of CCC. In case of ethephon treated plants, Mg\(^{2+}\) is decreased in all plant parts by low dosage while increased only in root and stem part due to high concentration of ethephon as compared to control.

As opined by Marschner, (1986) Magnesium is most important small, mobile, strongly electropositive and abundant divalent element in plant. It is an indispensable constituent of chlorophylls occupying central position in tetra pyrrol porphyrin structure of chlorophylls. The activities of photosynthetic enzymes such as RUBISCO, fructose 1-6 bisphosphatase and phosphoribulokinase are regulated by changes in concentration of Mg. The assembly of ribosomal subunit depends upon this specific element. According to Marschner (1986), the functions of Mg\(^{2+}\) in plants are related to its mobility within the cells, its capacity to interact with strongly nucleophilic ligands (e.g., phosphoryl groups) through ionic binding and to acts as a
RESULTS AND DISCUSSION

bridging element and/or to form complexes of different stabilities. The enzymes belonging to subgroup kinase have an absolute requirement of magnesium and formation of ADP-Mg complex is a pre-requisite in such reactions. Kinases in general and protein kinases in particular play an important role in different facets of cellular metabolism.

Uptake of Mg takes place both actively and passively (Russel and Clarkson, 1976 and Pitman, 1976). Magnesium is generally taken up by plants in lower quantities than Ca\(^{2+}\) or K\(^+\). The content of Mg\(^{2+}\) in plant tissues is usually in the order of ~ 0.5 % of the dry weight of the vegetative parts (Mengel and Kirkby, (1982) and Marschner, 1986) while according to (Munson, 1998) the sufficient or normal magnesium level in the plant tissue is estimated to be 0.25 to 1% on dry weight basis. In the chloroplast and cytoplasm high concentrations of Mg and K are required to maintain a high pH in between 6.5 to 7.5 as compared to much lower vacuolar pH of 5 to 6. Magnesium is a major cation in chloroplast so its deficiency is major factor behind leaf chlorosis. As suggested by Cakmak et al., (1994) and Hermans et al., (2006), Mg plays crucial roles in several processes like in partitioning of carbohydrates and dry matter production between roots and shoots, photosynthetic CO\(_2\) fixation and ROS formation and related photooxidative damage. So being vital element in growth and nourishment of plants, analysis of this mineral element status in the light of the influence of PGRs is worth.

As observed by Ries et al., (1993) in tomato, maize, and cucumber seedlings triacontanol increases L (+)-adenosine levels (Ries and Wert, 1988), and even picomole concentrations of (+)-adenosine can enhance Ca\(^2+\), Mg\(^2+\), and K\(^+\) concentrations. Mg increased by foliar spray of CCC in tomato plant (Gabr et al., 1985). Kutwal (1989) reported in groundnut pre-sowing soaking treatment with CCC increases Mg content of water stressed groundnut leaf, stem and root. Wang et al., (2010) in potatoes (Solanum tuberosum L.) studied the effect of exogenous chlorocholine chloride (CCC) foliar treatment significantly increased the contents of P, K, Ca, Mg, Fe, Mn, Zn and Cu in potato leaves. Stimulation in Mg content of sugarcane leaves pre-treated with CCC was reported by Patil (1995). It is noticed by Patil (2011) that CCC caused elevation in magnesium content of leaf of S. glauca when applied as a foliar spray.
RESULTS AND DISCUSSION

In the present investigation increased Mg content in leaf due to triacontanol and in root and stem due to all other PGRs as compared to all treatment will surely help plant in photosynthesis and other metabolic events due to scarcity of evidences the fate of decreased Mg content due to PGRs is unclear.

4. Influence of foliar spray of PGRs on micronutrients content of *Coleus forskohlii*

a. Iron

The influence of foliar spray of plant growth regulators triacontanol, brassinosteroids, CCC and ethephon on the Iron (Fe) content of leaf, root and stem of *Coleus forskohlii* is depicted in Table No. 35 and Fig. 87. It can be revealed from the figure that Fe content in leaf, root and stem part in response to all PGRs do not exhibit any trend. It is varied depending upon concentration of PGRs and part of plant. To summarize the effect of PGRs on Fe content, significant increase in root Fe content is noticed due to low doses of triacontanol, CCC and high dosage of BRs while in case of stem part no significant decrease or increase is seen due any concentration of any PGR tried except low dose of triacontanol it significantly increased. In case of leaf, Fe content is either at par with control or decreased due to all PGRs except for high dose of ethephon it is distinctly enhanced as compared to all treatments.

Iron is very crucial micronutrients involved in various metabolic processes in cell. It is important constituent of heme essential for different proteins like cytochrome and iron sulphur proteins ferredoxin with significant role in electron transport chain during respiration and photosynthesis. It has been reported that in the leaf 80% of Fe is located in the chloroplasts (Marschner, 1995). The number of antioxidant reactions carried out by enzymes like catalase, peroxidase cytochrome oxidase is activated by Fe$^{2+}$. Iron is a constituent of nitrogenase enzyme system. Iron element is a requisite for action of aconitase in the mitochondria and superoxide dismutase (Fe-SOD) in plastids. Enzymes nitrate reductase contains two metal ions, iron and manganese in each subunit (Campbell, 1999). Iron is also constituent of nitrogenase enzyme system in legume nodules. According to Kochian (2000), in plants, there are two definite strategies to solubilize and absorb iron from the soil plants because in soil solution iron exists in oxidized form so it should be first reduced (Fe$^{3+}$ to Fe$^{2+}$). Firstly, some plants (dicots) reduce ferric chelates at the outer surface of root, cell plasma membranes and absorb ferrous ions produced,
RESULTS AND DISCUSSION

whereas, others (grasses) excretes specific low molecular weight organic compounds phytosiderophores having high affinity for Fe$^{3+}$ which solubilize ferric ions and make them available for absorption. Once absorbed iron is transported and redistributed within the plants. As per the view of Stephan and Scholz (1993) nicotinamide is a mediator of iron transport within phloem tissue.

Srivastava and Sharma (1990) recorded that in opium poppy (*Papaver Somniferum* L.) the concentration of Fe is enhanced due to triacontanol at 0.01 and 0.1 mg/l of triacontanol. Miri *et al.*, (2015) studied the effect of Alfalfa plant extract on growth and uptake of micronutrients in sorghum. Their study reported that alfalfa extract, because of containing significant amounts of essential and beneficial nutrients for plant growth and likely growth stimulants (such as Triacontanol), can increase dry weight and uptake of micronutrients (Fe, Zn, Mn and Cu) in sorghum.

Vardhini *et al.*, (2012) found that EBR and HBR stimulated the growth of radish roots along with increased levels of iron and sodium. Similar high level of Fe is seen in roots in present plant. Wang *et al.*, (2010) in potatoes (*Solanum tuberosum* L.) studied the effect of exogenous chlorocholine chloride (CCC) foliar treatment increased the contents of P, K, Ca, Mg, Fe, Mn, Zn and Cu in potato leaves. In the present investigation iron contents are equivalent to control where as in root there is high accumulation of Fe which might be attributed to increased nitrogen metabolism and antioxidant system as Fe is important co factor for many antioxidant enzymes.

**b. Manganese**

The changes in Manganese (Mn) content in response to foliar application of PGRs from leaf, root and stem part of *C. forskohlii* is depicted in Table no. 36 and figure 88. It can be seen in figure that in leaf part, the Mn content is at par with control due to low dose of triacontanol, BRs and decreased due to all other PGRs except in high dose of ethephon it is increased. In root part, Mn content is distinctly increased due to low dose of triacontanol, CCC and high dose of BRs while it is decreased due all other PGRs treatments. Low dose of triacontanol, high dose of BRs and ethephon shows increased Mn content in stem part while all the other PGRs shows at par with control Mn content. In all, Mn content is increased in all plant parts due to low dose of triacontanol and high doses of BRs and ethephon as compared to all other PGRs concentrations.
Mn is an essential micronutrient as being structural part of photosynthetic proteins and enzymes in plants, it is required in both lower and higher plants for the Hill reaction. Mn plays a lead role in photosystem (II), where it is associated with the oxygen evolving complex bound to the reaction centre protein (D1) of PS II which catalyzes early stages of \( \text{O}_2 \) evolution (Goussias et al., 2002). Mn is engaged in biosynthesis of fatty acid, acyl lipids and proteins (Ness and Woolhouse, 1980), ATP synthesis process (Pfeffer et al., 1986), in RuBP carboxylase reaction (Houtz et al., 1988), in activation of many enzymes (Burnell, 1988), such as Mn-superoxide dismutase, Mn-catalase, pyruvate carboxylase etc. (Ducic and Polle, 2005). Mn can also perform as a Mg activator prerequisite of the enzymes, to appreciable extent as revealed from \textit{In vitro} studies of many enzyme systems. The availability of manganese (Mn) to plants is due to redox processes, which depend on soils Mn reserve, pH and the availability of electrons (Adams, 1981; Sparrow and Uren, 1987; Marschner, 1991 and Negra et al., 2005). According to Graham (1997), manganese is a free divalent cation absorbed mainly as Mn\(^{2+}\) and translocated predominantly from roots to shoots via xylem. Uptake of Mn in roots is a biphasic process. In the initial non metabolic phase, rapid and reversible uptake of Mn takes place with Mn\(^{2+}\) and Ca\(^{2+}\) or other cations being freely exchanged in the rizosphere while in second step Mn is less readily exchanged. In first step, negatively charged cell wall of apoplastic root spaces result in adsorption of Mn (Clarkson, 1988 and Humphries et al., 2007). The critical deficiency levels of manganese are between 10-20 mg g\(^{-1}\) dry weight in mature leaves and are surprisingly consistent regardless of the plant basis or cultivar or the prevailing environmental conditions (Marschner, 1986). In the view of Munson (1998), manganese concentration 2 - 30 mg 100 g\(^{-1}\) dry tissue, is optimal for plant growth. In the present investigation also in coleus leaf, root and stem Fe content ranges in between 2.5-26 mg 100 g\(^{-1}\) dry tissue. So, it indicates no deficiency or toxicity of Mn due to any PGR including control.

Srivastava and Sharma (1990) recorded that in opium poppy (\textit{Papaver Somniferum} L.), the concentration of Mn in shoots were maximum at 0.01 and at 0.1 mg/l of Triacontanol. Miri \textit{et al.}, (2015) studied the effect of Alfaalfa plant extract on growth and uptake of micronutrients in sorghum. They reported that alfalfa extract, containing significant amounts of essential and beneficial nutrients and triacontanol, can increase dry weight and uptake of micronutrients (Fe, Zn, Mn and Cu) in
sorghum. Wang et al., (2010) in potatoes (Solanum tuberosum L.) studied the effect of exogenous chlorocholine chloride (CCC) foliar treatment significantly increased the contents of Mn in potato leaves.

In the present investigation also in coleus leaf, root and stem Mn content (2.5-26 mg 100 g\(^{-1}\) dry tissue) ranges in between the values given by Munson (1998).

c. Copper

Influence of foliar spray of PGRs on Copper 'Cu' content of the leaf, root and stem of Coleus forskohlii is depicted in Table no. 37 and Fig. 89. It is clearly seen from the results that the level of Cu content is varying depending upon type, concentration of PGRs and type of plant part. In all, in leaf dry tissue Cu content is at par with control due to all PGRs except for CCC low dose Cu content is lowest in leaf while for ethephon high dose it shows highest value. In root dry tissue Cu content is at par with control or decreased due to all PGRs except for low dose of triacontanol and high dose of BRs and ethephon it is increased in root. Similar results are seen in case of stem dry tissue. High dose of ethephon shows highest Cu content in all plant parts.

Copper is one of the most essential micronutrient playing vital role in two important processes like photosynthesis and respiration being a vital constituent of chloroplast protein plastocyanin which forms part of the electron transport chain linking the two photosystems in light reaction of photosynthesis and also a part and parcel of cytochrome oxidase complex of mitochondrial electron transport chain. Copper also plays role in antioxidant enzymes like superoxide dismutase, ascorbate oxidase, phenolase, laccase, and amino oxidase. Out of these enzymes superoxide dismutase plays an important role in scavenging super oxide radical and preventing oxidative stress. According to Henriques, (1989) Cu plays an important role in maintaining membrane structure of thylakoids. Copper in a soil exists in organically complexed form as a root exudates and soil humus from which Cu ion is dissociated in soil solution and get absorbed as a Cu\(^{++}\). The root adsorption mechanism is complex process which takes place at three levels firstly on cell walls in the root free space, then across the plasmalemma into the cytoplasm of the cortical cells may be due to electrochemical gradient which is metabolically active process and can be affected by metabolic inhibitors as supported by Graham, (1981) and Loneragan, (1984). The normal range of copper content in agricultural crops is reported to be 5 to 30 mg kg\(^{-1}\) dry weight (Gupta, 1979) and found to be varied depending on the plant...
species, plant organ, developmental stage and nitrogen supply, this range can be larger (Robson and Reuter, 1981). In present investigation in Coleus leaf, root and stem Cu content ranges between 3 mg to 10 mg kg\(^{-1}\) dry weight due to all treatments.

Srivastava and Sharma (1990) recorded maximum concentration of Cu in shoots in opium poppy (Papaver Somniferum L.) at .01 and Zn at 0.1 mg/l Tria. Miri et al., (2015) reported positive effect of Alfalfa plant extract on growth and uptake of micronutrients in sorghum which contains significant amounts of essential and beneficial nutrients and growth stimulants specifically triacontanol, which increased the dry weight and uptake of micronutrients in sorghum. Wang et al., (2010) in potatoes (Solanum tuberosum L.) recorded significantly increased Cu in potato leaves due to chlorocholine chloride (CCC) foliar spray.

Overall high accumulation of Cu in root and stem than leaf in present studies in coleus indicate healthy root growth with improved antioxidant mechanism.

d. Zinc

Influence of foliar spray of PGRs on Zinc 'Zn' content of the leaf, root and stem of Coleus forskohlii is depicted in Table no. 38 and Fig. 90. It is clearly seen from the results that in leaf, root and stem dry tissue, Zn content is decreased due to all PGRs except for BRs low and ethephon high dose it is at par with control in leaf and increased in stem due to high dose of ethephon as compared to control. Maximum Zn content in all plant parts is found due to ethephon high dosage among all PGR treatments.

Zinc is a critical, divalent cation element, micronutrient required by plants. Zinc (Zn) is not found in oxidized or reduced forms while its functions as a mineral nutrient are primarily because of its divalent cation nature because of which it shows a strong tendency to form tetrahedral complexes (Clarkson and Hanson, 1980). The mechanism of zinc uptake has not been fairly understood and based on radiotracer studies, it is indicated that plants contain multiplicity of zinc transporters (Kochian, 2000). Bukovac and Wittwer (1957) stated that zinc is partially phloem mobile. Zn plays an important role in the process of transcription. It also acts as a cofactor of superoxide dismutase enzyme which is associated with oxidative stress tolerance. Zinc is also required for the activity of various types of enzymes, including dehydrogenases, aldolases, isomerases, transphosphorylases and RNA and DNA polymerases. A remarkably high concentration of zinc was reported by Kitagishi and
RESULTS AND DISCUSSION

Obata, (1986) in meristematic tissue of rice leaves where cell division as well as synthesis of nucleic acids and protein takes place. Pinton et al., (1994) suggested the role of Zn in membrane stabilization by controlling the level of oxidizing O$_2$ species. Marschner, (1986) reported that critical deficiency level of Zn is below 15- 20 mg Kg$^{-1}$ dry weight of leaves and the critical toxicity levels of zinc in leaves of crop plants are more than 400- 500 mg kg$^{-1}$ dry weight. The values of sufficient or normal zinc content in plants are in the range of 27 – 100 ppm on a dry weight basis (Munson, 1998). In the present investigation in Coleus plant parts, Zn content ranges in between normal range of Zn content given by (Munson, 1998). also since zinc plays an important role in plant metabolism there are few attempts have been made to study influence of PGRs. Srivastava and Sharma (1990) recorded that in opium poppy (Papaver Somniferum L.) the concentration of Zn in shoots were maximum at 0.1 mg/l Tria. Misra and Srivastava (1991) reported increased level of micronutrients (Fe, Zn, Mn and Cu) in lemongrass (Cymbopogon flexuosus Steud.) in response to Miraculan-triacontanol formulation 0.4 μg/ml concentration. Miri et al., (2015) tried Alfaalfa plant extract on growth and uptake of micronutrients in sorghum and reported that alfalfa extract, which contains significant nutrients and stimulants like triacontanol, increased the dry weight and uptake of micronutrients in sorghum. Wang et al., (2010) found enhanced content of Zn in potato (Solanum tuberosum) due to foliar application of CCC.

Overall increased level of potassium in all plant parts in response to all PGRs will be beneficial to plant in efficient nutrient uptake, increased photosynthesis and other several metabolic activities leading to increased growth of the plant and all the other yield attributes. Calcium is important for membrane permeability and integrity. Enhanced calcium content will certainly play a key role in activating various enzymes of several metabolic process and will boost the overall growth and health of the Coleus plant. The increased Mg content in leaf due to triacontanol and in root and stem due to all other PGRs as compared to all treatment will surely support plant in photosynthesis and other metabolic events. Being critical micronutrient, iron is involved in various metabolic processes. Thus, accumulation of Fe due to PGRs might be attributed to increased nitrogen metabolism and antioxidant system as Fe is an important co factor for many antioxidant enzymes. Mn content was increased in all plant parts due to low dose of triacontanol and high doses of BRs and ethephon as
RESULTS AND DISCUSSION

compared to all other PGRs concentrations. Overall accumulation of Cu in root and stem than leaf in coleus indicates healthy root growth with improved antioxidant mechanism. In leaf, root and stem dry tissue, Zn content was found decreased due to all PGRs except for BRs low and ethephon high dosage in which it was at par with control in leaf and increased in stem due to high dose of ethephon. Thus, it can be concluded that in present study application of PGRs induced increased content of K, Ca, Mg, Cu, Fe and Mn which might be advantageous for plant growth and yield.