The aim of primary study was to determine the response of *Withania somnifera* to abiotic stress (temperature and light) to its morphological, physiological, biochemical parameters and therapeutically active constituents. The seeds were sown in the nursery beds in 2011 and 2012, in the nursery area of Shoolini University, Solan. One month old seedlings with three leaves and 8 inch length were transferred in the pots (12 inches) filled with 6 kg soil mixture containing soil: sand : farm yard manure (FYM) in 1:1:1 ratio. One seedling per pot in replicates of three were used for each treatment. No inorganic fertilizer and pesticide were use during the experiment. Temperature stress (8°C, 18°C, 38°C, 48°C and 58°C) and light stress (8hrs, 12 hrs, 16 hrs, 20 hrs and 24 hrs) were applied on seedlings for two months. The pots were arranged in completely randomized design and positions of pots were changed weekly to avoid a position effect. The findings of the study are summarized below.

### 6.1 Growth

Seedling growth due to light and temperature stress was adversely affected. Stem length and root length decreased both at low and high temperatures. However plants under light stress showed interesting behavior as long photoperiod (16hrs, 20hrs and 24hrs) decreased stem length but increased root length. Plants under short photoperiod (12hrs and 8 hrs) enhanced the stem length due to etiolation effect. However, root length decreased with short photoperiod. Maximum reduction in stem length was observed at 58°C and at 24 hrs photoperiod.

### 6.2 Biomass and Productivity

The stress treatments affected biomass and productivity. The leaf area increased with the growth of plants but decreased in temperature stress (low and high) treated plants compared to control plants. In light stress leaf area increased in plants kept under short photoperiod and decreased in long photoperiods. After 60 days of stress treatments fresh weight and dry weight decreased except in plants kept in short photoperiods (12hrs and 8hrs) where it increased marginally. Membrane stability index increased with an increase in temperature and decreased with decrease in temperature compared to control plants. In light stressed plants membrane stability index decreased with decrease as well as increase in photoperiods compared to control plants.
plants. The relative water content, a consistent increase was found with low temperature and long photoperiod while it decreased at high temperature and short photoperiods.

6.3 Physiochemical parameters

Carbohydrate content increased in all stress treatments except 38°C where it decreased by 21.43% compared to control plants. Maximum increase was at 58°C where it increased by 51.77%. Protein content increased with increase and decrease in temperature from control condition. In light stress plants, protein content increased in short photoperiods and decreased in long photoperiods. The maximum increase (323%) in protein content was in plants kept at 58°C.

6.4 Non enzymatic Antioxidants

The non-enzymatic antioxidants like chlorophyll, carotenoids, ascorbic acid, tocopherol, phenol, flavonoids were studied in the present investigation. Quantitatively chlorophyll content increased with decrease in temperature and photoperiod but it decreased with an increase in temperature and photoperiod from the plants growing in control condition. Carotenoids and tocopherol content decreased in plants kept at low temperature and short photoperiod but increased with an increase in temperature and photoperiod. Alkaloids followed the same pattern as that of carotenoids and tocopherol except in high temperature growing plants where alkaloids decreased. Ascorbic acid, flavonoids and phenol contents also increased in all the applied stresses. However, phytosterol and saponin content increased in high temperature and long photoperiods and decreased in low temperature and short photoperiods kept plants.

6.5 Enzymatic Antioxidants

The enzymatic antioxidants analyzed in the leaves revealed significant increase in the content of SOD and POD in all the applied stress treatments in *Withania somnifera* plants. The increase was maximum in plants kept at 24 hrs stress light conditions. Catalase content also increased in all the stress plants except in plants kept at long photoperiods. Ascorbate peroxidase content increased in plants kept at high temperature and long photoperiod conditions and decreased in low temperature and short photoperiods conditions. Glutathione reductase decreased in all the
applied stresses. However, GST showed variable behavior due to treatments. It increased in high temperature and short and long photoperiods and decreased in low temperature conditions.

### 6.6 Phytochemistry

In this study three neuroactive compounds i.e withanolide A, withaferin A and withanolide D were identified and quantified. Identification was done with the help of Thin Layer Chromatography and IR spectroscopy. Thin layer chromatography showed the presence of three compounds in hexane: chloroform: methanol solvent system at different $R_f$ values i.e. withanolide A at 0.58, withaferin A at 0.41 and withanolide D at 0.19. IR spectroscopy confirmed the presence of withanolides at 1719:98:968 (cm$^{-1}$) in *Withania* root extracts. Quantification was done with HPLC where formic acid and H$_2$O acted as mobile phase at 227nm. HPLC chromatograms of three compounds showed their presence as three dominant peaks and these were: withanolide A at $R_t$= 17.8, withaferin A at 10.6 and withanolide D at 20.9. Withanolide A, withaferin A and withanolide D content varied with respect to plants treated with temperature and light stress. From the study it was interpreted that withanolide A showed highest concentration in the root extract followed by withaferin A and withanolide D. Withanolide A content decreased by 5.6% in 8°C and increased by 4.9% in 58°C treated plants. In light stress plants, it decreased by 5% in 8hrs and increased by 12% in 24 hrs photoperiod conditions. Withaferin A and withanolide D content showed the similar pattern as it decreased in low temperature stress and short photoperiods plants and increased in high temperature and long photoperiod plants compared to control plants.

### 6.7 Herbicidal effect

The impact of extract of leaves, stem and roots of *W. somnifera* was tested in four weeds i.e. *Chenopodium album*, *Achyranthus aspara*, *Ageratum coenyzoides* and *Parthenium hysterophorus*. *Withania* extract affected germination and growth of studied weeds and leaf extract had toxic effect. Leaf extract suppressed germination by 50% in *Chenopodium album*, by 65% in *Achyranthus aspara*, by 70% in *Ageratum coenyzoides* and in *Parthenium hysterophorus*. A considerable reduction in the stem length, root length, fresh weight and dry weight was reported in *Withania* extract treated weeds. *Withania* extract decreased number of shoot as well as root branches in all the studied weeds.
Conclusion

Plant growth in *Withania somnifera* was affected due to applied abiotic stresses i.e. temperature and light stress. Phytochemical contents were also influenced in stress treated plants indicating that abiotic stress affect the physiological processes in the treated seedlings. There was an increase in enzymatic and non-enzymatic antioxidants indicating that the plant has the ability to scavenge or control the level of cellular ROS and can be grown successfully under stressful conditions. Withanolide A, withaferin A and withanolide D content increased in high temperature and continuous light condition, however, it decreased in low temperature and short photoperiod conditions. The study revealed the tolerance ability of *Withania somnifera* and strong antioxidant defense mechanism in temperature and light stress. The herbicidal effect of *W. somnifera* against *Chenopodium album, Achyranthus aspara, Ageratum coenyzoides* and *Parthenium hysterophorus* was found in the present study. The results of present study can be used as a baseline for further study involving screening and selection of tolerant genotypes and using these traits in breeding programs. Herbicidal property of *Withania somnifera* could be used in weed management in the Himalayan region.