DISCUSSION

Swertia chirayita (Roxb. ex Fleming) Karst. of Family Gentianaceae, is a high value Himalayan medicinal herb. The population of the plant is depleting from its natural habitat due to anthropogenic interference as well as certain deleterious features of the plant itself. There is no established agro-technique for promoting its cultivation (Joshi and Dhawan, 2005; S. Pant, 2007). However, the massive use of this plant in different traditional as well as in the modern medicines generated a great domestic and international market (M. Karan *et al.,* 1996). Keeping in mind such vital problems of this promising plant, an attempt was made in this investigation to invigourate the plants by chemical manipulations which include application of plant growth regulators (PGRs). PGRs influence growth and development when applied in very minute quantity. There are many reports which indicate that application of growth regulators enhanced plant growth and crop yield (Hernandez, 1997; Ashraf *et al*., 1987, 1989). Further, potential performance of the plants were analyzed by a number of physiological and biochemical tests. A regular monitoring was done for the principle active compound constituents at different treatments.

In the present investigation, two types of PGRs were used- growth promoters and growth retardants. The growth promoters used were GA_3, IAA and KIN; while the retardants used were MH, SADH and ABA. These PGRs have well documented literature in scientific application for the betterment of plant growth, metabolism and yield (Hernandez, 1997; Ashraf *et al*., 1987, 1989; Amanullah *et al*., 2010). Considering the efficacy in chemical manipulation development, they were used with a view to obtaining the desired modifications of growth, metabolism and yield of a vanishing yet promising Himalayan medicinal plant *Swertia chirayita.*

After initial screening of matured chirata seeds from the different natural habitats of Darjeeling Hills, determining the different phases in the life cycle of this species and selecting of optimum concentration range of the growth substances suitable for this plant, application were timed at three different developmental stages of the plant *viz.,* rosette, sapling and pre-flowering stages. The promoter as well as retardant induced changes of some growth and biochemical parameters were analysed at different developmental stages of the plant and reflection of such changes on yield attributes was recorded. The results obtained in this investigation were discussed at length from the available literature in this field and allied field of research.

The present investigator, after the collection of matured chirata seeds from the different natural habitats of Darjeeling Hills, found that the plant seeds from Permaguri (Sukhia Pokhri) were much superior to others with respect to general vigour and yield of plant biomass. Thus, the chirata seeds of this particular place i.e., from Permaguri were collected locally and used as experimental material. Important events in the life cycle of the experimental plant were analysed and was found that the plant stayed at the first stationary phase of growth for a prolonged period of time when it reached 135-240 days. Active growth of the plant was stopped and it remained in 'rosette' form. Formation of internodes and branching was initiated when the plant reached 245-320 days. Active apical growth occurred during this period and this period was determined as the log phase of plant growth. At around 325-360 days, bud formation started which later gave bisexual flowers. As the plant matured seed pods formed with minute seeds. At around 415-435 days, gradual drying up of the whole plant took place and the plant finally died and completed its life cycle within 525-550 days (Table 1.1).

Results of foliar treatment with PGRs at rosette stage revealed that the chemicals, irrespective of their concentrations, failed to induce any permanent effect on modification of growth, metabolism and yield of chirata plant. Data on PGRs-induced biochemical changes in leaves in chlorophyll (Table 1.5), protein and free amino acids (Table 1.6), soluble and insoluble carbohydrate (Table 1.7), nucleic acids (Table 1.8), lipid and total phenol content (Table 1.9) were recorded at the rosette, sapling, pre-flowering, fruiting and senile stages of plant development. The tables revealed that the increase was noticed till sapling stage and in some cases, upto pre-flowering stage of plant development and subsequent changes were found to be statistically at par with that of control values. Out of three promoters applied, GA_3 was found to have a more pronounced effect with a significant increase in soluble carbohydrate at the sapling stage as compared to control.

Likewise, the activity of catalase remained subdued in the PGRs-treated samples only upto sapling stage (Fig. 4.1). This effect seemed transient and its activity increased significantly upto the pre-flowering stage with a decline at the later stages. In contrast the activity of peroxidase was found to be enhanced significantly at the rosette stage with the highest activity at the pre-flowering stage. GA_3 was shown to have a marked effect on the activity of peroxidase with a significant increase in the level of the enzyme as compared to control (Fig. 4.2). RNase (Fig. 4.3), α-amylase (Fig. 4.4) and IAA-oxidase (Fig. 4.5) also showed a transitory increase upto sapling stage, after which the activities declined.

Regarding the total percentage of bitter principle content in dried chirata plant, it was found maximum in root than in other parts of the plant. It was noted that the bitter principle compounds remained more or less constant in all the treated plants and was recorded that, the efficacy of PGRs, on synthesis of secondary metabolites was less significant.

Growth parameters like height of the plant, stem circumference, number of leaves, branches and nodes and internodal distance were found to increase considerably upto pre-flowering stage in the promoter-treated plants; GA_3 showing the greatest increase. Similar increase was observed with the retardants (Tables 1.15, 1.16 and 1.17). Thereafter, all changes were found insignificant. Concomitant with the increase in the growth parameters, an augmentation in the yield data as well was observed with the increase in the number of flowers, total length of the plant, fresh weight and dry weight of the plant.

These results are indicative of the fact that PGRs possibly affected the biosynthetic processes of the macromolecules which actually occur at the early stage of plant development, but with the progress of the plant age the effects were nullified because of diminished action of the PGRs and consequent revival of the biosynthetic machinery of the cellular components. The resultant biochemical changes were correspondingly reflected in the plant growth as evident from the changes in the stem length and stem circumference recorded at different stages.

Reports from the different literature suggest that PGRs temporarily exert acceleratory and inhibitory effects on growth and metabolism (Knyll and Chylinsha, 1972; Ben-Gad *et al.*, 1979; Bhattarjee, 1984, 1986). Ben-Gad *et al.*, 1979 observed that elongation of *Citrus* seedlings was initially retarded by SADH treatment but vigorous growth was resumed thereafter. Similar retardant-induced inhibition followed by rapid growth was also observed by Monselise *et al.* (1966), Sachs and Mock (1975), Bhattarjee (1984) and many others who reported such effects using conventional retardants like CCC, SADH, MH etc.

In the present investigation, results of foliar treatment with promoters (GA_3, IAA and KIN) and retardants (MH, SADH and ABA) at the sapling stage (245-320 day old plants) revealed that the PGRs induced increase in chlorophyll (Table 1.18), protein and free amino acids (Table 1.19), soluble and insoluble carbohydrates (Table 1.20), RNA (Table 1.21) levels, which perpetuated only upto the pre-flowering stage. The changes in the level of lipids and total phenol content (Table 1.22) was similar to that of the plants treated at rosette stage: the change in the level of lipid remained insignificant, while that of total phenol decreased with the progress in plant age. In case of insoluble carbohydrate (Table 1.20), after a transient increase from sapling to pre-flowering stage, the PGRs-induced changes recorded at fruiting and senile stages were insignificant.

Activities of catalase (Fig. 4.6) and peroxidase (Fig. 4.7), RNase (Fig. 4.8) and IAA-oxidase (Fig. 4.9) enzymes in leaves were found to increase only upto pre-flowering stage. Such effects were arrested shortly and the levels of these enzymes were found to decline till the plant reached their senile phase of growth. Peroxidase and α-amylase activity (Fig. 4.10) was however seen to decrease from the initial observation period of sapling stage only in the promoter-treated plants. However, GA_3 at 200µg/ml and IAA at 200µg/ml were found to slightly increase the activity of α-amylase from sapling to pre-flowering stage. With the advancement of plant age, it again declined.

Effects of PGRs were observed in the change in bitter principle content of plants treated at sapling stage All the plant growth substances used *viz.*, promoters as well as retardants showed almost an equal change in the concentration of principle bitter compounds in different plant parts. However, GA_3 in particular was found to increase the bitter principle content more inn comparision to control and other treatments.

Regarding the change in growth parameters, height of the plant (Table 1.27), increased considerably with the advancement in plant age. GA_3, in particular, seem to have a tremendous effect as compared to control. Retardant-induced changes were not found to be that prominent. The increase in stem circumference (Table 1.27) with both the promoters as well as the retardants was recorded throughout the observation periods. The number of leaves was found maximum at pre-flowering stage, with an insignificant change thereafter. The number of branches (Table 1.28), nodes and internodal distance (Table 1.29) increased with the increase in height of the plant.

In this study, with positive effect on the growth parameters, concomitant increase in the yield attributes was observed with an increase in the number of flowers, total length of the plant, fresh weight as well as dry weight of the plant (Table 1.30). Compared to others, GA_3 showed a marked increase in fresh weight of the treated plants.

Results of foliar application of the PGRs at the sapling stage of 245-320 days old plants thus indicate that higher levels of chlorophyll, protein, free amino acids, soluble carbohydrate, and nucleic acids, as well as enhanced activities of the anabolic enzymes like catalase and peroxidase, RNase and IAA-oxidase in PGRs-treated plants maintained vital functional life of the plant for longer duration. All the treated plants revealed higher metabolic status and showed enhanced plant potential throughout its life span. In almost all the cases, the regulatory effect of the promoters was found at the observation period of treated stages. On the other hand, a transient set-back with respect to potential performance of the species at sapling stage was observed on retardant-treated plants.

The initial inhibitory effect of the retardants may be justified by an immediate strong retardation action rendered by the chemicals on plant metabolism which started relinquishing with the progress of plant age which was not the case with the promoters. The regulatory action of the promoters was manifested early on in the development of the plant. The adverse effects of retardants, however, did not at all persist for a longer duration. A perpetuating promoting action of the promoters was clearly reflected on the overall growth of the chirata plant as manifested from the augmentation in the morphological features. On the contrary, the effect of the retardants at later stages of plant development was evident from the lesser increase in plant height (as compared to promoters) with a concomitant enhancement of radial growth of stem which persisted till the senile stage of plant growth.

Increased crop yield by the PGRs can be substantiated from the enhanced plant potential as evident from the biochemical analyses of this investigation. Foliar treatment with the PGRs at the sapling stage was found more effective than the treatment done at rosette stage. Plants treated at rosette stage may attain sapling stage earlier, i. e., development of apical growth may occur, shortening the life cycle of the plant. But the vegetative growth later is less vigorous than the plants treated at sapling stage. In the latter case, the vegetative growth of the plant is more prominent.

Experiments on foliar treatment with plant growth substances at pre-flowering stage showed that all the chemicals used caused to decrease the levels of chlorophyll (Table 1.31), protein (Table 1.32), soluble carbohydrate (Table 1.33), nucleic acids (Table 1.34), as well as the activities of catalase, peroxidase, RNase, IAA-oxidase and α-amylase enzymes (Figs. 4.11, 4.12, 4.13, 4.14 and 4.15). This decrease perpetuated till the senile phase of plant growth. As regards the level of free amino acids (Table 1.32), insoluble carbohydrate (Table 1.33) and total phenol (Table 1.35), a transient increase was observed at the pre-flowering stage; although the change seemed insignificant. The change in the content of lipid (Table 1.35) and bitter principle compounds (Table 1.38) was insignificant.

Biochemical changes in leaves were associated with the changes in the growth parameters. The height of the plant remained unaffected (Table 1.39) with both the promoters and retardants. Stem girth (Table 1.39), number of leaves and branches (Table 1.40), number of nodes and internodal distance (Table 1.41) however increased till the plant reached fruiting stage. A decrease in internodal distance was observed with the retardants.

Regarding yield parameters, no noteworthy differences were noticed in the number of flowers, total length of the plant, fresh weight as well as dry weight of the plant as compared to control (Table 1.42).

Unlike the results of foliar treatment of the PGRs at sapling stage, the consistent decrease of chlorophyll, protein, soluble carbohydrate, nucleic acids as well as in the activities of catalase, peroxidase, RNase, IAA-oxidase and α-amylase enzymes, which persisted till senile stage, indicate that their application at the pre-flowering stage seemed to be of not much importance as manifested also in negligible changes in the growth and yield parameters. A slight increase in free amino acids, insoluble carbohydrate and total phenol was too small to be noticed as are the changes in lipid and principle bitter compounds.

Reports exist in the literature that application of PGRs enhanced plant growth and crop yield (Hernandez 1997; Ashraf *et al*., 1987, 1989). GA_3 was most effective on stem height, leaf area, and shoot fresh and dry weights of almond (Mobli and Baninasab, 2008). IAA exerts influence on plant growth by enlarging leaves and increasing photosynthetic activities in plants and also activates the translocation of carbohydrates during their synthesis (Awan *et al.,* 1999; Ritenour *et al.,* 1996). Cytokinins enhanced the cell expansion in soybean (Makarova *et al.,* 1988) and increased stem thickness while kinetin reduced shoot length but increased the fresh weight by increasing stem diameter in morning glory (Kaul & Farooq, 1994) and in okra (Chaudhry & Khan, 2000). Abdel-Latef, 2003; Abou Al-Hamd, 2007 showed that plant growth regulators (IAA, GA_3 and Cytokinins) induced a marked accumulation of protein content and carbohydrates content.

Mostafa and Alhamd (2011) found out that the seeds of *Balanites aegyptiaca* soaked in GA_3 and IAA solutions showed significant increase in the germination percentage, plant height, number of branches and leaves, total chlorophyll content, dry weight and protein, carbohydrates, alkaloids, tannins and saponins. Das *et al*. (2002) reported that foliar application of kinetin increased chlorophyll content and leaf yield of mulberry. Increase of yield of some legumes by Cytokinins (kinetin or benzyladenine) was reported by Zhlobak (1986) on pea, Salem (1989) on soybean, Khalil and Mandurah (1990) on cowpea. Spraying of MH at 500ppm increased the number of lateral branches and leaves compared to control in chrysanthemum (Beach and Leopold, 1963), carnation (Dubey, 1972), china aster (Reddy and Sulladmath, 1983) marigold (Lal and Mishra, 1986, Singh, 2004). ABA treatments increased the fresh weight, dry weight, root growth, total chlorophyll, protein and amino acid content, while it decreased the stem length (Gopi and Panneerselvam, 2011).

The increase in seed germination percentage as a result of the exogenous application of GA_3 and IAA was positively correlated with the decrease in total phenols. This result agrees with those of Baskin and Baskin (1998) and Araby *et al.,* 2006. Total phenolic and total anthocyanin contents in red lettuce treated with ABA were significantly higher than in controls, whereas no significant differences were observed in green lettuce.

There are reports that GA_3 and IAA induced a marked accumulation of protein and carbohydrates content which might be linked with the efficiency of photosynthetic apparatus, which leads to increase in plant productivity and dry matter production (Azooz *et al.,* 2004). Application of IAA resulted in maximum promotive effect on number of tubers, fresh weight and dry weight, followed by GA_3 and Kinetin as compared to control. Foliar application of cowpea plants with Indole Acetic Acid (IAA) at three concentrations (12.5, 25 & 50ppm) induced increments of the plants height, fresh and dry weights, number of branches and number of leaves per plant as well as yield components (pods per plant, seeds per pod, weight of pod, weight of seeds per plant and weight of seeds per feddan). Application of IAA at the rate of 10^{-5}M increased grain yield and biological yield in wheat (Arif *et al*., 2001).

Similarly, Zahir *et al*., (2000) reported up to 50% increase in fresh biomass of soybean by the application of L-tryptophan (precursor of IAA). Increase in the number of flowers by Cytokinins was recorded in other plants such as beans and soybean (Dubing and Westgate, 1996; Lynas, 1981; Mansour *et al.,* 1994). Dry weight of shoot was increased with 500ppm of MH but it was reduced with higher concentrations (Shanmugam *et al*., 1973). Application of growth retardants may also enhance the chlorophyll content of leaves which helps to increase the functional life of the source for a longer period leading to improved partitioning efficiency and increased productivity (Kashid *et al*., 2010). ABA significantly induced the accumulation of chlorophyll b and total carotenoids in lettuces and it elevated the content of individual phytochemicals in red lettuces (Li *et al.*, 2010).

Studies have shown that the use of PGRs increases the secondary metabolites of medicinal plants. GA application resulted in higher percentage of total alkaloids accumulated in leaf, stem, and root as reported in morphine yield of opium (Khan *et al.,* 2007), *Solanum khasianum* (Gowda, 1986), IAA and NAA treatments in *Andrographis paniculata* (Gudhate *et al*., 2009), IAA application in *Solanum nigrum* (Bhatt *et al*., 1983). In the present study, PGRs treatment on production of secondary metabolites in chirata plant was manifested by the increase in bitter principle content in the plants treated at sapling stage. The same treatments were also effective for improving fresh and dry biomass of the whole plant. This suggests that the improvement in quality as well as quantity can be achieved with the use of foliar application of PGRs, which can be a good approach of secondary metabolite improvement for growers. Reports in *Solanum jaminoides* (Sahoo *et al*., 1999) *Solanum khasianum* (Bores *et al*., 2001) had also shown positive influence of IAA on secondary metabolite production. The existing literature pointing to the PGRs-induced effects on the changes in growth and metabolism thus, corroborate the overall findings of this investigation done with a different plant species.

In this study, PGRs also showed a tendency towards deferring plant senescence and increasing crop yield as well as significant senescence delaying effects of kinetin (200μg/ml) which can be substantiated from the enhanced plant potential as evident from the biochemical analyses of this investigation. Unlike treatment at rosette stage, foliar treatment with PGRs at the sapling stage enhanced the levels of intercellular components like chlorophyll, protein, insoluble carbohydrate and others. PGRs, in general, delay the onset of senescence in plants (Orchard and Lovett, 1976; Weaver, 1972; Bhattacharjee, 1984; Bhattacharjee *et al.,* 1976). PGRs, especially, retardants-induced delaying in seed senescence and consequent enhancement of seed potential in some species have been established (Bhattacharjee and Gupta, 1985; Chettri *et al.,* 1993; Rai *et al*., 1995; Maity *et al.,* 1999). Deferral of senescence in vegetables, cut flowers and even in mushroom and the resultant yield augmentation have also been documented (Halevy and Wettever, 1966). Nooden *et al.* (1939) concluded that the prevention of the internally progressive degeneration might open a way to yield improvement. In the present study, thus, the augmented yield in the treated plants can be justified from the reported observations.

The growth retardant succinic acid 2, 2-dimethylhydrazide (SADH) reduces both vegetative and fruit growth of apples (Batjer *et al.*, 1964) and induces similar growth responses in many other plant species (Cathey, 1964). It is used commercially on apples to increase fruit firmness and red colour; to delay development of water core; and to decrease fruit size and vegetative growth on young vigorous trees (Bartram, 1960). SADH treatments of eight apple cultivars induced reductions in catalase activity and iso-peroxidase spectrum during growing season which were associated with reduced vegetative growth and enhanced fruit bud formation. SADH was found to delay ripening of apples (Looney, 1967), advance maturity and improve fresh color of peaches and sour cherries (Unrath *et al*., 1969). Astacio and Iersel (2011) demonstrated in tomatoes (*Solanum lycopersicum*) that ABA drenches rapidly close stomata, limit transpirational water loss, and can extend the shelf life of retail plants by up to 8 days, which exemplifies its potential as a commercially applied plant growth regulator.

In the present work, potentiation of source system was well documented where the treated plants got rejuvenated as evident by visual appearance, i. e., augmentation in the growth and yield parameters and this was biochemically substantiated by analyzing some variables like enhancement of chlorophyll, protein, carbohydrates, nucleic acids as well as activities of certain enzymes. Hormone-directed translocation of assimilates is a well established phenomenon (Patrick; 1979; Taiz and Zeiger, 1998). It is generally accepted that actively growing meristems and reproductive organs are the potential sinks for photosynthetically produced assimilates, and that activation or repression of apical sink and/or reproductive sinks may result in corresponding changes in growth and yield of plants (Moorby, 1977; Wareing and Patrick, 1975).

Treatment at rosette stage may shorten the time to attain sapling phase with the initiation of apical growth. But the overall development of the plant was not found to be augmentative. Foliar application at sapling stage resulted in positive changes as manifested by vigorous growth of the plant and increase in the final yield. Treatment at pre-flowering stage was found to have an insignificant change in biochemical, growth as well as yield parameters. The increase and decrease in the different parameters was not noteworthy. Out of all the growth promoters used, GA_3 seemed to be the most effective, especially at a concentration of 100µg/ml. On the other hand, all the growth retardants used seemed to have similar effects on the growth, development and yield of chirata plant. Hence, the application of GA_3 was found to be the most effective and out of three stages of treatment, sapling stage was found to be the most beneficial.