SUMMARY & CONCLUSIONS

The increase in atmospheric concentration of chlorofluorocarbons, methane, nitrous oxide and other gases due to man made activities is responsible for the depletion of stratospheric O\textsubscript{3} layer, which is sole attenuator of solar ultraviolet BC (Liv-B, 280-320 nm) radiation. The reduction in the stratospheric O\textsubscript{3} leads to an increase in UV-B radiation reaching the Earth’s surface. (Anderson etal., 1991). Stratospheric O\textsubscript{3} reduction is one of the pressing global concerns of climate change. The concern derives from potential photo biological consequences of increased ultraviolet- B radiation flux reaching the Eolith’s surface. Stratospheric O\textsubscript{3}reduction only affects a solar wave bond of approximately 25 mm (290-315 mm) within the UV-B band, and the remainder of the solar spectrum is for the purpose of photo biological phenomena, and is essentially. Unaffected (Caldwell et.al., 1993): Recent evidences indicated that there has been a significant up word trend in solar UV-B radiation at middle and high latitudes in the northern hemisphere (Herman et.al., 1996; Pyle, 1997; Caldwell et.al., 2003; McKenzie et.al., 2003).

UV-B has the potential to disproportionately affecting the metabolic processes in the plants, and elicits plant responses in many different ways. For example, UV-B may cause damage to DNA, proteins and lipids one may modify photosynthesis, growth and development (Bornman, 1989; Teramura and Sullivan, 1994; Jansen et.al., 1998). Both the primary events of photochemistry in photo system II and reaction of CO\textsubscript{2} assimilation are affected by elevated levels of UV-B radiation (Middlenton and Teramura, 1993; Nogues and Baker, 1995: Kakani et.al., 2003 b).

Although the biological significance of stress resistance or developmental responses in term of effect on overall plant growth, can be determined quantitatively in the laboratory- But plants are generally much more sensitive to UV-B under controlled environment chamber or glasshouse conditions. Demonstrating the usefulness of genetically isolated physiological characteristics
or control mechanism in a more natural setting should enhance the efficiency with which one can distinguish between environmental responses what are recognizable only in an artificial, optimized laboratory environment and those that are critical to plant growth and yield in the field. In addition, plant sensitivity to UV-B is differing not only at species level, but also at the cultivars level of the similar species.

Therefore, the present study was conducted in pots under natural levels of two different doses of UV-B radiation e.g. ambient and ambient supplemental (7.1 KJm-1, simulating 20% ozone depletion at Allahabad, 20.47’N) on Withania somnifera to evaluate the sensitivity of plant.

A field condition in which the test plants experience all growth and metabolic requirements in optional levels is a rare situation. The multiple stress responses in plants may be additive, synergistic or antagonistic. The effect of supplemental UV-B radiation on plants can be modified by other co-occurring stresses. There is a noteworthy study on soybean, in which Murali and Teramura (1986) and Teramura et. al., (1990) examined the potential for alteration in the yield and seed quality of soybean grown for six years in the field, and suggested the necessity for multiple year experiments to increase the understanding of the interaction between UV-B radiation and other environmental stresses to assess the potential consequences of stratospheric O3 depletion. There is also a dearth of information concerning the sensitivity of tropical plants to stress under field conditions.

The present investigation was, conducted in pots to evaluate the effect of supplemental UV-B and heavy metals applied individually as well as in combination on Withania somnifera to see inter and intraspecific response.

The present study describes the results of different experiments conducted to assess the individual and combined effects of supplemental UV-B radiation and heavy metals (Cd and Ni) on morphological, physiological and biochemical
characteristics and yield parameters of *Withania somnifera*. Results and discussion on these aspects have been described in detail in previous chapters.

In this chapter, an attempt has been made to collate the results and to discuss some of the emerging issues.

*Withania somnifera* showed variations in response to supplemental UV-B radiation.

Physiological and biochemical characteristics of *Withania somnifera* were negatively influenced by the supplemental UV-B radiation. Chlorophyll ‘a’ content increased with increasing age in all the treated plants. However, chlorophyll ‘b’ content reduced in all treated plants at all ages as compared to their respective controls. In case of individual reduction in chlorophyll ‘b’ content. Decrease in chlorophyll content due to sUV-B radiation was 0.386, 1.536, 2.814 (Table – 4.10) and 1.299, 1.450, 2.879 at 30, 60 and 90 DAS (Table – 4.10).

Antioxidant system was activated in response to UV-B radiation in all the test plants. Superoxide dismutase (SOD) activity increased in all the treated plants. Peroxidase and catalase activities showed an inverse response under influence of UV-B radiation. Peroxidase activity increased, whereas catalase activity decreased in all the treated plants as compared to their respective in all the treated plants as compared to their respective controls. The changes in these endogenous levels of antioxidants suggest generation of free radicals and H$_2$O$_2$ due to sUV-B and heavy metals Maximum increased in SOD activity was observed in *Withania somnifera* was 0.823, 0.945 at 90 DAS(Table – 4.15). Decreased in catalase activity at 90 DAS was 29.073, 30.103, whereas increase in peroxides activity was 3.645, 2.288 in first and second trial at 90 DAS due to sUV-B exposure at last sampling date (Table – 4.16, 4.17).

Secondary metabolites such as flavonoids and related phenol are well known for their UV-B absorbing capacity. Disparities in UV-B sensitivity among species of different growth form and functional types have mainly been attributed
to the efficiency) of foliage at screening harmful UV-B radiator. One important predictor of this is the increase in the concentrations of secondary metabolites in the exposed leaves. Foliar flavonoids and phenol contents were always higher in all test plants of *Withania somnifera* under sUV-B treatment. Increase in falconoid content due to sUV-B exposure in *Withania somnifera* were 2.283 in first trial and 2.383 in second trial at 90 DAS. (Table – 4.14) Similarly, increase in foliar phenol content due to sUV-B exposure in *Withania* was 42.300, 43.303 at 90 DAS (Table – 4.19) Similarly, anthocyanin showed marked increase in plants treated with sUV-B recitation in all the test plants. Maximum foliar anthocyanin content was observed in *Withania somnifera* due to sUV-B exposure were 2.173 in first trial and 2. in second trial at 90 DAS (Table – 4.13).

Protein content in *Withania somnifera* showed significant decline under sUV-B treatment. The reduction in protein content is especially detrimental as the plant need more protein for repair under stress, whereas reduction in ascorbic acid content was always higher in sUV-B treated plants. Reducing sugar content decreased in UV-B treated plants than their respective controls. Changes in these primary metabolites suggest adverse, effect of sUV-B radiation on *Withania somnifera*.

Plant growth parameters such as shoot length, leaf area, Dry weight, member of leaves, number of branches etc. also reduced in UV-B exposed plants. Reduction in yield is a typical index of sensitivity of plants to any stress Supplemental UV-B radiation severely affected and growth parameters. Studied in the present investigation shoed the differential response of *Withania somnifera*.

**Differential Responses of Withania somnifera to heavy metal stress.**

Most of the parameters analyzed to assess the sensitivity of *Withania somnifera* to heavy metal stress were unfavorably attired due to heavy metal treatment. Concentrations of photosynthetic pigments (chlorophyll and carotenoids) decreased under individual treatment of both Cd and Ni, however, combined application of Cd and Ni produced less than additive effects on photosynthetic
pigments. Less than additive effects on photosynthetic pigments. Reduction in photosynthetic pigments led to reduction in biomass accumulation. Highest reduction in all photosynthetic pigments was due to combined treatment of Cd and Ni, followed by was due to combined treatment of Cd and Ni. Antioxidant enzymes and metabolites of lest plants were enhanced in response to heavy metal stress. Similar to the UV-B exposure, superoxide dismutase and peroxidase activity increased due to heavy metal treatments, whereas catalase activity decreased in all the plants. Maximum increase of SOD activity was observed in plants treated with Cd and Ni together followed by individual treatments of Cd and then Ni. Increase in peroxidase activity was 1.929, 1.258, 1.941, 1.266 and 1.423, 1.292 in first and second trial in Cd, Ni and Cd+Ni treatment, respectively. 

In *Withania* at 90 DAS Decrease in catalase activity at 90 DAS was 35.38, 36.73 in combined treatment of Cd+Ni. Decrease in low molecular weight antioxidant, ascorbic acid was also observed antioxidant, ascorbic acid was also observed in heavy metal treated plants. (Table – 4.18).

Unlike sUV-B exposure, flavonoids and anthocyanin contents did not increase significantly in response to heavy metal treatment. Protein contents decline significantly in heavy metal treated *Withania somnifera* plants. Reducing sugar content also decreased to their respective controls.

Heavy metal stress also adversely affected all the growth parameters. Shoot length, Root length, Dry weight, fresh weight, Number of branches, Number of leaves reduced in all heavy metal treated plants. Leaf area and Leaf Area Index also reduced under heavy metal treatments. Yield was the most important parameters from agriculture point of view. Reduction in yield was observed in all the test plants due to heavy maximum under combined treatment of Cd+Ni, however, reduction was less than additive. Reduction in yield was 71.30, 74.67, 68.33, 72.33 and 50.33, 54.33 in *Withania somnifera* due to Cd, Ni and Cd+Ni treatments, at 90 DAS, respective, (Table – 4.9).

In present investigation all the biochemical and growth parameters, showed differential responses in *Withania somnifera* to heavy metal.
The study showed that combined application of sUV-B radiation and heavy metal caused more adverse effect on the growth and development of plants than their individual treatment. However, the response is always less than additive. Chlorophyll and protein contents also showed higher magnitude of reduction, but the effect is less than additive. Under combined treatment of sUV-B and heavy metals as compared to individual treatments. Flavonoids are considered as UV-B screen for protection the plants from UV-B damage. In combined treatments of heavy metal and UV-B, flavonoids contents decreased. The possible reason of this reduction was that the UV-Screening pigments might decrease in presence of heavy metals due to the synthesis of phytochelatins. Lower content of flavonoids make the plants more susceptible to harmful effect of UV-B radiation.

Plants height again decreased under combined treatment of sUV-B radiation and heavy metals. This decrease was resulted due to suppression in photosynthesis caused by reduction in chlorophyll content. Interactions of sUV-B and heavy metals adversely affected the physiological, biochemical and growth parameters of all the test plants which ultimately leads to reduction in yield. However, interactions of sUV-B and heavy metal showed less than additive response. Reduction in yield was 22.33, 26.33, 17.33, 21.33 and 8.33, 12.33 in first and second trial at 90 DAS in Withania somnifera due to sUV-B+Cd, sUV-B+Ni and sUV-B+Cd+Ni treatment respectively (Table – 4.9).

The present study led to the following conclusions :-

- sUV-B radiation and Cd and Ni heavy metal treatments caused significant reductions in photosynthetic pigments, biomes accumulation and yield of Withania somnifera plants in general showed an increase due to sUV-B exposure and heavy metal treatments. Antioxidants capacity of plants increased due to sUV-B exposure and heavy metal treatments. Defense mechanism was also activated as shown by the increase in the levels of enzymatic and non-enzymatic antioxidant system. Various growth indices also showed adverse impact of sUV-B radiation and heavy metals on the growth patterns Withania somnifera plants. Combined treatment of sUV-B radiation
(120 mins) and Cd and Ni heavy metals showed more negative effects on the plants than their individual effects.