Print ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Temperature stress mediated oxidative and antioxidant defense in *Withania somnifera* L. Dunal

Mamta Sharma*, Sunil Puri
School of Biological and Environmental Sciences has been added, Shoolini University, Solan, HP, India
***Corresponding author: E-Mail: diamondmamta@gmail.com, Mobile: 09805330803**

ABSTRACT

W*ithania somnifera*, a multipurpose medicinal plant of the Himalayan region possesses antioxidant, antitumor, anti-inflammatory, antistress, immunomodulatory, hematopoetic, anti-ageing, anxiolytic, anti-depressive rejuvenating properties and influence various neurotransmitter receptors in the central nervous system. Withanolides, secondary metabolites present in *W. somnifera*, have neuron regenerative property. In order to evaluate heat stress mediated morphological, physiochemical, oxidative stress and antioxidant defence in the *Withania somnifera*, plant was subjected to varied temperature conditions. For temperature treatments the seedlings were exposed to five temperature conditions (8˚C, 18˚C, 38 ˚C, 48˚C and 58˚C). Seedlings grown at 22˚C were treated as control plants. Temperature treatments caused significant decrease in stem length, root length, fresh weight and dry weight in all the treatments. Changes in leaf area, membrane stability and relative water content was also observed and these protein, carotenoids, tocopherol, ascorbic acid and alkaloids decreased. The antioxidant enzymes like superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione–S-transferase, DPPH (1, 1-diphenyl-2-picrylhydrazyl) and ABTS (2, 2'-azino-bis-3ethyl benzthiazoline-6-sulphonic acid) enhanced due to stress treatments. Withanolides were found to be higher in high temperature treated plants. *W. somnifera* was found to have protective mechanism against oxidative damage by maintaining higher enzymatic and non-enzymatic antioxidants.

KEY WORDS: Temperature stress, secondary metabolites, withanolides, *Withania somnifera*.

1. INTRODUCTION

Medicinal plants are essential natural resource which constitutes one of the potential sources of new products and bioactive compounds for drug development. *Withania somnifera* (L.) Dunal, a prominent herb of lower Himalaya is commonly known as "Ashwagandha", "Asgandh" and "Winter Cherry". It belongs to Solanaceae family and has 1250 species. *Withania somnifera* shows presence of several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannin etc. which give the plant material values. At present, more than 12 alkaloids, 40 withanolides and several sitoindosides have been isolated and reported from aerial parts, roots and berries of *Withania* species (Mir, 2010; Kumar, 2011).

Plants growth, productivity and secondary metabolites are affected by biotic and abiotic factors. High temperature is one of the most significant abiotic stresses that affect plant growth and development (Ramakrishna and Ravishankar, 2011). It results in reduced available water in the soil to such critical levels and atmospheric conditions adds to continuous loss of water. Increase in temperature often causes oxidative stress and change in the physiology of the plant. Similarly, low temperature affect plant growth and productivity. Plants growing in low temperature conditions adapt by adjusting their metabolism during autumn, increasing their content of a range of cryoprotective compounds to maximize their cold tolerance (Ramakrishna and Ravishankar, 2011). The temperature stress can affect secondary metabolites and other compounds that plants produce, which are usually the basis for their medicinal activity. Generally when plants are stressed, secondary metabolites production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed not allocated to growth instead allocated to secondary metabolites. Some report that secondary metabolites increase in response to elevated temperatures while others report that they decrease (Snow, 2003). Therefore, studies on abiotic stress in medicinally important plant of the Himalaya needs greater attention to understand the underlying phenomenon.

2. MATERIAL AND METHODS

Study Site: The seeds of *Withania somnifera* were obtained from Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP). Seeds of *Withania somnifera* were sown in the nursery beds for which land was prepared by mixing farm yard manure (FYM), soil and sand in the ratio of 1:1:1. One month old seedlings were transplanted in pots and kept in growth camber for stress (temperature) study.

Induction of Heat stress (HTS) and Low temperature stress (LTS):

Control (22˚C): The control plants were exposed during the day at 18˚C for 1h, 20˚C for 3 hrs, and then 22˚C for 8 hrs and finally ramped back to 20˚C for the remaining 4 hrs of daylight. Night temperature remained constant at 18˚C for 8 hrs.

HTS of 38˚C: In this treatment during day time plants were kept at 22˚C for 1h followed by 28˚C for 3 hrs, then at 38˚C for 8 hrs and ramped back to 22˚C for the remaining 4 hrs of daylight. Night temperature remained constant at 18°C for 8 hrs.

HTS of 48˚C: In this treatment during day time plants were kept at 28˚C for 1h followed by 38˚C for 3 hrs and then 48˚C for 8 hrs and ramped back to 38˚C for the remaining 4 hrs of daylight. Night temperature remained constant at 18˚C for 8 hrs.
HTS of 58˚C: In this treatment daytime temperature was kept at 38°C for1 h, followed by 48°C over 3 hrs, maintained at 58°C for 8 hrs then ramped back to 48°C for the remaining 4 hrs of daylight. Night temperature remained constant at 18˚C for 8 hrs.
LTS of 18˚C: During daytime plants were kept at 22˚C for1hr, followed by 20°C for 3hrs, and at 18˚C for 8 hrs which was then ramped back to 20˚C for 4 hrs of daylight. Night temperature remained constant at 18˚C for 8 hrs.
LTS of 8˚C: In this treatment during day plants were first exposed at 22˚C for 1hr, followed by 15˚C for 3 hrs, and then at 8˚C for 8 hrs which was ramped back down to 15˚C for the remaining 4 hrs of daylight. Night temperatures remained constant at 18˚C for 8 hrs.
Morphological and Physiochemical Parameters: Morphological parameters of three months old plants were recorded by measuring stem length (cm), root length (cm), fresh weight (g) and dry weight (g) of whole plant. Leaf area, relative water content, membrane stability index was also observed. Phytochemical study was also carried out by carbohydrate estimation and protein estimation. Status of antioxidants were assessed by quantifying alkaloids, flavonoids, saponins, phytosterols, chlorophyll, carotenoids, ascorbic acid and tocopherol. The Enzymatic Antioxidants were analyzed in the leaves of *Withania somnifera* were superoxide dismutase, catalase peroxidase ascorbate peroxidase (Nakano and Asada, 1981), glutathione reductase glutathione-S-transferase. Evaluation of radical scavenging effects was done DPPH scavenging effects of Mensor (2001) and ABTS Scavenging Effects of
Phytochemical Extraction & Purification: Roots (25g) of *Withania* were extracted with 250 ml methanol through Soxhlet for 48 hrs. The defatted Methyl alcohol extract was again evaporated and partitioned by adding 10 ml $CHCl_3$ into two layers. One layer is of chloroform soluble fraction and other is of aqueous layer. Chloroform soluble fraction (pH 6.8) was loaded on a silica gel plate (TLC).
Thin layer Chromatography (TLC): A drop of 2 ml extract was loaded on a silica gel plate with mobile phase of hexane, chloroform and methanol. Extract showed best fractionation with ratio of hexane: chloroform: methanol in 50:48:2 ratio. Presence of withanolides was detected with the help of chromatographic reagent Dragendroff's reagent. Confirmation of extracted compounds was done by comparing Rf values of compounds with standard withanolide A, withanolide D and withaferin A (Chromodex).
Infrared Spectroscopy (IR): The solid extracted compound was observed in FTIR and observation of peak confirmed the presence of withanolides in the sample.
High-performance liquid chromatography (HPLC): Withanoides (withnolide A, withaferin A and withanolide D) were quantified using HPLC system of Agilent technologies composed of bin pumps combined with Agilent technologies ALS along with Agilent photodiode array detector with column: hypersil BDS C-18 bonded with 5 µm (4.6 x 150 mm) coupled with EZ-Chrom software recording. Stock solutions of withanolides (98% pure Chromadex) 5mg/10ml were prepared in solvent system (formic acid: methanol in water (HPLC grade) 60:40) and stored at 2–8°C until used.

3. RESULTS

Growth Parameters: Stem length increased with successive growth stages. However, seedlings kept at high temperature (38˚C, 48˚C, and 58˚C) as well as at low temperature (18˚C, 8˚C) had reduced stem length as compared to control plants (22˚C). Similar to shoot length, root length also decreased on exposure to different temperature conditions. Degree of inhibition of root length was maximum at 8˚C which was 59.3% in comparison to control plants (Fig.1.)

Figure.1. Variation in growth of *Withania somnifera* plants grown under different temperature conditions. W represents control, W-1: 8°C, W-2: 18°C, W-3: 38°C, W-4: 48°C,W-5: 58°C.

Biomass and Productivity: A reduction in fresh weight was found in seedlings treated with different temperature compared to control plants. Dry weight in *Withania somnifera* increased with time in treated and untreated plants. Decrease in dry weight was maximum at high temperature (58˚C). Leaf area showed inconsistent variation with growth and treatment. Maximum reduction in leaf area was at 58˚C which was 32.1% in comparison to control. The decrease in leaf area due to temperature followed pattern as: 8˚C (69.2%) <48˚C (78.7%) <18˚C (81.3%) <38˚C

(86.1%) < 58 ˚C (32.1%) against control. Variation in temperature from control condition considerably decreased the membrane stability both in low and high temperature. The decrease in membrane stability was more prominent at low temperature than at high temperature. In 8˚C was 30.7% less compared to control plants. Variation in relative water content was found in all the treated plants compared to control plant. Relative water content consistently increased with decrease in temperatrure (8˚C >18˚C >22˚C). Reduction in RWC was observed at high temperature. Minimum RWC content was reported in 58˚C where decrease was 29.1% compared to control plants.

Physiochemical Parameters: Total carbohydrate content under different temperature conditions increased except in 38˚C plants where it decreased by 21.43% compared to control plants. Protein content increased with an increase in temperature and decreased as temperature decreased as compared to control plants. Maximum protein content was found in plants growing at 58˚C (0.5 mg/g FW) and it was 326% higher from control plants. The interactions has shown that there was variation in physiological parameter of treated plants from untreated plants, which rejected null hypothesis.

Table.1. Variation in the stem length, root length, fresh weight and dry weight in temperature and light treated plants of *Withania somnifera* (Values are of mean ± standard error)

Treatment	Stem length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control	42.29±0.35	42.66±0.88	57±0.57	5.53 ±0.21
8°C	19.70±0.44	17.33±0.37	34.06 ±0.55	1.91±0.04
18°C	23.81±0.38	23.33±0.04	43.98±2.30	3.71±0.01
38°C	41.60±0.50	37.66±0.26	54.61±0.83	5.36±0.17
48°C	29.39±0.62	30.66±0.45	36.92±1.31	2.64±0.02
58°C	16.51±0.53	19±0.15	24.4±1.05	1.32±0.01
8 hrs	44.20±0.04	35±2.08	61.5±0.86	5.94±0.26
12 hrs	43.46±0.22	36.66±0.23	59.33±4.17	5.60±0.04
16 hrs	40.63±0.16	40.66±0.12	53.9±0.86	4.23±0.27
20 hrs	31.41±0.17	43±0.57	47.5±0.86	2.81±0.03
24 hrs	28.68±0.55	44±0.22	40.95±0.62	2.5±0.23

Non-enzymatic antioxidants: Chlorophyll content decreased as the temperature was increased. The decrease in chlorophyll content follow the pattern: 38˚C (1.5%) < 48 ˚C (21%) < 58˚C (35%) in comparison to control plants. Reduction in the carotenoid content was found in high temperature condition (38˚C>48˚C> 58˚C). Similarly it decreased with decrease in temperature (18˚C > 8˚C). Temperature stress adversely effected the alkaloids content as it decreased in low temperature and high temperature growing plants (Table 1.2). The alkaloid content reduced by 58% in 58˚C, by 42% in 8˚C, by 26% in 18˚C, by 23% in 48˚C, by 7.6% in 38˚C. Flavonoid content increased by 16% in 58˚C, by 15% in 48 ˚C, by 13.7% in 18˚C, by 12% in 38˚C, in comparison to control plants. It is evident from the results that ascorbic acid content increased in all the stressed plants compared to control plants. The results revealed that stressed plants produced high ascorbic acid compared to normal plants. Tocopherol content under temperature stress was observed in the leaves of *Withania somnifera*. Tocopherol content decreased with decrease in temperature (18°C > 8˚C). However, an increase in its content was reported with high temperature (38˚C<48˚C<58˚C). It is evident from the results that phenol content was higher in all the treated plants compared to control plants. The pattern of increased content of phenol was: 58°C (11.7 %)> 48°C (11.5%) >8°C (11.3%) >38°C (11%) >18°C (10.5%) in comparison to control plants. Total phytosterol increased with an increase in temperature and decreased with low temperature conditions. Phytosterol content increased by 59% in 58°C, by 31% in 48°C, whereas it decreased by 18°C in 26% and by 35% in 8°C compared to control plants.

Decreased by 34% in 8°C, by 22% in 18°C compared to control plants. Saponin content varied in treated plants compared to control plants. It decreased when the plants were subjected to low temperature whereas it increased in high temperature. Maximum content was found at 58°C. (2.68mg/gFW) compared to control plants (1.98mg/FW). Saponin content increased by 90% in 58°C, by 57% in 48°C.

Table.2. Variation in the phenol, flavonoids, phytosterol, saponin, tocopherol and ascorbic acid in treated and untreated plants of *Withania somnifera* (Values are of mean ±standard error)

Treatment	SOD µmol/g	CAT µmol/g	POD µmol/g	APX µmol/g	GR µmol/g	G-S-T µmol/g
Control	1.26±0.02	38.27±1.24	0.91±0.06	1.34± 0.01	2.35±0.02	1.34±0.02
8°C	2.06±0.05	59.67±0.47	1.88±0.04	1.03± 0.02	2.04±0.01	1.03± 0.04
18°C	1.62±0.10	46.48±0.44	1.47±0.05	1.14± 0.01	2.18±.0.03	1.23±0.01
38°C	1.87±0.05	41.53±0.71	1.06±0.02	1.44±0.02	2.12±0.08	1.37±0.03
48°C	2.16±0.06	61.94±0.81	1.99±0.02	1.56± 0.04	1.94±0.03	1.43± 0.04
58°C	2.18±0.08	75.32±0.39	2.34±0.08	1.68±0.23	1.83±0.07	1.56±0.01

Table.3. Variation in the superoxide dismutase, catalase, peroxidase and ascorbic acid in treated and untreated plants of *Withania somnifera* (Values are of mean ±standard error)

Treatment	Phenol mg/g FW	Flavonoids mg/g FW	Phytosterol mg/g FW	Saponin mg/g FW	Tocopherol mg/g FW	Ascorbic acid µmol/g FW
Control	1.48±0.01	0.62±0.05	1.48±0.19	0.33±0.01	1.98±0.14	0.26±0.01
8^0C	1.68±0.03	0.99±0.01	0.97±0.05	0.22±0.04	1.28±0.01	0.63±0.01
18°C	1.56±0.04	0.84±0.02	1.10±0.06	0.26±0.06	1.73±0.04	0.47±0.01
38°C	1.64±0.07	0.76±0.01	1.54±0.09	0.30±0.06	2.23±0.12	0.37±0.01
48°C	1.71±0.02	0.94±0.02	1.95±0.07	0.52±0.05	2.56±0.24	0.51±0.04
58°C	1.76±0.04	0.99±0.03	2.36±0.07	0.96±0.07	2.68±0.17	0.82±0.07

Enzymatic Antioxidant: Enzymatic antioxidants analysed were SOD, CAT, POD, APX, GR and GST in the leaves of *W. somnifera*. Activity of superoxide dismutase significantly increased due to temperature treatments compared to control plants. The increase in SOD in different treatments followed the pattern: 73% higher in 58°C, 71% higher in 48°C, 63% higher in 8°C, 48% higher in 38°C and 28% higher in 18°C compared to control. Peroxidase enzymes activity was found to be quantitatively higher in all the treatments compared to control plants. The increase was variable in all the treatment that the POD content increased by 157% in 58°C, by 118% in 48°C, by 106% in 8°C, by 61% in 18°C and by 16% in 38°C. Catalase activity increased in the treated plants. It increased by 96% in 58°C by 61% in 48°C, by 55% in 8°C, by 21% in 18°C, by 8% in 38°C as compared to control plants (Table.3). Ascorbate peroxidase content decreased in the plants germinating in low temperature but it increased with an increase in temperature. APX content increased by 25% in 58°C, by 16% in 48°C, by 7% in 38°C and decreased by 24% in 8°C, by 15% in 18°C compared to control plants. Glutathione reductase content decreased in all the treated plants. Varied temperature conditions decreased the glutathione reductase content in condition dependent manner i.e. by 23% in 58°C, by 18% in 48°C, by 8% in 38°C, by 10% in 18°C, by 16 % in 8°C compared to control. Glutathione-S-transferase was found to be higher in plants growing at high temperature compared to control plants. GST decreased in plants growing at low temperature treatment. The GST content increased by 16.4% in 58°C, by 6.7% in 48°C, by 2.2% in 38°C whereas it decreased by 23 % in 8°C and by 8% in 18°C in comparison to control plants.

DPPH & ABTS: DPPH scavenging activity increased inhibition in all the applied stress treated plants in comparison to control plants of *Withania somnifera.* After 60 days of stress treatment percentage inhibition was maximum in plants kept at 58°C (18%), 8°C (17%), 48°C (15%), 12 hrs (14 %), 18°C (12%), 38°C (11%) in comparison to control plants. ABTS radical scavenging activity increased in all the applied stress as comparison to control plants of *Withania somnifera*. ABTS activity was found minimum in 18°C (10.4 µmol/g). The inhibition of ABTS follow the pattern: 51% in 58°C, 25.3% in 8°C, 18.2% in 38°C, 10 % in 18°C in comparison to control plants.

Thin Layer Chromatography: Withanolides were present in stress treated and control plants in a solvent system comprising of hexane: chloroform: methanol in ratio of 50:48:2. When plates were run TLC profile of the control plants showed withanolides as evident from Fig. 4.18 (lane D). The R_f values for withanolide A, withaferin A and withanolide D was 0 .58, 0.41 and 0.19, respectively. The plants growing under different temperature and light conditions were also studied for the presence of withanolides and the results are shown in Figs.1. 2 &1.3.

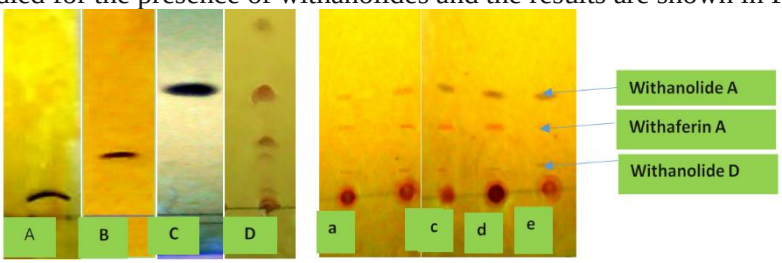

Figure.2. TLC profile of comounds extrcated from roots of *Withania somnifera.* Lane A- withanolide D, lane B-withaferin A, lane C-withanolide A and lane D –compounds from the root extract of plant. Lane a: 8°C, lane b: 18°C lane c : 38°C, lane d : 48°C and lane e:58°C.

IR spectroscopy: The withanolides (withanolide A, withanolide D and withaferin A) being steroidal lactones showed similar pattern and presence in the range of carbonyl group between 1700-1730 cm^{-1}.

IR cm^{-1} (Standard withanolide): 3350: (O-H) stretch, 1953: (C-H) stretch, 1707: (C-H) C=O stretch vibration, 1644: (C=O) stretch, 1413: (C-H) asymmetric vibration, 1056: (C-H) deformation.

IR cm^{-1} (Extracted withanolide): 3353: (O-H) stretch, 1710: (C-H) C=O stretch vibration, 1640: (C=O) stretch, 1413:(C-H) asymmetric vibration, 1056:(C-H) deformation.

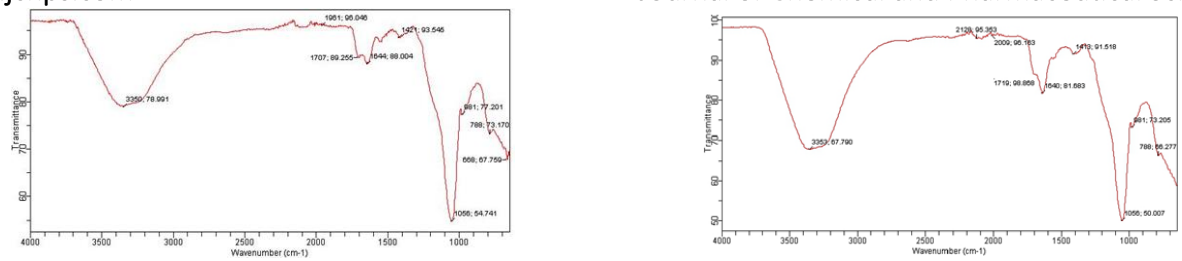

Figure.3. IR - Chromatogram of standard marker withanolide (A) and extracted withanolide (B) from *Withania somnifera*. FTIR graph showing presence of withanolides at wavenumber 1707 (cm^{-1}) (A) and 1719(cm^{-1}) (B)

Withanolides quantification: In stress treated plants (temperature), chromatograms of methanolic extract showed homogenous pattern of three main peaks. In root extract of 120 days old plants growing under 8°C, 18°C, 38°C, 48°C and 58°C revealed presence of three dominant peaks- A, B and C. Peaks A^a, A^b, A^c A^d, A^e referred to withanolide A in 8°C, 18°C, 38°C, 48°C and 58°C chromatograms. Similarly, B^a, B^b, B^c, B^d, B^e and C^a, C^b, C^c, C^d, C^e are referred to withaferin A and withanolide D, respectively. Withanolide A was 42.56 mg/g DW in untreated plants. It decreased by 1.8% in 18°C growing plants, by 5.7% in 8°C growing plants, however increased by 1.9% in 38°C growing plants, by 2.6% in 48°C growing plants and by 4.9% in 58°C growing plants compared to control growing plants. Similar pattern was observed by withaferin A and withanolide D. A decrease was reported in 12°C (B^c by 3%, C^c by 8%) and 8°C (B^b by 4%,C^b by 17%) whereas a small increase was reported at high temperature condtions i.e. in 38°C (B^d by 0.9%,C^d by 0.8%), in 48°C (B^e by 2.4%,C^e by 8%) and in 58°C (B^f by 4.6%,C^f by 4.2 %) as is evident from Figs.4, 5 and 6.

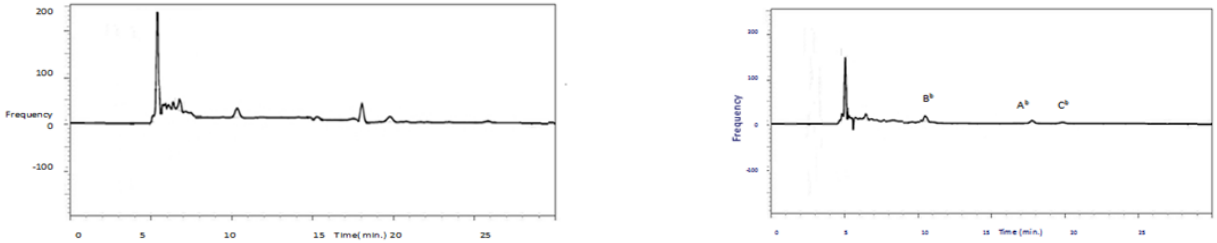

Figure.4. HPLC graph of root extract of withanolide A (WD-A), withaferin A (WF-A) and withanolide D (WD-D) under control and 8^0C condition after 120 days of germination.

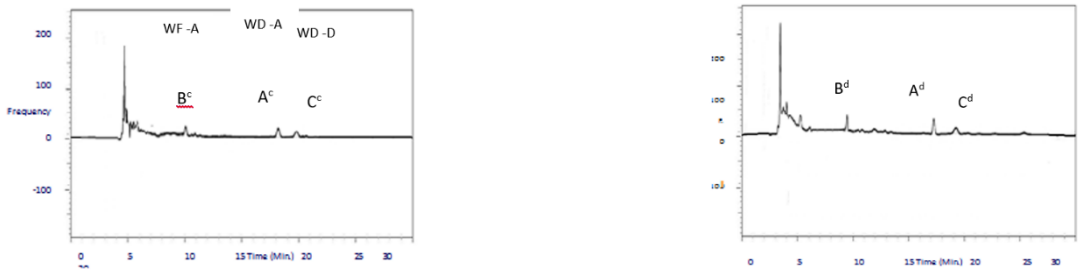

Figure.5. Withanolides content in the root extract of *Withania somnifera* under 38^0C and 18^0C temperature conditions after 120 days of germination. In graph A : Withanolide A, B :Withaferin A and C : Withanolide D peaks

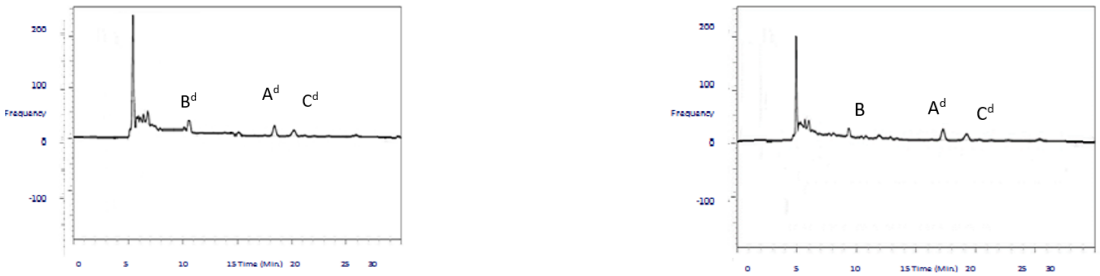

Figure.6. Withanolides content in the root extract of *Withania somnifera* under 58^0C and 48^0C temperature conditions after 120 days of germination

DISCUSSION

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. Varied temperature significantly affected stem growth as stem length reduced significantly in both low and high temperature. These results are in agreement with

those of Shah (2011), for rice plants, Young, (2004) for *Brassica napus*, Arves (2013), for *Pisum sativam* and Iloh (2014), for Nigerian cereal crops: maize, rice and sorghum. 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. Similarly, the effect of cold stress depends on the degree of severity and the time of exposure. 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. Root length decreased in *Withania somnifera* decreased in seedlings treated with temperature stress (high and low). As evident from the literature that similar results were found in maize (*Zea mays* L) by Herrero and Johnson (1980), in bentgrass by Huang,(1998), in Sorghum plantlets and maize by Iloh (2014), in wheat and ryegrass by Sultana (2013), in *Caragana korshinskii* by Lai (2014) and in *Plantago psyllium*, *Althaea officinalis* and *Nigella sativa* by Jamian (2014). Fresh weight and dry weight in seedlings of *Withania somnifera* declined in temperature treatment. Earlier a significant negative linear relation between shoot and root biomass and increased temperature was found in wheat and in *Arachis hypogaea* L. 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. Cold stress severely affected fresh weight and dry weight as it significantly reduced cell division and cell elongation, which eventually resulted in stunted growth. A significant reduction in fresh weight was reported in wheat in corn, in watermelon (Bradow, 1990), in cucumber and squash in muskmelon Markhart (1986) found that extended periods of cold stress significantly delayed seedling growth and damaged photosynthetic apparatus. Variation in temperature from control condition considerably decreased the membrane stability both in low and high temperature. 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. Farooq (2008), observed a gradual increase in leakage of ions from the cells upon prolongation of chilling exposure in maize hybrids. Similarly, change in the cell membrane properties due to cold stress resulted in loss of membrane semipermeability, and alteration in membrane transport properties. 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. RWC is the major tool for assessing changes in plant water relations for studying plant responses to stress and subsequent relation to stress tolerance. Farooq, (2009) found that RWC was influenced by leaf temperature. 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. Earlier studies revealed that high temperature altered carbohydrate partitioning in potato (*Solanum tuberosum* L.) plants from tubers to shoots and reduced overall plant yield. Liu and Bingru (2013) reported that reduction in carbohydrate concentrations in shoots was more pronounced than that of roots. 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. Carotenoids are isoprenoid molecules synthesized by all photosynthetic and non –photosynthetic organisms. Duvivier (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*. Similarly, 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. Temperature 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. In the present study phytosterol and saponin content increased with high temperature and decreased with low temperature in comparison to control plants in *Withania somnifera*.

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 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. Effect of temperature was investigated on litchi and asparagus where increased amount of peroxidase activities was confirmed in these species. Liu (2013), reported that low temperature induced increased activity of POD in *Avena nuda*. Catalase is the principle enzyme that scavenges harmful oxygen species in plants. In *Avena nuda* CAT activity was higher under the cold treatment than normal temperature (Liu, 2013). A decrease in CAT activity was reported by many researchers when the plants were subjected to abiotic stress (Fu and Huang 2001; Jung, 2003). Ascorbate peroxidase exists as isoenzymes and plays an important role in the metabolism of H_2O_2 in higher plants. It eliminates ROS through multiple mechanisms and is also donor of electron for APX-mediated H_2O_2 detoxification. 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. 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. The glutathione-S-transferases (GST) represent a major group of detoxification enzymes. 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). Inferred that GST would play the protective role under heat stress by inactivating the excessive metabolic toxicants.

Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions (Ramakrishna and Gokare, 2011). A reduction in withanolides quantity was evident in low temperature (short photoperiods) whereas it increased significantly in high temperature. Bilal Ahmad Mir (2014), reported that in *Withania somnifera* leaves high temperature increased production of withanone and withaferin A and these withanolides may have a role in combating oxidative stress. A study carried out by Kumar (2012), were of view that seasonal temperature played a key role in increasing secondary metabolites (withanolides) rather than the phenological stage of the plant. 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, 2007). Some report that secondary metabolites increase in response to elevated temperatures while others report that they decrease (Snow, 2003). 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 (2010), reported that cold stress could impose osmotic injury and desiccation of plants. 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*.

4. CONCLUSION

Plant growth in *Withania somnifera* was affected due to applied abiotic stresses i.e. temperature 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 however, it decreased in low temperature conditions. The study revealed the tolerance ability of *Withania somnifera* and strong antioxidant defense mechanism in temperature stress.

5. ACKNOWLEDGEMENT

For this work my sincere thanks to faculty, researchers and staff of Shoolini University, Solan, H.P. and NIPER, Chandigarh.

REFERENCES

Arve LE, Terfa MT, Gislerod HR, Olsen JE, Torre S, High relative air humidity and continuous light reduce stomata functionality by affecting the ABA regulation in rose leaves, Plant Cell and Environment, 36, 2013, 382 – 392.

Barbara, Alina, Włodzimierz, Jolanta, Effect of differential temperature and photoperiod on growth of *Ocimum basilicum*, Agriculture, 2011, 98,104.

Beck EH, Fetitig S, Knake C, Hartig K, Bhattarai T, Specific and unspecific responses of plants to cold and drought stress, J Biosci, 32 (3), 2007, 501-510.

Bhagirath Ram, Meena HS, Singh VV, Singh BK, Nanjundan J, Kumar Arun, High temperature stress tolerance in Indian mustard (*Brassica juncea*) germ plasm as evaluated by membrane stability index and excised-leaf water loss techniques, Journal of Oil seed Brassica, 5 (2), 2014, 149-157.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA, Plant drought stress, effects, mechanisms and management, Agronomy for Sustainable Development, 29, 2009, 185-212.

Iloh AC, Omatta G, Ogbadu GH, Onyenekwe PC, Effects of elevated temperature on seed germination and seedling growth on three cereal crops in Nigeria, Scientific Research and Essays, 9 (18), 2014, 806 - 813.

Jamian Saba Siadat, Mehrani Sadegh, Asilan Kamal Sadat, Tabrizi Avishan Taherkhanchi, Goharian Alireza, Responses of seedling growth and germination parameters in three medicinal plants under drought stress, International Journal of Agriculture and Crop Sciences, 7-4, 2014, 191-195.

Janska A, Marsik P, Zelenkova S, Ovesna J, Cold stress and acclimation-what is important for metabolic adjustment? Plant Biol, 12, 2010, 395-405.

Jochum GM, Mudge KW, Thomas RB, Elevated temperatures increase leaf senescence and root secondary metabolite concentrations in the understory herb *Panax quinquefolius* (Araliaceae), American Journal of Botany, 94, 2007, 819-826.

Lai Zongrui, Zhang Yuqing, Wu Bin, Qin Shugao, Feng Wei, Liu Jiabin, Impacts of morphological traits and temperature on fine root respiration during dormancy of *Caragana korshinskii*, Ecol Res, 30 (2), 2015, 337-345.

Liu Li, Wangli, DengFei, HuangYun, Liud ai-yin, Renwan-jun, Response of Osmotic Regulation Substance Content and Protective Enzyme Activities to Shading in Leaves of Different Rice Genotypes, Rice Science, 20 (4), 2013, 276−283.

Milner MJ, Kochian LV, Investigating heavy-metal hyper accumulation using Thlaspi caerulescens as a model system, Annals of Botany, 102, 2008, 3–13.

Mori K, Goto-Yamamoto N, Kitayama M, Hashizume K, Loss of anthocyanins in red wine grape under high temperature, Journal of Experimental Botany, 58, 2007, 1935- 1945.

Noohi KE, Fatahi, Kamali GHA, Heat stress effects analysis on wheat crop in southern provinces, Geophysical Res Abstracts, 11, 2009, 4441.

Ntatsi G, Savvas D, Ntatsi G, Klaring HP, Schwarz D, Growth, yield and metabolic responses of temperature-stressed tomato to grafting onto rootstocks differing in cold tolerance, Journal of the American Society for Horticultural Science, 139 (2), 2014, 230-243.

Passardi F, Zamocky M, Favet J, Jakopitsch C, Penel C, Obinger C, Dunand C, Phylogenetic distribution of catalase-peroxidases, are these patches of order in chaos? Gene, 397, 2007, 101-113.

Ramakrishna Akula, Ravishankar GA, Influence of abiotic stress signals on secondary metabolites in plants, Plant Signal Behaviour, 1, 2011, 6-11.

Shah F, Huang J, Cui K, Nie L, Shah T, Chen C, Wang K, Impact of high-temperature stress on rice plant and its traits related to tolerance, Journal of Agricultural Science, 149, 2011, 545-556.

Sharkey TD, Zhang R, High temperature effects on electron and proton circuits of photosynthesis, International Journal of Drug Development and Research, 52, 2010, 712–722.

Sharma PK, Dudhe R, Singh S, Singh G, Biological activities of *Withania somnifera*, Annals of Biological Research, 1 (3), 2010, 56-63.

Wahid A, Gelani S, Ashraf M, Foolad M, Heat tolerance in plants, An overview, Environmental and Experimental Botany, 61, 2007, 199-223.