In nature plant grow in an inconstant environment that frequently impose constraints on its growth and development upon exposure to a broad range of abiotic stresses. Among abiotic stresses, temperature and light affect plant growth most. The plants respond to temperature and light stress with number of physiochemical and developmental changes. The present study examined the effect of temperature stress (high and low temperature stress) and light stress (duration of lights) on the morphology, physiochemical constituents and production of secondary metabolites in *Withania somnifera*. In addition a study of herbicidal role of *W. somnifera* was undertaken to determine the role of plant extract on the germination and seedling growth of four weeds, viz., *Ageratum coenyzoides*, *Achyranthus aspara*, *Parthenium hysterophorus* and *Chenopodium album*. The significant observations and results are discussed hereunder.

**5.1 Plant Morphology**

**5.1.1 Stem length**

It is a well-known fact that seedling growth is dependent upon temperature and light. Varied temperature (low and high) and light conditions (short and long photoperiods) significantly affected stem growth as stem length reduced significantly in both low and high temperature (Fig.4.1). The degree of stem length reduction increased both with an increase and decrease in temperature as compared to control temperature condition (22°C). These results are in agreement with those of Patel and Golakia (1988) for *Albizzia* seedlings, Shah *et al.* (2011) for rice plants, Young *et al.* (2004) for *Brassica napus*, Arves *et al.* (2013) for *Pisum sativum* and Iloh *et al.* (2014) for Nigerian cereal crops: maize, rice and sorghum. Myster and Roarmoe (1995) showed that when there was big difference between day and night temperature plant growth was affected and resulted in stunted plants. Similarly, Barbara *et al.* (2011) found that differential temperature and photoperiod affected the growth of *Ocimum basilicum*. The reduced stem length due to high temperature is attributed to high transpiration rate and loss of water (Barbara *et al.*, 2011). Investigations carried out by Pollet *et al.* (2009) revealed that high temperature inhibited cell elongation. Reduced stem length in *Withania somnifera* could be because of high transpiration rate and dehydration in plant in high temperature condition which ultimately reduced cell division and cell elongation. Stem extension growth is a product of both cell elongation and cell division and stems developed under positive DIF (differentiation in day and night temperature) have more
cells and longer cells compared to negative DIF (Thingness et al., 2003). Moreover plants growing under positive DIF had higher content of plant hormone gibberellin as shown by Stavang et al. (2005). Similarly, the effect of cold stress depends on the degree of severity and the time of exposure. The seedling stage of plants is most sensitive to cold. The potential chilling symptoms are: stunted stem length, surface lesion, a water-soaked appearance of the tissue, discoloration, desiccation, tissue breakdown, accelerated senescence, ethylene production, and faster decay due to leakage of plant metabolites (Sharma et al., 2005). Reduced stem length in *W. somnifera* in low temperature is in agreement with these findings. Beck et al. (2007) emphasized that of the several primary effects at the cellular level the temperature (low and high) caused increase of membrane viscosity, retard metabolism, delay energy dissipation and radical formation and oxidative stress which reduced stem length. In addition to this, low non-freezing temperature also cause dehydration, mainly due to reduction in water uptake by roots. At freezing temperature, membrane damage is caused by severe cellular dehydration, associated with ice formation. The accumulation of ice in the intracellular spaces caused the physical disruption of cells and tissues. Freezing temperature also caused protein denaturation and precipitation of solutes as a result of freezing induced dehydration and ultimately reduction in stem length (Uemura et al., 1995; Salinas, 2002).

Light stress similar to temperature stress significantly influenced stem length in the present study. *W. somnifera* exposed to short photoperiod had increased stem length while those exposed to long photoperiods had decreased stem length. This is attributed to etiolation effect which resulted in long stem length in short photoperiods. Silva et al. (2006) found shoot etiolation, large internodal spaces and a small number of leaves in *Passiflora edulis* seedlings grown at 70% shading in comparison with plantlets maintained under full-sunlight condition. However, continuous light reduced stem growth in *Jatropha curcus* (Wadhwa et al., 2010) and in Cowpea (*Vigna unguiculata*) (Adelusi and Aileme, 2006). In the classical experiments by Went (1937) and Vanderhovef (1981) the excessive elongation of mesocotyl was correlated to higher auxin production in coleoptile tips of plants kept in continuous darkness in comparison with plants which received occasional light. The impact of light stress resulted in retarding growth, yellowing of the leaves and finally plant death in *Sorghum* in continuous light (Elfadil and Abdallah, 2013).

5.1.2 Root length

In order to know the effect of variation in temperature and photoperiods in *W. somnifera,*
root length was measured after 60 days of stress treatment. Root length in *Withania somnifera* decreased in seedlings treated with temperature stress (high and low) and short photoperiods (8 hrs, 12 hrs) whereas in long photoperiods (16 hrs, 20 hrs and 24 hrs) an increase in root length was found in the present study (Fig.4.1). As evident from the literature that similar results were found in maize (*Zea mays* L) by Herrero and Johnson (1980), in bentgrass by Huang *et al.* (1998), in Sorghum plantlets and maize by Iloh *et al.* (2014), in wheat and ryegrass by Sultana *et al.* (2013), in Caragana korshinskii by Lai *et al.* (2014) and in *Plantago psyllium*, *Althaea officinalis* and *Nigella sativa* by Jamian *et al.* (2014). Effects of temperature on the growth and morphology of roots in oilseed rape (*Brassica napus* L.) and barley (*Hordeum vulgare* L.) was studied by Macduff and Wild (1986) and it was found that root temperature affected root extension, mean radius, root surface area, numbers and length of root hairs. Decreased root length due to high temperature in *Withania somnifera* is in consistent with many previous studies. Kannan and Kulandaivelu (2011) attributed that reduction in root length in high temperature treated plant was associated with decrease in cytokinin transport from roots to shoot or an increase in the amount of phytohormone abscisic acid (ABA). The hormone imbalance led to changes in the cell wall extensibility and decline in the concentration of photosynthetic enzymes which resulted in reduced growth. Low temperatures favor decreased roots production and its elongation rate was linearly related to changes in soil temperature (Hasanuzzaman *et al.*, 2013). At soil temperatures less than 17°C, temperature was found dominant factor affecting rate of growth, but at temperatures greater than 17°C soil water potential became the important factor. Decrease in root elongation rate had been observed by Teskey and Hinckley (1981), Hund *at al.* (2004) and Pregitzer *at el.* (2008). Lavisolo and Schuber (1998) attributed decreased root length to the blocking up of xylem and phloem vessels in low temperature hindering any translocation in plants.

Photoperiod is an important factor in plant growth and development (Lennart Eliasson, 2006; Reich *et al.*, 2006; Zakizadeh, 2013). In the present study, long photoperiods affected root length more than short photoperiods. Variation in physiological and morphological traits among tree species had been related to their differences in light availability (e.g. Loach,1970; Bazzaz,1979; Pompa and Bongers, 1988; Walters *et al.*,1993; Jones *et al.*,1994; Kitajima,1994; Walters and Reich, 1996; Dash and Shree, 2013). Despite growing underground, largely in darkness, roots are sensitive to light. In *Arabidopsis*, continuous illumination of roots speeds-up root growth via
reactive oxygen species-mediated and F-actin dependent process and also activated phytohormones (Yokawa et al., 2014). This could be a reason for slightly increased root length in Withania somnifera in long photoperiods. In rice seedlings, strawberry plantlets, sprouting broccoli, grapes, roses and cymbidium plantlets it was observed that limited supply of light reduced root length and root biomass in seedlings (Chen et al., 2014). Reduced root length in short photoperiods in W. somnifera is in agreement to many studies. Stunted growth in short photoperiod was attributed to the low rate of photosynthesis and low carbohydrate content by Lambers et al. (1981) and Lambers (1992).

5.1.3 Fresh weight and dry weight

Fresh weight and dry weight in seedlings of Withania somnifera declined in temperature and light stress conditions except in short photoperiod where fresh weight and dry weight increased slightly in comparison to control plants in the present study (Fig.4.2). Earlier a significant negative linear relation between shoot and root biomass and increased temperature was found in wheat (Mitchell et al., 1993; Ferris et al., 1993; Batts et al., 1998) and in Arachis hypogaea L. (Wheeler et al., 1997). Gunawardhana and Silva (2011) attributed that decline in fresh weight and dry weight was due to high respiratory and transpiration rate in high temperature stress. Long exposure to high temperature cause severe water loss in crops and increased rate of respiration which ultimately resulted in decrease in fresh and dry weight (Lavy and Veilleux, 2007). Mayland (1968) found that enzymatic respiration and thermochemical degradation in alfalfa caused loss of dry matter.

In the present study low temperature decreased fresh weight and dry weight of Withania somnifera plants. Cold stress severely affected fresh weight and dry weight as it significantly reduced cell division and cell elongation, which eventually resulted in stunted growth (Miedema et al., 1982). A significant reduction in fresh weight was reported in wheat (Davis et al., 1988; Mohammadi et al., 2007; Jenner, 1991; Jenner, 1994), in corn (Wolfe, 1991), in watermelon (Bradow, 1990), in cucumber and squash (Reyes and Jennings, 1994), and in muskmelon (Korkmaz and Dufault, 2001). Markhart (1986) found that extended periods of cold stress significantly delayed seedling growth and damaged photosynthetic apparatus. Loss of photosynthesis reduced CO₂ uptake, loss of chlorophyll and impaired oxidation-reduction chain linking Photosystem II to Photosystem I. Similarly, Hassell (1979) reported that ‘Sugar Baby’ water-melon seedlings, exposed continuously to 1°C or 4.4°C for up to 60 hrs, became chlorotic.
and some seedlings died. Hassell (1979) opined that severe leaf yellowing and lowered chlorophyll levels suggest that low temperature stress might have interfered with chloroplast function and ultimately decreased fresh weight and dry weight. It has also been found that heat shock protein (HSP) involved in the development of thermotolerance, reduced the primary root growth which in turn decreased fresh weight in maize (Nieto-Sotelo et al., 2002). Longenberger (2005) found that consistent heat stress significant by reduced root growth, fresh weight and dry weight at the high temperature, i.e., 36/32°C day/night.

Decreased fresh and dry weight was reported in long photoperiod conditions whereas it increased in short photoperiods in comparison to control in the present study. Similarly, in *Anoectochilus formosanus*, dry weight was higher in plants growing under low light conditions whereas fresh weight was lowest for the plants growing under high light conditions (Stancato et al., 2002). An increase in light intensity increased the ratio of dry weight to fresh weight (Zengqiang et al., 2010). The plants that grew under higher light intensity showed decreased fresh weight and dry weight (Mackay et al., 1990). According to Eliasson (2006) increase in dry weight was strongly dependent on light as etiolation effect at low light increased cell division and cell elongation which in turn increased fresh and dry weight. However, continuous light for 24 hrs decreased growth and yields of tomato and sweet pepper plants because of chlorosis and blistering of leaves (Verzina et al., 1991; Sysoeva et al., 2010), which ultimately decreased fresh and dry weight of plant.

5.1.4 Leaf area

In the present study the plant leaf area under different stress conditions of temperature showed a progressive decrease (Fig. 4.3). However, in light stress plant leaf area increased in short photoperiod and decreased in long photoperiods. The literature is replete with reports where reduction in leaf area due to temperature variation and light stress cause reduced crop yield through reduction in photosynthesis. High temperature causes loss of water in the cells and the total leaf area per plant decreased significantly due to heat stress (Warrington et al., 1977; Noohi et al., 2009; Saini et al., 1988; Acevedo et al., 1990). However, contradictory results were presented by Tadesse et al. (2000) who reported that reduction in temperature increased leaf area. Leaf area decreased curvilinearly in *Withania somnifera* exposed to low temperature in the present
Similar findings for decrease in leaf area were reported in corn (Wolfe, 1991), cucumber (Cucumis sativus L.) (Bulder et al., 1987), and muskmelon (Korkmaz and Dufault, 2001). Dash and Shree (2013) observed that the prolonged exposure of leaves to low temperature resulted in selective inactivation of the oxygen evolving system in cucumber, bean, and tomato. Wolfe (1990) reported in Pisum sativum that 40–50% of leaf area reduced due to cooler temperature.

Many researchers have found that leaf attributes, such as leaf life span, leaf weight ratio, specific leaf area, and leaf area ratio vary significantly with light (Poorter et al., 1990; Lambers and Poorter, 1992; Reich et al., 1992; Walters et al., 1993; French and Noore, 2003). Leaf area index was affected by light, apparently because of morphological plasticity (increased leaf length and width), increased photosynthetic efficiency, and increased chlorophyll concentrations under low light (Mascarini et al., 2001). Butler (1961) found reduction in leaf area and attributed it to the effect of light which may be divided into two phases, a sensitive effect on cell multiplication and a less sensitive one on cell enlargement. Growth in 0.1 f.c. results in an increase in leaf area over that attained in darkness mainly due to an increase in the number of cells. Further and more marked increases in area at 100 f.c. and 1,000 f.c. were due to effect on cell size. Low light in short photoperiods reduced internode length, owing to an effect on cell multiplication. Both internode length and the number of cells in leaves were gradually further reduced as the intensity is increased to 1,000 f.c. in the seedling (Butler, 1961). In Withania somnifera reduced leaf area in short and long photoperiods may be due to restricted cell multiplication and cell elongation.

5.1.5 Membrane stability

Cell membranes are probably the first line of defense against the adverse environmental or abiotic stresses. Variation in temperature from control condition considerably decreased the membrane stability both in low and high temperature in the present study (Fig. 4.8). A major impact of plant environmental stress is cellular membrane modification, perturbed function or total dysfunction. The cellular membrane dysfunction is well expressed in increased permeability and leakage of ions. Lukatkin (2003) and Farooq et al. (2008) observed a gradual increase in leakage of ions from the cells upon prolongation of chilling exposure in maize hybrids. Similarly, change in cell membrane properties due to cold stress resulted in loss of membrane semipermeability, membrane rupture and alteration in membrane transport properties (Palta, 2014). Injury to membranes from a sudden heat stress even may result from either denaturation of the membrane...
proteins or from melting of membrane lipids which leads to membrane rupture and loss of cellular contents (Ahrens and Ingram, 1988). Heat stress may be an oxidative stress (Lee et al., 1983), and peroxidation of membrane lipids has been observed at high temperatures (Mishra and Singhal, 1992; Upadhyaya et al., 1990), which is a symptom of cellular injury. In addition, by causing injuries to the cell membrane, organization of microtubules and ultimately to the cytoskeleton, heat stress changes membrane permeability and alters cell differentiation, elongation, and expansion (Smertenko et al., 1997; Potters et al., 2009; Rasheed, 2009). High temperature stress tolerance in *Brassica juncea* germplasm was due to high membrane stability index (Bhagirath Ram, 2014). Membrane disruption due to high temperature, alter water, ion and organic solute movement, photosynthesis and respiration (Christiansen, 1978). In order to tolerate high temperatures, plants must maintain membrane fluidity within a biologically functional range. The degree to which membrane fluidity increases with temperature is dependent on membrane composition (Raison et al., 1982; Los and Murata 2004; Larkindale and Huang 2004; Sharkey, 2005).

Light stress significantly affected the membrane stability in present study as it decreased in short photoperiods as well as in long photoperiods. Tardy and Havaux (1997) reported that when barley leaves exposed to long photoperiods led to massive conversion of the xanthophyll and violaxanthin to antheraxanthin and zeaxanthin. The light-induced membrane rigidification was proportional to the amount of zeaxanthin present in the membranes which slowly reversed in the dark. The amount of xanthophyll-cycle pigments found in photosystem II was observed to significantly decrease after illumination (Tardy and Havaux, 1997). Membrane stability reduced in high light whereas low light had no significant effect on the membrane stability as reported by Yang et al. (2008) in *Picea asperata*.

### 5.1.6 Relative water content

Relative water content (RWC) is a measure of plant water status in terms of the physiological consequence of cellular water deficit. RWC is the major tool for assessing changes in plant water relations for studying plant responses to stress and subsequent relation to stress tolerance. Both temperature and light treatments given to *Withania somnifera* considerably affected relative water content in the present study. Farooq et al. (2009) found that RWC was influenced by leaf temperature. In dry environments, higher temperatures led to higher vapour
pressure deficits which drove higher evapotranspiration. As soil water is depleted, RWC and water potential of leaf decreased. However, evaporation from the leaf surface enhances leaf and canopy cooling, so overheating may be ameliorated by higher rates of transpiration (Martinez and Ballesta, 2004; Machado and Paulsen, 2001; Almeselman et al., 2009 and Sairam et al., 2000). In the present study, high temperature decreased relative water content as soil water decreased in W. somnifera. Transpiration rates also increased with increasing temperature but only for certain limit beyond which transpiration rate decreased due to water deficiency and with a concomitant increase in leaf temperature (Machado and Paulsen, 2001). It has been observed that the species which are better adapted to dry environment have higher relative water content at given water potential (Jarvis and Jarvis, 1963). Leaf relative water content reduced in response to soil cooling. According to Anderson and McNaughton (1973) there was a critical relative water content above which both transpiration and photosynthesis are insensitive to water reduction. Root chilling which limits photosynthesis is responsible for the reduction of relative water content in low temperature.

The effect of variation in light from control condition considerably decreased relative water content in the present study. The relative content of water in the leaves depend on light conditions of growing plant (Ivanov, 2010). Relative water content markedly increased in light treated plants and reduced in drought in Spilanthes acmella (Reshmi and Rajalaxmi, 2012). In light tolerant genotype the water potential was reduced in 84%, however in the susceptible genotype the reduction was 26% (Nava et al., 2013). The decreased relative content of water in the leaves greatly depended on light conditions of growing plant. Relative water content decreased with short photoperiods due to etiolation effect. (Ivanov, 2010).

5.2 Biochemical Parameters

5.2.1 Carbohydrates

An alteration in carbohydrate contents under abiotic stress has been reported by a number of researchers (Tammam et al., 2008; Hasaneen et al., 2009). In the present study temperature treatment (high & low) increased the carbohydrate contents except in 38°C plants where it decreased by 21.43% compared to control plants (Fig.4.6). Earlier studies revealed that high temperature altered carbohydrate partitioning in potato (Solanum tuberosum L.) plants from tubers to shoots and reduced overall plant yield (Wolf et al., 1990; Stancato et al., 2001). Liu
and Bingru (2000) reported that reduction in carbohydrate concentrations in shoots was more pronounced than that of roots. It has been suggested that high carbohydrate availability, particularly glucose and sucrose, during heat stress was an important physiological trait associated with heat-stress tolerance in creeping bentgrass (Madan et al., 2014). Similar to our results, carbohydrates content in the leaves was elevated significantly under heat stress but a dramatic decrease in the sucrose content was reported upon exposure to 40°C and 45°C in *Capsicum annum* L. (Sepulveda and Kliwer, 1986) and to 40°C in grapevines (Wahid, 2007). Long exposure of high temperature may reduce photosynthesis ultimately carbohydrates but increases respiration, leading to an imbalance between the two processes as reported by Carrow (1996); Huang and Gao (1999); Huang et al. (1998) and Liu and Huang (2000). According to Ipek (2007) decrease in sucrose concentration at 40°C resulted from reduction in the activity of invertase which breaks down sucrose into glucose and fructose. Additionally, the reduced carbohydrate levels between 35°C to 40°C in both shoots and roots may also be caused by increase in photorespiration under heat stress, which reduced photosynthetic efficiency (Nilsen and Orcutt, 1996). Change in carbohydrates due to temperature stress in *W. somnifera* could be because of the same reasons as revealed by previous researches. However, contradictory to our findings, no significant difference was observed in carbohydrates content under heat stress and normal conditions in wheat grain (Zamani et al., 2014). Low temperature in the present study significantly decreased carbohydrates content in *W. somnifera*. According to Saghfi and Eivazi (2014) leaf soluble carbohydrates, glucose, ramnose, mannose and fructan decreased in leaves exposed to cold stress as reduction in photosynthesis and respiration has been observed which supported the low content of carbohydrates in cold stressed plants.

A decrease in photoperiod from 14 hrs to 8 hrs, carbohydrate content decreased whereas carbohydrates content increases in small quantity as photoperiod increased from 16 hrs to 24 hrs. The light stressed seedlings showed an elevated content of sugars in comparison with dark grown seedlings (Gill et al., 2001). Similarly, presence of high amount of sucrose has been observed under high light by Kozak (1991). Light intensity had significant effect on the enzymes involved in starch synthesis in grains as well as the translocations and quantity which affected the carbohydrate content in rice (Qi-hua et al., 2014). A reduction in the photosynthesis in long photoperiods was reported by Stutte et al. (1996) who attributed that increased amount of
carbohydrates is because of high starch accumulation in leaves, suggesting that photosynthetic production (source) was greater than photosynthate utilization (sink).

5.2.2 Protein

Abiotic stresses usually cause protein dysfunction. Maintaining proteins in their functional conformations is important for cell survival under stress (Feller et al., 2008). Protein content increased with increase as well as decrease in temperature from normal condition in the present study. High temperature was reported to increase the proportion of gliadins to glutenins in wheat varieties (Daniel and Triboi, 2000; Dupont and Altenbach, 2003; Martre et al., 2006). An increase in protein content under induced temperature stress is attributed to escalation in biosynthesis of heat shock proteins, along with antioxidant, metabolites like polyphenols (Rutuja and Mala, 2014). According to Baruah et al. (2005) protein content in field-grown plants, undergoing natural temperature stress was greater in open sun than shaded environments. A study conducted by Inbaraj and Muthuchelian (2011) revealed that interaction of light and temperature significantly influenced protein accumulation in field-grown plants. Proteins synthesized in response to high temperature are called dehydrrins (Close and Chandler, 1990) and accumulates in a wide range of plant species under dehydration condition caused by heat stress. The accumulation of dehydrin like proteins was detected in the roots and leaves of heat-stressed plants, which could protect plants from further dehydration damage (Bewley et al., 1983; Mohammadkhani and Heidari, 2008). Increased content of protein in W. somnifera could be attributed as tolerance to heat stress in the present study. However, contradictory to above results Castro et al. (2009) reported that heat stress did not noticeably influence the protein quantity of wheat genotypes. Sharma et al. (2013) showed that osmotic and temperature stress generated significant reduction in total protein contents in Anabaena.

In the present study light stress decreased protein content in long photoperiods (16 hrs and 24 hrs) and increased in short photoperiod (12 hrs and 8 hrs). Similar results were reported by Geetika and Sumit (2014) in Brassica juncea. Variation in light from control condition resulted in the release of reactive intermediates of reduced dioxygen such as superoxide radicals, hydroxyl radicals, hydrogen peroxide or singlet oxygen (Adamska, 2006). In order to maintain their normal function under light stress conditions, chloroplasts develop multiple repair and protection systems. The induction of specific light stress proteins, the ELIPs (for early light-induced proteins), are
considered to be part of these protective responses and its accumulation is correlated with the photoinactivation of PSII, degradation of the D1-protein of PSII reaction centre and changes in the level of pigments (Adamska, 2006). Like our results, Wanjun et al. (2014) reported reduced amount of protein under low light condition compared to control.

5.3 Antioxidant system (Non-enzymatic)

5.3.1 Chlorophyll

Abiotic stresses (temperature and light) affect the chlorophyll content and limit plant growth (Levitt, 1980). In the present study, chlorophyll content increased with decrease in temperature and decreased with increase in temperature compared to control conditions. Berry and Bjorkman (1980) reported that photosynthesis and chlorophyll content is most sensitive to high temperature stress. Inhibition of photosynthesis due to high temperature stress is a common phenomenon in tropical and subtropical plants when exposed periodically to high temperatures (Larcher, 1995). Heat stress induces changes in respiration and photosynthesis and shortened life cycle and diminished plant productivity (Barnabas et al., 2008). The early effects of thermal stress comprise of structural alterations in chloroplast protein complexes and reduced activity of enzymes (Ahmad et al., 2010). The photochemical modifications in the carbon flux of the chloroplast stroma and those of the thylakoid membrane system are considered the primary sites of heat injury (Wise et al., 2004), as photosynthesis enzymes of the Calvin–Benson cycle, including rubisco and rubisco activase, were severely inhibited even at low levels of heat stress (Maestri et al., 2002; Morales et al., 2003). By increasing chlorophyllase activity and decreasing the amount of photosynthetic pigments, heat stress ultimately reduces the plant photosynthetic activity (Todorov et al., 2003; Sharkey and Zhang, 2010). In Withania somnifera (present study) decreased amount of chlorophyll in long photoperiods conditions could be attributed to reduced photosynthetic activity. Chlorosis of leaves is the first visual symptom of heat stress leading to senescence (Fletcher and Hofstra, 1988) and is associated with a concomitant decline in concentration of photosynthetic pigments (Fletcher and Hofstra, 1990; Goasavi et al., 2014). Cold stress in wheat seedlings increased chlorophyll a amount indicating that chlorophyll participated in the resistance to low temperature (Pyatygin, 2008; Stanetska et al., 2011; Babenko et al., 2014). However, contradictorily, reduced chlorophyll content in cold stress was reported by Qi Zhang et al. (2014) which may be due to injury to photosynthetic apparatus.
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Variation in chlorophyll content in light stress is due to onset of protective mechanisms in plants (Phonguodume et al., 2012). In the present study chlorophyll content decreased with decrease as well as increase in photoperiod from control conditions. Phonguodume et al. (2012) reported that variation in the chlorophyll content in two light intensities was observed where high light intensity decreased chlorophyll content (21%). The decrease in the amount of chlorophyll was due to increase in chlorophyllase activity in low light conditions whereas in high light damage to structural component of chlorophyll was the reason as reported by Jeanty (2008). Our results are in agreement with Jenabiyan et al. (2015) who found that increased light intensity decreased chlorophyll content.

5.3.2 Carotenoids

Carotenoids are isoprenoid molecules synthesized by all photosynthetic and non – photosynthetic organisms (Andrew et al., 2008). Abiotic stresses applied (temperature and light) affected the carotenoid content as high temperature as well as low temperature reduced it in the present study. Duivier et al. (2013) reported that total carotenoid content decreased in plants subjected to low temperature (25°C) and at high temperature (75°C) than control conditions in Ipomoea batatas. According to Fletcher and Hofstra (1990) the leaves of corn plants after high temperature stress were found chlorotic and carotenoids markedly decreased. Carotenoids in addition to function as an accessory pigment, act as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them (Havaux, 1993). The levels of α-carotene and β-carotene were significantly reduced under cold stress in rice (Du et al., 2012). However, Ntatsi et al. (2014) in tomato (Solanum lycopersicum cv. Kommeet) found that low and intermediate temperature increased carotenoid content in roots to higher levels. Webb and Fletcher (1996) reported that phytoene synthase, the first committed step in carotenoid biosynthesis, decreased at high temperature. Similarly, carotenoid decreased in high temperature and low temperature treated seedlings of wheat (Webb and Fletcher, 1996). As the phytothene synthase formation is low in low temperature treated plants, carotenoids production decreased. In Nannochloropsis oceanica it was found that continuous light stress resulted in reduction of pigments, especially carotenoids which is an adaptive mechanism to cold stress tolerance (Solovchenko, 2014). Carotenoid content decreased in both short as well as long photoperiods in the present study.

5.3.3 Alkaloids
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Discussion

Alkaloids are naturally occurring, complex organic molecules that contain nitrogen and have physiological effect on organisms through wide range of effects. Varied light and temperature conditions affected the alkaloidal content in the present study. It decreased in both low and high temperatures. Similar results were reported by Jansen at el. (2009) where influence of high temperature on alkaloid content in rice was studied and found to be increased in the seeds but decreased in seedlings. However, increase in indole alkaloid accumulation in high temperature was found in *Catharanthus roseus* (Tovonen et al., 1992; Amirjani, 2013). Hoff et al. (1996) reported that heat stress reduced photosynthesis in plants and allocated almost equal proportions of leaf nitrogen to alkaloids in *Shorea javanica*. In cold treatment a significant reduction in the biosynthesis of tropane alkaloids in *Datura innoxia* was found which was due to cell injury due to crystal formation in cold stress (Yadav, 2010).

Light play a significant role in synthesis of alkaloidal content in plants. Hoff et al. (1996) found that high light intensity lowered alkaloid content but promoted growth. In present study alkaloidal content decreased in short photoperiods and increased in long photoperiod conditions in comparison to control conditions. Ralphas et al. (1998) found that alkaloidal concentration was lower in larkspur plants growing beneath forest canopy and in potted plants kept in shade (70% reduction in sun light) than plants growing in open sunlight. Photoperiods directly influence photosynthesis, respiration and other metabolic process which indirectly triggered alkaloidal production. As photosynthesis is reduced in continuous light, leaf nitrogen increased which ultimately increased alkaloidal content (Ralphas et al., 1998). The *Catharanthus roseus* was subjected to different photoperiod conditions and three alkaloids viz., vinblastine, vindoline, and catharanthine, increased in long photoperiods (Jaleel et al., 2014).

5.3.4 Flavonoids

Flavonoids content increased in *Withania somnifera* in temperature as well as light stresses in the present study. As evident from literature high temperature had a rapid positive effect on isoflavone concentration in Soybean (Zhang et al., 2010). Tang et al. (2014) reported that in *Vigna radiata* lavone, isoflavone, flavonoids, and isoflavonoids increased in abiotic stress. Mori et al. (2007) reported that *Vitis vinifera* when subjected to high (35°C in daytime/25°C in night time) and low (25°C in daytime/20°C in night time) temperatures showed significantly increased in anthocyanin concentrations in the berry skins in low temperature whereas anthocyanin
concentrations were almost stable at high temperature. The increase in flavonoids content could be a defense mechanism against heat stress as supported by Tarara et al. (2008) and Spayd et al. (2002). The flavonoid content increased in short as well as long photoperiods in *Withania somnifera* in the present study. It was reported that the expression of flavonol synthase genes was induced by light significantly (Downey et al., 2004; Fujita et al., 2006). Yamamoto et al. (2011) reported that temperature and light influence the anthocyanin and total phenolic concentration of fruits. The increased flavonoid content may be attributed to increased activities of enzymes involved in flavonoid production in plants in light stress condition (Hall and Stark, 1972).

### 5.3.5 Phenol

In the present study, foliar phenol contents were higher in all the treated plants (variation in temperature and light regimes) as compared to control. Akowuah et al. (2009) opined that the higher the temperature greater is the total phenol content. The metabolism of soluble phenolics is regulated by the activity of various enzymes. The synthesis of the phenyl-propanoid skeleton in higher plants is the deamination of the L-phenylalanine catalysed by the enzyme PAL, which is commonly considered the principal enzyme in the biosynthesis of phenolic compounds. It has been demonstrated that heat and cold stress induced the production of soluble phenolics and thereby increased PAL activity. In addition, the metabolism of phenolic compounds also includes the action of oxidative enzymes such as POD (peroxidase) and PPO (polyphenol oxidase), which catalyze the oxidation of phenols to quinones (Rivero et al., 2001). Increased amount of phenol in *W. somnifera* could be attributed to increased PAL (Phenylalanine ammonia lyase) activity in temperature stress condition which is an adaptive mechanism to stress tolerance. Bennici et al., (2005) reported in *Sarcoscypha occidentalis* that leaves accumulate phenolic compounds as a function of the length of the daily light period. In the light, significant amount of phenol accumulated over a temperature range of 12°C -28°C was observed while in the dark accumulation exhibited a monotonic relationship with temperature Bennici et al., (2005).

### 5.3.6 Phytosterol and Saponin

Abiotic stress has adverse impact on the phytosterol and saponin content in plants. Phytosterols are present as free sterols or in conjugated forms (steryl esters, acyl steryl glycosides, and steryl glucosides) which are enzymes, channels, and receptors or other components of signal transduction pathways (Schaller, 2004). In the present study phytosterol and saponin content increased with high temperature and long photoperiods and decreased with low temperature and
short photoperiods in comparison to control plants in *Withania somnifera*. According to Lee *et al.* (2004) the overexpression of Panax ginseng squalene synthase gene (PgSS1) due to high temperature resulted in remarkable increase of phytosterols as well as ginsenosides (Jiewen *et al.*, 2014). Total phytosterol levels and composition changed, with greater percent of campesterol and lower percent stigmasterol and β-sitosterol at higher temperatures in Soyabean. *Vigna radiata* showed variation in phenol and saponin content under abiotic stress as studied by Tang *et al.* (2014). Roche *et al.* (2010) reported that high temperature treatment to sunflower induced in total sterol concentration up to 35%. The variation in temperature during the plant growth in *Dioscorea pseudojaponica* affected the saponin level (Ramakrishna and Ravishankar, 2011). The highest level of saponins was detected with the high temperature whereas it slightly decreased in low temperature condition.

The availability, intensity and quality of light are major components of the environment that can influence the saponin content of plants. The level of soya saponins in germinating Glycine max seeds was found to be considerably higher in seeds subjected to day/night periods than in seeds germinating in total darkness, although light irradiation did not produce an equal effect on the biosynthesis of all the tested compounds (Shimoyamada and Okubo, 1991). The effect of light on ginsenoside content was investigated in *Panax quinquefolius* which showed increased content in continuous light (Ramakrishna and Ravishankar, 2011). Exposure to more than 36% of the solar radiation resulted not only in reduced ginsenoside accumulation, but also caused photoinhibition, photobleaching and if the light conditions persisted, premature leaf death. Variation in light availability inside a tree crown influenced the amount of saponins was demonstrated for the fruiting African tree *Diospyros abyssinica* by Szakiel *et al.* (2011).

### 5.4 Enzymatic antioxidants

#### 5.4.1 Superoxide dismutase (SOD)

SOD is one of the ubiquitous enzymes in aerobic organisms and play a key role in cellular defense mechanism against reactive oxygen species (ROS). In our findings SOD increased with variation in temperature and light from control condition. It is evident from the literature that increased SOD enzyme activity under low and high temperature stresses was reported by number of researchers. Schoner and Krause (1990) reported that only cytosdolic SOD mRNA level
significantly increased in *Nicitiana plumbaginifloia* exposed to high temperature. Similarly, mRNA level of chloroplastic SOD raised at combined effects of chilling and intense light conditions in *N. plumbaginifloia* (Kels and Oncel, 2000). A significant increase was found in the activity of SOD due to high temperature in five wheat genotypes viz., PBW 343, PBW 175, HDR-77, HD 2815 and HD 2865 (Almeselmani *et al.*, 2006). Superoxide dismutase converts one form of ROS (O$_2$) to another equally toxic one (H$_2$O$_2$). However higher activity of SOD alone is not sufficient for providing heat stress tolerance, as the enzymes for H$_2$O$_2$ scavenging (APX, GR, CAT and POX) are lacking in PBW 343(wheat genotype) and other susceptible genotypes. Thus, the higher SOD under very high temperature condition in PBW 343 is of little consequence in the absence of inadequate H$_2$O$_2$ scavenging mechanism. Variable degree of stimulation was also reported in the activities of SOD in leaves of *Arachis hypogaea* L. irradiated with selected doses of light (Sreedhar M. *et al.*, 2013). Exposure of plant cells to light leads to the formation of ROS and SOD removes superoxide formed during radiation exposure and also inhibits formation of more reactive pro-oxidants (Sreedhar *et al.*, 2013).

### 5.4.2 Peroxidase (POD)

Peroxidases are enzymes involved in several reactions such as indoleacitic acid oxidation, lignification, phenol oxidation, pathogen defense and cell wall elongation in plants (Urs *et al.*, 2006; Passardi *et al.*, 2007). In the present investigation peroxidase activity was found to be higher in all the treatments of temperature and light stresses. Effect of temperature was investigated on litchi and asparagus where increased amount of peroxidase activities was confirmed in these species (Rodrigo *et al.*, 1996; Mizobuts *et al.*, 2010). Liu *et al.* (2013) reported that low temperature induced increased activity of POD in *Avena nuda*. It is attributed that low temperature affected RNA transcription and translation and enhanced the synthesis of POD (Liu *et al.*, 2013). Benedict (1971) studied influence of light on peroxidase activity in tomato (*Lyeopersicon esculentum* Mill. cultivar Heinz) and found high level of enzymatic activity with high light. Light and temperature variations influenced antioxidant activity in *Raphnus sativum* (Wang *et al.*, 2009), in *Beta vulgaris L.* (Panagopoulos, 1990) and in *Spinacia oleracea* (Penel and Greppin, 1979) which supported our present investigation.
5.4.3 Catalase (CAT)

Catalase is the principle enzyme that scavenges harmful oxygen species in plants. In the present study catalase activity increased in temperature (high and low) and light (short and long) treatments suggesting that it protects against oxidative damage due to light and temperature stresses. In *Avena nuda* CAT activity was higher under the cold treatment than normal temperature (Liu et al., 2013). Catalase activity is also associated with the scavenging of H$_2$O$_2$ and an increase in its activity is related with increase in stress tolerance (Almeselmani et al., 2006). In *Eupatorium adenophorum*, increase in activities of antioxidant enzymes was effective in protecting the plant from the accumulation of active oxygen species (AOS) at low temperature, but the activities of catalase (CAT) were not accompanied by increase of superoxide dismutase (SOD) during the heat treatments (Lu et al., 2008). Catalase eliminates H$_2$O$_2$ by breaking it down directly to form water and oxygen. Interestingly, in leaves of *E. adenophorum*, CAT activity was depressed by high temperature and increased to a high level in the low temperature treatment. However, in leaves of *E. odoratum* the response pattern of CAT activity was reversed, CAT activity enhanced by high temperature and the plants were unable to maintain normal activity when subjected to low temperature. A decrease in CAT activity was reported by many researchers when the plants were subjected to abiotic stress (Saruyama and Tanida 1995; Fu and Huang 2001; Jung, 2003). Feierabend et al. (1992) found that CAT activity is photoinactivated under low and high temperature stresses and CAT activity varies with plant species. At high light period the increased catalase activity was reported but it decreased drastically with further increase of light duration in *Lactuca sativa* L. (Fu et al., 2012). Effects of light intensity on *Calathea* showed that under low light catalase activity increased significantly (Vanhuylenbroeck et al., 2000). Although in the present study H$_2$O$_2$ or ROS was not measured, increase in catalase was likely to have happened due to responses of antioxidant enzymes as suggested by Olmas et al.(2003).

Antioxidant enzymes play a crucial role in detoxification of ROS and generation of antioxidants in response to prevailing stress. Accumulation of the ROS, H$_2$O$_2$ induced by various environmental stresses result in the combined activity of CAT and POD (Blokhina et al., 2003). Our results are not in agreement with heat stressed mustard (Dat et al., 1998) and drought stressed pea (Moran et al., 1994) which exhibited a significant increase in endogenous H$_2$O$_2$ and marked decline in CAT. Increased activity of CAT was suggested by
Agarwal and Pandey (2004) as an adaptive mechanism to reduce the H$_2$O$_2$ and offer protection against oxidative damage in *Cassia angustifolia*.

**5.4.4 Ascorbate peroxidase (APX)**

Ascorbate peroxidase exists as isoenzymes and plays an important role in the metabolism of H$_2$O$_2$ in higher plants. It eliminates ROS through multiple mechanisms and is also donor of electron for APX-mediated H$_2$O$_2$ detoxification (Navabpour *et al.*, 2003). In the present study, ascorbate peroxidase content decreased in the plants growing in low temperature and short photoperiod but it increased with an increase in temperature and photoperiod (Fig 4.14). Similar results were reported by Almeselmani (2006) where it was found that effect of high temperature stress on the antioxidant enzyme activity in five wheat genotypes resulted in an increase in APX content. Tolerance to high temperature stress in crop plants has been reported to be associated with an increase in antioxidant enzymes activity. Heat inducible transcriptional activation of cytosolic ascorbate genes corresponds with an increase in APX activity (Almeselmani, 2006). Remarkable increase in APX levels indicated the induction of antioxidant mechanism such as ascorbate cycle in French bean (Halliwell and Gutteringe, 1989; Koca *et al.*, 2007). In *Eupatorium adenophorum*, increase in the activities of antioxidant enzymes including APX was effective in protecting the plant from the accumulation of active oxygen species (AOS) at low temperature and during the heat treatments (Lu *et al.*, 2008). Effects of light intensities on antioxidant enzymes in *Phalaenopsis* was reported by Ali *et al.* (2007). Loewus (1980) reported an increase in APX activity in leaves up to 50% in long photoperiods compared to *in vitro* grown plantlets where no changes were observed. Significant enhancement of APX indicated the oxidation by molecular oxygen of ascorbate peroxidase to dehydroascorbate with the formation of H$_2$O (Esaka *et al.*, 1992). Light stress may modify the APX levels and maintains the redox cellular balance, which provides additional support to the plants against the oxidative stress (Potters *et al.*, 2000).

**5.4.5 Glutathione reductase (GR)**

Glutathione reductase is a flavoprotein that catalyzes reduction of oxidized glutathione. The enzyme maintains adequate levels of reduced cellular glutathione which is essential for protection against oxidative stress. Among the various antioxidant examined, glutathione
reductase decreased slightly in all the stress treatments of temperature and light in the present study. Our observations are in contrast to those reported during low temperature stress in pea and maize, wherein a rise in the GR activity was reported (Edwards et al., 1994; Prasad et al., 1995). Kocsy et al. (2002) revealed that Glutathione is an important component of the ascorbate-glutathione cycle and participates in the removal of hydrogen peroxide which may be accumulated during high temperature-induced oxidative stress. Nieto-Sotelo (1989) reported an increase of GSH content and a decrease of glutathione reductase content in maize subjected to heat stress. In wheat reduced GSH due to GR activity in the frost-sensitive variety during heat stress was reported. Kocsy et al. (2000) reported during low and high temperature stress the glutathione reductase affected wheat GSH and hmGSH accumulation and oxidative stress. High and low temperature stress caused increase in GSH and hmGSH contents in the frost-tolerant wheat variety. Similarly, decreased glutathione reductase and increased glutathione were reported in durum wheat and mustard (Paolacci et al., 1997; Dat et al., 1998). Exposure of Arabidopsis plants that were maintained under low light to excess light for 1 hr caused photooxidative burst of hydrogen peroxide (H₂O₂) associated with photoinhibition of photosynthesis, resulted in a rapid increase in glutathione and reduction in glutathione reductase.

5.4.6 Glutathione –S-transferase (GST)

The glutathione-S-transferases (GST) represent a major group of detoxification enzymes. It decreased in all the treated plants in the present study as shown in the Fig.4.14. GSTs are involved in response to oxidative stress including temperature, drought, light and heavy metals. Under oxidative stress, excessive reaction oxygen species (ROS) induce decrease in GST which metabolize the toxic products and damage DNA and other products (Liu et al., 2012). The low temperature decreased GST specific activity and glutathione (GSH) pool size in resistant and susceptible Alopecurus myosuroides biotypes and it deactivated the ROS generated oxidative stress (Milner and Kochian 2007). Zuo et al. (2000) and Yung and Lv (2006) inferred that GST would play the protective role under heat stress by inactivating the excessive metabolic toxicants. Heat treatment significantly elevated mRNA level of GST (Yao et al., 2011). In the present study GST content decreased in high temperature and light stress conditions (short photoperiods and long photoperiods). Green and Fluhr (1995) opined that high light generate oxidative stress through reactive oxygen species in plants leading to the production of oxidative damage. GSTs
participate in the detoxification of products created by oxidative damage and thereby protect cells against such stress (Dixon et al., 1998; May et al., 1998). Loyalla-Vargas et al. (2007) disapprove the possibility of GST action due to high light.

5.5 Secondary Metabolites

Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions (Ramakrishna and Gokare, 2011). The temperature and light stress can affect secondary metabolites and other compounds that plants produce which are usually the basis for their medicinal activity (Zobayed et al., 2005; Salick et al., 2009). In the present study, variation in the withanolides quantity was noticed in different temperature and light conditions. A reduction in withanolides quantity was evident in low temperature and low light (short photoperiods) whereas it increased significantly in high temperature and high light conditions (long photoperiods). Results revealed that secondary metabolites content was more influenced by variation in light regimes in comparison to varied temperature conditions. Bilal Ahmad Mir et al. (2014) reported that in Withania somnifera leaves high temperature increased production of withanine and withaferin A and these withanolides may have a role in combating oxidative stress. The three withanolides (withanolide A, withaferin A and withanolide D) showed insignificant and irregular fluctuations in content in W. somnifera during the period of temperature stress in the present study. A study carried out by Kumar et al. (2012) were of view that seasonal temperature played a key role in increasing secondary metabolites (withanolides) rather than the phenological stage of the plant.

Temperature is one of the most important environmental factor that affects growth and development of plants (Noohi et al., 2009). High temperatures can result in repression of several important cellular pathways by repressing the synthesis and activities of protein and secondary metabolites involved. Several studies have examined the effects of increased temperatures on secondary metabolite production of plants (Jochum et al., 2007). Some report that secondary metabolites increase in response to elevated temperatures (Litvak et al., 2002), while others report that they decrease (Snow et al., 2003). Withanolides content increased in high temperature and decreased in low temperature conditions in the present study. Temperature strongly influences metabolic activity and plant ontology, and high temperatures can induce premature leaf senescence (Morison and Lawlor, 1999). Jochum et al. (2007) reviewed that elevated temperatures increased
leaf senescence and root secondary metabolite concentrations in *Panax quinquefolius* because elevated temperatures reduced photosynthesis and biomass production. Gera *et al.* (2007) reported that temperature variations had multiple effects on the metabolic regulation, permeability, rate of intracellular reactions in plant cell cultures and subsequently affect growth and secondary metabolite production. Temperature and light quality influenced on production of ginsenoside in *Panax ginseng* (Yu *et al.*, 2005). In *Eleutherococcus senticosus* low (12°C and 18°C) and high (30°C) temperature caused significant decrease in total eleutheroside accumulation while low temperature increased it. However, according to Gera *et al.* (2007) despite the negative effect of elevated temperatures on root biomass at the end of the growing season, the concentration of total storage root ginsenosides was 49% higher when plants were grown at higher rather than lower temperatures. Generally when plants are stressed, secondary metabolite production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed not allocated to growth is instead allocated to secondary metabolites (Mooney *et al.*, 1991). Increased withanolides content in *Withania somnifera* could be attributed to allocation of carbon to formation of secondary defense instead of biomass production in plant in high temperature stress in present study. Low temperature is one of the most harmful abiotic stresses affecting temperate plants. Janska *et al.* (2010) reported that cold stress could impose osmotic injury and desiccation of plants. Some researchers emphasized on increased production of secondary metabolites in low temperature condition i.e. Pedranzani *et al.* (2003) in *Pinus pinaster*, Lei *et al.* (2004) in carrot, and Hummel *et al.* (2004) in *Pinus antiscorbutica*. However, withanolides content decreased in *W. somnifera* in present study in low temperature conditions. Cold temperatures can induce ice formation in plant tissues, leading to cellular dehydration, imbalanced osmotic homeostasis which could be a reason for decreased content of withanolides in *Withania somnifera*. Our present results were supported by Uemura *et al.* (1995), Prasad (1996), Sharma *et al.* (2005) and Chinnusamy *et al.* (2007).

Light as an energy source for plant life is known to affect plants dually. It affects photosynthetic rate and assimilate accumulation, thereby playing a substrate role, but also controls plant growth and development, in that way playing a regulatory role (Sysoeva *et al.* 2010). While plant growth in short-day and long-day conditions showed variation than in control condition (Adams and Langton, 2005). Withanolides content increased in long photoperiods whereas decreased in short photoperiods in comparison to control condition in *Withania somnifera* in the present study. Increased content of withanolides could be attributed to the fact that in long...
photoperiods light energy is not exclusively used for photosynthesis. Chlorophyll molecules absorb light and enter to triplet state, increasing protection of radical scavenging system of plant which ultimately leads to formation of withanolides for protection purpose (Niyogi, 1999; Mittler, 2002). Supporting this a positive correlation between increasing light intensity and levels of secondary metabolites was reported by Chalker et al.(1989). Fett-Neto et al. (1995) reported the effect of white light on taxol and baccatin III accumulation in Taxus cuspidate. Catharanthus roseus plants, exposed to light showed significant increase in the production of vinblastine and vincristine (Bernard et al., 2009). Similarly, increased endogenous indoleamines (serotonin and melatonin) in long photoperiods in Dunaliella bardawil was found (Ramakrishna and Gokare, 2011). In short photoperiods, withanolides content decreased in the present study. Observation was supported by Larsson et al. (1986) where content of phenolic glycosides decreased in shaded willow foliage.

5.6 Herbicidal Effect

Aqueous extracts of Withania somnifera exhibited phytotoxic activity against germination and early seedling growth of the noxious weeds, i.e., Chenopodium album, Achyranthus aspara, Ageratum coenyzoides and Parthenium hysterophorus. The leaf extract of Withania was comparatively more inhibitory to germination and seedling growth of the test weeds than corresponding stem and root extract in laboratory and foliar spray bioassays.

5.6.1 Phytotoxicity of Withania extracts at seed germination level

Germination is the most critical stage in the establishment of weeds (Rohni and Rao, 2000a). Withania extract has shown negative effect on the germination of studied weeds in the present investigation. Herbicidal role of medicinal plants was studied by various researchers. Zhang et al. (2005) studied the inhibitory effect of Lantana camera extracts on germination of Eichhornia. Prasad et al. (2006), Thapar and Singh (2006) and Kumar (2006) noted the effects of Cassiata and many other weeds on the germination, growth and metabolism of Parthenium hysterophorus. Among four weeds studied, many researchers have studied Parthenium hysterophorus. Inhibition in the germination of Parthenium seeds by aqueous leaf extract of tree species i.e Azadirachta indica (L.).A. Juss., Ficus bengalensis L., and Melia azaduarachta L. were studied by Shafique et al.(2005). The inhibitory effects of Cassia on Parthenium hysterophorus and its suppression in nature was reported by Joshi (1991), Kandasamy et al. (1999) and Tefera
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(2002). Thapar and Singh (2003) studied the herbicidal role of *Avena viridis* on *Parthenium hysterophorus* which helped in its management. Rahman (2006) reported reverse trend and indicated that *Parthenium hysterophorus* had negative influence on different species of *Cassia*, through seed germination bioassay studies. Golisz *et al.* (2007) observed the negative effect of buckwheat on germination and growth of associated weeds. The extract of plant could suppress the seed germination of weeds for longer period, which might be due to chemical inhibitors or allelochemicals existing in them (Chepil, 1946). The results of present investigations are in agreement with the above findings as *Withania somnifera* possess variety of allelochemicals in leaves, stem and roots.

In the present study, germination of all the studied weeds were effected by all the *Withania* extracts whereas leaf extract was more inhibitory. The greater inhibitory effect of aqueous extract of aerial parts on germination and growth of test species has also been reported in other plant species by Kil and Yun (1992) and Noor and Khan (1994). Effect of leaf extract was more inhibitory to germination and growth in *Ageratum coenyzoides* in comparison to other weeds. Leaf and stem extracts of *Withania* were more phytotoxic compared to root extracts. In *Chenopodium album*, *Achyranthus aspara* and *Parthenium hysterophorus* leaf affected stem length than root length whereas in *Ageratum coenyzoides* root length was inhibited to greater extent by leaf extract. In laboratory bioassay, it was evident that minimum fresh weight was found in seedlings treated with leaf extract. The germination and seedling growth response of different weeds to aqueous extracts of *Withania* was different. This unequal susceptibility to weeds could be due to inherent differences in the phytochemicals involved in the process. The species specificity of phytotoxicity was demonstrated earlier by Shukla *et al.* (1987) and Noor and Khan (1994). Leaf and stem extract of *W. somnifera* were more inhibitory to germination and seedling growth of studied weeds than root extract indicating that leaves and shoots contain more of inhibitor than the roots. As stated earlier that phytochemicals like flavonoids, alkaloids, phytosterols, saponin etc. were quantitatively different in different organs of *Withania* which could have variable inhibitory effect on the seed germination.

5.6.2 Phytotoxicity at Seedling stage

Foliar spray bioassay on weeds seedlings also supported the earlier results. The stem and root length of weeds was also negatively influenced by aqueous extract of *Withania*. Leaf extract
was more toxic to stem length than root length. Similarly fresh weight and dry weight of seedling was severely influenced by leaf extract. Withania extract also reduced number of shoot and root branches in all the studied weeds. Ageratum coenyzoides was more susceptible to Withania extract. Likewise Javaid et al. (2009) conducted studies on effect of Withania somnifera on Rumex dentatus, Parthenium hysterophorus and Tagetes erectus L and found similar results as in the present study. Similarly, Shafique and Shafique (2011) on the Parthenium hysterophorus have provided very encouraging results. The effect of grasses on the germination of Parthenium hysterophorus as reported by Javaid and Anjum (2006) showed reduction in seedlings root and shoot length and attributed it to reduce rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extract (Buckolova, 1971). Many seedlings lost their ability to develop normally as a result of reduced radical elongation and root nerosis (Belel and Rahimatu, 2012).

Root growth was inhibited by aqueous extracts of many plants (Chung and Miller, 1995). Aqueous extracts of plants generally had more pronounced effect on radicle growth than the hypocotyl growth (Turk et al., 2005). This may be attributable to the fact that roots come in contact earlier with the extracted phytochemicals than the other organs of plants (Abdul et al., 2012). Root growth was more sensitive to the phytotoxin present in the aqueous sorghum and sunflower extract than was the shoot growth (Abdul et al., 2012). The greater inhibitory effect of aqueous extract of aerial part on the germination and growth of the test species than the effect of sub aerial parts had also been reported in other plants (Kil and Yun, 1992; Noor and Khan, 1994).

The four weeds studied are the prominent weeds of mid Himalayan region and the fact that many plants (especially with medicinal values) can be used as herbicide to control such weeds as well as invasive weeds. Earlier initiatives has been proved successful, for examples, Artemisinin, a sesquiterpenes lactone from the Artemisia annum L. is a patent plant growth inhibitor (Ditomaso and Duke,1991). Leptospernone is a known allelochemical from which the triketone class of herbicides was produced (Mitchill et al., 2001). Similarly 1, 8, coneole, a monoterpen, has been identified as one of the most potent allelochemical released by Artemisia spp. and a synthesize analog, cinnmethylinia, being sold as a herbicide in Europe (Duke et al., 2002). As medicinal plants like Withania somnifera are rich repository of phytochemicals and have herbicidal effect over weeds, there is a possibility to commercialise its extracts as herbicide which will prove a milestone in the weed management in the Himalayan region.