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TEMPERATURE STRESS MEDIATED CONSEQUENCES ON PHYSIOLOGY AND SECONDARY METABOLITES OF *DATURA STRAMONIUM* (L.)

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ABSTRACT: *Datura stramonium* (L.), a multipurpose plant of Himalayan regions which possess antiseptic, sedative, antidiarrhoeal properties used for treating various diseases which include treatment of ulcer, skin disorder, jaundice, fever, heart diseases, etc. To evaluate the temperature stress-mediated morphological, physiochemical, and antioxidative defense in *Datura stramonium*, plants were subjected to different temperature conditions (High temperature and Low temperature). The seedlings were exposed to five temperature conditions (50 °C, 40 °C, 30 °C, 20 °C, and 10°C). Seedlings grown at 25 °C were treated as control. Temperature treatment significantly decreased stem length, root length, fresh weight, dry weight, and leaf area both in high temperature as well as low-temperature stress. Phytochemicals as carbohydrate, protein, alkaloids, flavonoids, ascorbic acid, tocopherol and enzymatic antioxidants, i.e. superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione-s-transferase, and DPPH increased tremendously with both temperature stresses in comparison to the control. Carotenoids and chlorophyll found increased in low temperature whereas decreased in high temperature stressed plants. HPLC analysis of secondary metabolites revealed higher alkaloids (Atropine, Scopolamine) content in high temperature and low temperature treated plants. Results depicted that plant adopt protective mechanism against oxidative damage by maintaining a higher quantity of phytochemicals, enzymatic, and non-enzymatic antioxidants. Increased secondary metabolites also provide a defense mechanism against the adverse condition.

INTRODUCTION: Medicinal plants are natural resources used widely in Herbalism, development of modern drugs and other pharmacological products¹. Medicinal property to the plants is attributed by secondary metabolites which are used by plants themselves for many biological functions and defense against unfavorable conditions.

Datura stramonium (L.) is a prominent annual herb of the Himalayas commonly known as “Thorn Apple,” belongs to family Solanaceae which is also entitled as Nightshade family. *D. stramonium* contains sixty-four tropane alkaloids². It is a rich source of alkaloids including atropine, hyoscyamine, and scopolamine out of which, hyoscyamine is the chief component and found in maximum concentration³.

The plant contains various medicinal properties and useful in the treatment of skin disorders, ulcers, bronchitis, jaundice, heart diseases, fever, and piles⁴. Climate changes occurring in the environment are somehow important for plants as these alter the

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production of secondary metabolites for adaptation against adverse conditions. Biotic and abiotic environmental factors affect the plant growth, productivity, and production of secondary metabolites. Temperature is the key abiotic factor that regulates plant growth and development throughout its whole life.

It is mainly required for the germination of seeds, primary growth of seedlings, and also in the capacity and germination rate of seeds⁵. Both high and low-temperature stress conditions may lead to various alterations in the plant such as morphology, physiology, and biochemical activities of plants. Photosynthesis is one of the most sensitive processes which get affected by high temperature before the detection of any other symptom⁶. Low temperature also inhibits the growth and photosynthetic processes of chilling-tolerant plants. For response and susceptibility of leaves to chilling stress, root temperature is the major factor⁷. Therefore, the present study was focused on studying the effect of temperature stress on morphological and physiochemical parameters of the plant along with the antioxidant activity.

MATERIAL AND METHOD: The experimental work for the present study was carried out in the year 2017 in the laboratory of Shoolini University, Solan (H.P). Seeds of *Datura stramonium* (L.) were collected from Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP) and surface sterilized with 0.1% HgCl₂ before sowing in the nursery. Seeds were then allowed to grow in pots containing soil, sand, and manure in the equal ratio (1:1:1) in the nursery area. The plant was identified with authentication number SUBMS/BOT/1961.

After one month the plants of *Datura* were collected from nursery and transferred to other pots in a growth chamber for 60 days of stress induction.

Induction of Temperature Stress: Temperature stress was divided into two parts: High-temperature stress and low-temperature stress.

25 °C (Control): In controlled temperature condition plants were kept at 18 °C for 1 h followed by 22 °C for 3 h, 25 °C for 8 h and then again 22 °C for 4 h in a day.

High-Temperature Stress:

30 °C: In this treatment, plants were allowed to grow at different temperature conditions in a day which involves: 22 °C for 1h followed by 28 °C for 3h, then at 30°C for 8h and then again 28 °C for 4h.

40 °C: In this treatment, plants were allowed to grow at different temperature conditions in a day which involves: 28 °C for 1 h followed by 38 °C for 3 h, 40 °C for 8 h and then again 38 °C for 4 h.

50 °C: In this treatment, plants were allowed to grow at different temperature conditions in a day which involves: 38 °C for 1 h followed by 48 °C for 3 h, 50 °C for 8 h then again 48 °C 4 h.

Low-Temperature Stress:

20 °C: In this treatment, plants were allowed to grow at different temperature conditions in a day which involves: 25 °C for 1 h, 22 °C for 3 h, 20 °C for 8 h and then 22 °C for 4 h.

10 °C: In this treatment, plants were allowed to grow at different temperature conditions in a day which involves: 22 °C for 1 h, 15 °C for 3 h, 10 °C for 8 h and then 15 °C for 4 h.

Morphological and Physiochemical Parameters:

Morphological parameters of the plants were recorded after 60 days of stress induction by analyzing stem length, root length, fresh weight, dry weight, leaf area, relative water content, and membrane stability whereas, the phytochemical study was done by analyzing the carbohydrate and protein content. Quantitative analysis for antioxidants was done for alkaloids, flavonoids, chlorophyll, carotenoids, ascorbic acid, and tocopherol. Analysis of enzymatic antioxidants was done for superoxide dismutase, catalase, peroxidase, glutathione reductase, and glutathione-s-transferase.

Phytochemical Extraction and Purification:

Phytochemical analysis was done on leaves of *Datura stramonium*. The extract was prepared using properly washed and dried leaves, crushed with mortar and pestle. Methanol (HPLC Grade) was used for extract preparation. 2 g leaf sample (powdered) was added into 20 ml methanol of HPLC grade and placed on a shaker for 24 h. The extract formed was filtered using Whatman filter paper, to the residue more 20 ml methanol was

added and repeated the procedure. The extract formed was again filtered and used for HPLC analysis.

High- Performance Liquid Chromatography (HPLC): Atropine and scopolamine were quantified using HPLC system of Agilent technologies composed of Waters 515 HPLC pump combined with DAD detector with mobile phase acetonitrile: water (60:40) at wavelength 254 nm. The injection volume was 5 μ l, with a runtime of 20 min.

Data Analysis: Data was analyzed using absorbance values obtained by using spectrophotometer and then calculated mean and standard error mean of values. Further, the values

of mean and standard error mean were used for analysis in Prism software followed by One-way ANOVA and Tukey's Multiple Comparison Test.

RESULTS:

Growth Parameters: Stem length increased with successive growth stages whereas plants kept at high temperature (50 °C, 40 °C and 30 °C) and low temperature (20 °C and 10 °C) reported with reduced stem length.

Similar to the stem length, root length was also reported decreasing with both increase and decrease in temperature. Maximum inhibition of stem length was at 50 °C whereas root length at 10 °C and observed 25.8% and 47.97% respectively in comparison to control **Fig. 1**.

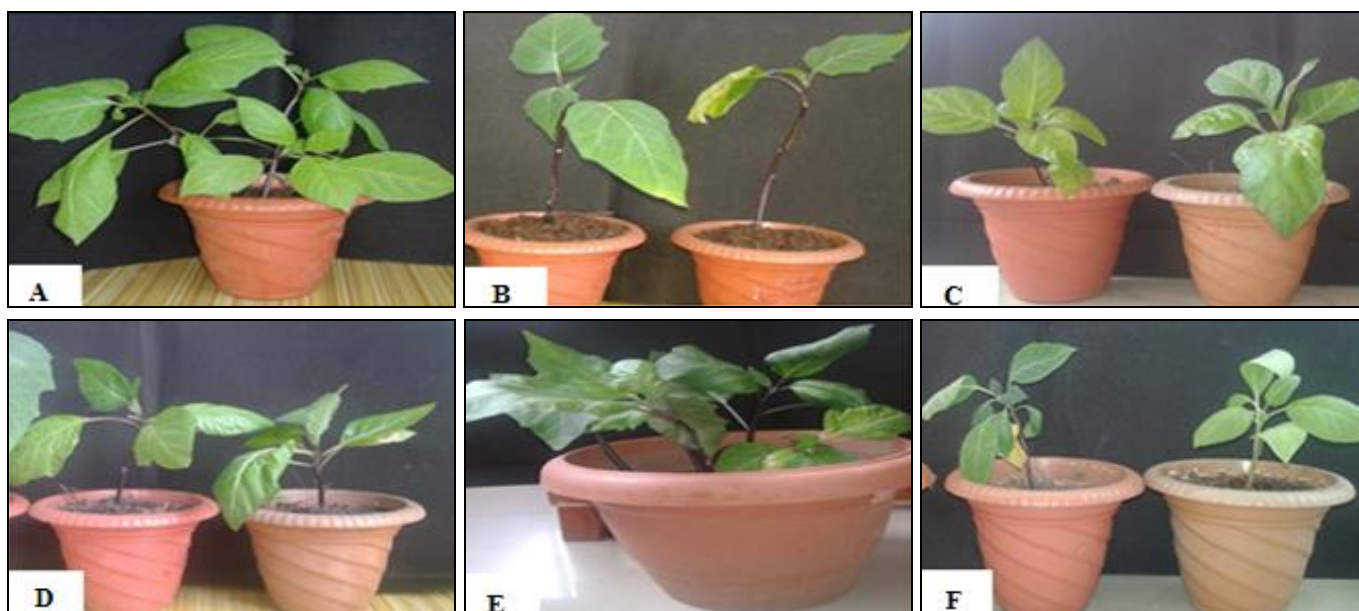


FIG. 1: PLANTS OF DATURA STRAMONIUM GROWN IN GROWTH CHAMBER UNDER DIFFERENT TEMPERATURE CONDITIONS A: CONTROL, B: 50 °C, C: 40 °C, D: 30 °C, E: 20 °C AND F: 10 °C.

Biomass and Productivity: Fresh weight, dry weight, and leaf area were found reduced with increase and decrease in temperature. The

maximum decrease in fresh weight and dry weight was in plants treated with 10 °C and found 16.7% and 8.3% in comparison to control.

TABLE 1: VARIATIONS IN THE STEM LENGTH, ROOT LENGTH, FRESH WEIGHT AND DRY WEIGHT IN TEMPERATURE TREATED PLANTS OF DATURA STRAMONIUM

Treatment	Stem length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control	13.55 \pm 1.25	8.40 \pm 0.65	16.03 \pm 0.65	2.62 \pm 0.24
10°C	04.30 \pm 0.23	4.03 \pm 0.29	02.68 \pm 0.53	0.22 \pm 0.05
20°C	10.08 \pm 1.19	5.25 \pm 0.39	04.69 \pm 0.35	0.77 \pm 0.08
30°C	12.18 \pm 1.29	6.30 \pm 0.92	13.90 \pm 0.71	1.54 \pm 0.13
40°C	11.98 \pm 0.84	5.90 \pm 0.81	7.27 \pm 1.22	1.09 \pm 0.25
50°C	03.50 \pm 1.04	4.50 \pm 0.21	04.29 \pm 0.72	0.33 \pm 0.05

Leaf area was also reported to be decreased with both decrease and increase in temperature, and

maximum inhibition was found at 50 °C with 14.7% in comparison to control. Relative water

content was reported minimum in plants treated with low temperature 10 °C where it was 26.08% whereas and membrane stability was reported minimum in 10 °C (54.2%) in comparison to control **Table 1**.

Physiochemical Parameters: Carbohydrate content was reported increasing with increase and decrease in temperature in comparison to control except 30 °C where it was 56.03%. Protein content was reported increasing with increase and decrease in temperature. Protein was found maximum in plants kept at 50 °C, and it was 207.32%.

Non-enzymatic Antioxidants: Alkaloid content was found maximum in plants treated with 50 °C where it was 354.54% in comparison to control followed by 10 °C (345.45%) > 20 °C (263.63%) > 40 °C (254.5%) > 30 °C (227.27%). Flavonoid content was reported maximum in plants treated with 50 °C and 10 °C with percentage 202.12% in comparison to control. Chlorophyll content was reported maximum in plants kept at 10 °C which was 130.43% followed by 20 °C (117.39%) > 30 °C (108.69%) > 40 °C (69.56%) > 50 °C (65.21%) in comparison to control. Carotenoid content was found maximum at 10 °C (166.6%) followed by 20 °C (156.25%) > 30 °C (120.83%) > 40 °C (83.3%) > 50 °C (58.3%) as compared to control.

Maximum ascorbic acid content was found in 50 °C with an increase of 337.5% followed by 10 °C (262.5%) > 40 °C (216.6%) > 20 °C (200%) > 30 °C (154.16%). Tocopherol content was found

maximum in plants treated with 50 °C with an increase of 201.72% followed by 40 °C (192.24%) > 30 °C (113.79%) > 20 °C (104.31%) and minimum in 10 °C (95.6%) as compared to control as shown in **Table 1**.

Enzymatic Antioxidants: Enzymatic antioxidants analyzed were SOD, CAT, POD, APX, GR and GST. SOD (Superoxide dismutase) was reported maximum at temperature 50 °C with an increase of 470.21%, compared to control followed by 10 °C (446.80%) > 40 °C (442.5%) > 20 °C (340.43%) > 30 °C (219.14%). Similar as SOD catalase was also reported high in plants kept at 50 °C with an increase of 154.84% followed by 40 °C (145.10%) > 10 °C (141.84%) > 20 °C (132.6%) > 30 °C (120.6%) compared to control. Similarly, peroxidase was found maximum in 50 °C (360.65%) followed by 40 °C (303.27%) > 10 °C (285.24%) > 20 °C (206.55%) > 30 °C (142.62%) as compared to control. Ascorbate peroxidase was reported maximum at 50 °C with an increase of 138.52% followed by 40 °C (132.78%) > 30 °C (105.73%) > 20 °C (97.54%) > 10 °C (94.26%) in comparison to control. Maximum GR was found at 50 °C (186.36%) followed by 40 °C (161.03%) > 10 °C (137.01%) > 20 °C (134.41%) > 30 °C (111.03%) and glutathione-S-transferase was maximum at 50 °C (293.4%) followed by 10 °C (285.5%) > 40 °C (282.60%) > 20 °C (257.97%) > 30 °C (223.18%) in comparison to control **Table 2**. Increased amount of enzymatic antioxidants revealed defense mechanism against abiotic stress.

TABLE 2: VARIATIONS IN THE ALKALOID, FLAVONOID, CHLOROPHYLL, AND CAROTENOID IN TEMPERATURE TREATED PLANTS OF DATURA STRAMONIUM

Treatment	Alkaloid	Flavonoid	Chlorophyll	Carotenoid
Control	0.11 ± 0.05	0.47 ± 0.04	0.23 ± 0.02	19.2 ± 1.60
10 °C	0.38 ± 0.01	0.95 ± 0.01	0.30 ± 0.01	32.0 ± 1.33
20 °C	0.29 ± 0.06	0.60 ± 0.12	0.27 ± 0.06	30.0 ± 2.00
30 °C	0.25 ± 0.03	0.55 ± 0.04	0.25 ± 0.08	23.2 ± 1.22
40 °C	0.28 ± 0.08	0.62 ± 0.05	0.16 ± 0.06	16.0 ± 1.33
50 °C	0.39 ± 0.03	0.95 ± 0.01	0.15 ± 0.06	11.2 ± 0.53

TABLE 3: VARIATIONS IN THE SOD, CAT, POD, APX, GR, GST IN TEMPERATURE TREATED PLANTS OF DATURA STRAMONIUM

Treatment	SOD	CAT	POD	APX	GR	GST	DPPH
Control	0.47 ± 0.15	1.84 ± 0.21	0.61 ± 0.09	1.22 ± 0.05	1.54 ± 0.04	0.69 ± 0.01	0.26 ± 0.03
10 °C	2.10 ± 0.13	2.61 ± 0.07	1.74 ± 0.03	1.15 ± 0.01	2.11 ± 0.02	1.97 ± 0.01	0.62 ± 0.02
20 °C	1.60 ± 0.24	2.44 ± 0.08	1.26 ± 0.15	1.19 ± 0.05	2.07 ± 0.05	1.78 ± 0.01	0.37 ± 0.01
30 °C	1.03 ± 0.24	2.25 ± 0.04	0.87 ± 0.02	1.29 ± 0.05	1.71 ± 0.04	1.54 ± 0.01	0.41 ± 0.01
40 °C	2.08 ± 0.17	2.67 ± 0.06	1.85 ± 0.03	1.62 ± 0.06	2.48 ± 0.05	1.95 ± 0.03	0.50 ± 0.02
50 °C	2.21 ± 0.22	2.85 ± 0.05	2.20 ± 0.15	1.69 ± 0.03	2.87 ± 0.03	1.79 ± 0.03	0.68 ± 0.02

Radical Scavenging Activity: After 60 days of stress treatment, the DPPH activity was maximum in plants treated with 50 °C with an increase of 261.53% followed by 10 °C (238.46%) > 40 °C (192.3%) > 30 °C (157.64%) > 20 °C (142.3%) in comparison to control **Table 3**.

High-Performance Liquid Chromatography: Quantification of secondary metabolites was done using High-Performance Liquid Chromatography. Atropine and scopolamine were identified and found to be increased as the temperature increases and decreases **Fig. 2**.

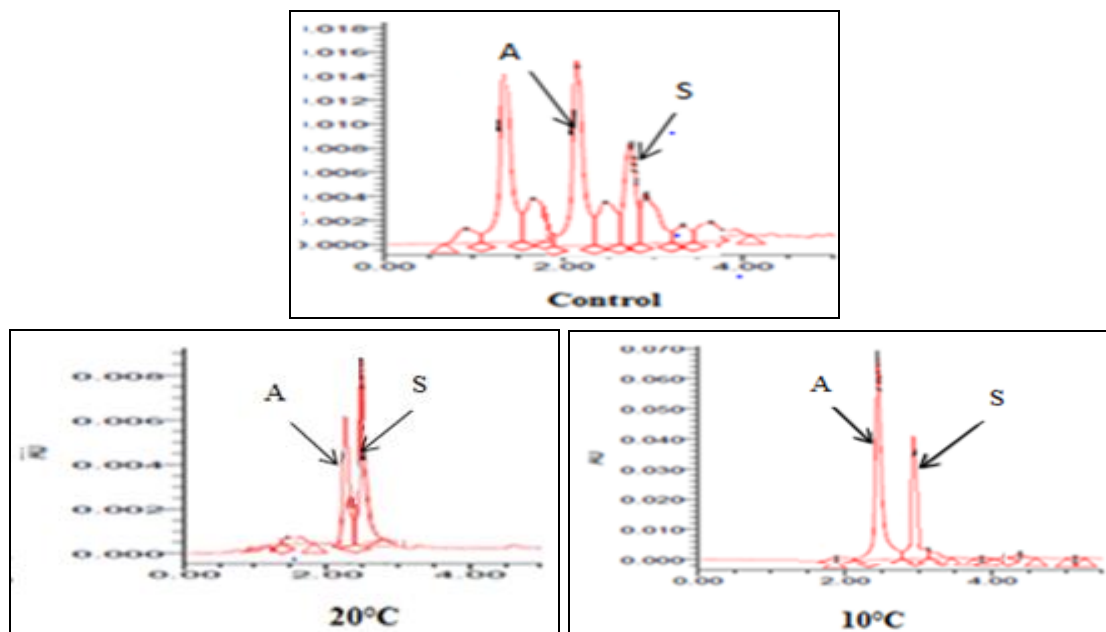


FIG. 2: HPLC GRAPH OF LEAF EXTRACT OF *DATURA STRAMONIUM* TREATED WITH LOW-TEMPERATURE STRESS 10 °C AND 20 °C IN COMPARISON TO CONTROL, A: ATROPINE, S: SCOPOLAMINE

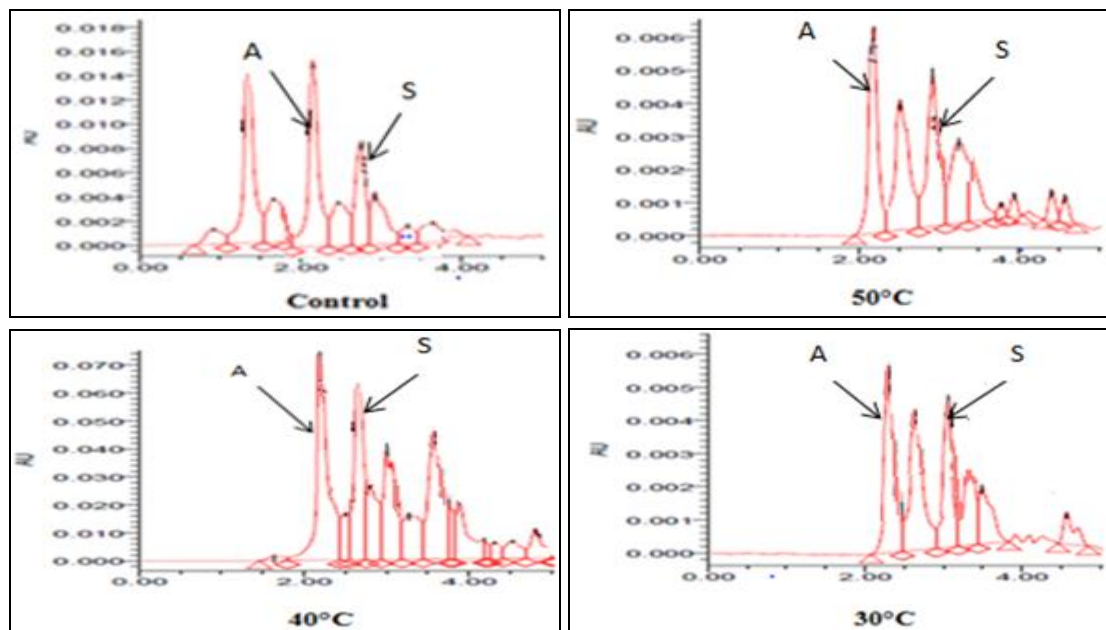


FIG. 3: HPLC GRAPH OF LEAF EXTRACT OF *DATURA STRAMONIUM* TREATED WITH HIGH-TEMPERATURE STRESS 50 °C, 40 °C AND 30 °C IN COMPARISON TO CONTROL

The maximum quantity of atropine and scopolamine was reported at 50 °C and 10 °C.

DISCUSSION: Temperature is the main environmental factor required for the growth and development of plants. Stem length gets reduced at

high and low temperature. A study on rose concluded that stem length decreases with increase in temperature. Root length was also found reduced with high and low temperature, similar results were reported in Sorghum and maize by Iloh *et al.*,

(2014)⁸ and in wheat and ryegrass by Sultana *et al.*, (2013)⁹. High and low-temperature results into reduced fresh weight and dry weight and similar findings were observed which shows that the high temperature leads to reduced fresh and dry weight of plants. Mohammadi *et al.*, (2007)¹⁰ reported that low temperature leads to reduced cell division and cell elongation resulting into reduced fresh and dry weight in wheat.

Carbohydrate and protein content was found high in plants treated with high and low temperature. Similar results were reported by Richardson *et al.*, (2007)¹⁴ in kiwi fruit and concluded that heating leads to a reduction in the carbohydrate content. In the present study, alkaloid and flavonoid content was found higher in plants treated with high and low temperature than in control. Kexuan *et al.*, (2014)¹⁶ reported that the flavonoid content was higher in *Vigna radiata* under abiotic stress. Holopainen and Gershenzon (2010)¹⁷ reported that there is an increase in alkaloid content under stress. Carotenoid content was found higher in plants treated with high and low temperature, and similar results were reported by Duvivier *et al.*, (2013)¹⁸ in *Ipomoea batatas* plant. Chlorophyll was high in plants treated with high and low temperature, a decrease in photosynthetic activity due to heat stress leads to an increase in the activity of chlorophyllase.

In the present study, ascorbic acid and tocopherol content were found increased with increase and decrease in temperature. Ascorbic acid help to protect the rice plants from chilling environment Guo *et al.*, (2005)²⁰ and heat stress in mungbean²¹. Collakova and Della Penna (2001)²² reported that heat stress in *Arabidopsis* leads to an increase in the content of tocopherol 18 folds. Superoxide dismutase, catalase, and peroxidase were found maximum in high and low temperature treated plants. Increase in catalase is directly associated with stress tolerance and also increase in superoxide dismutase activity due to high temperature in wheat genotypes (Mamta and Puri, 2017). Liu *et al.*, (2013)²⁴ reported that synthesis of peroxidase get enhanced in *Avena nuda* when exposed to low temperature. Ascorbate peroxidase was found increased with increase in temperature. Similar findings were reported by Almeselmani *et al.*, (2006)²³, that high-temperature results in

increased ascorbate peroxidase content in five genotypes of wheat. Glutathione-s-transferase was found increased with increase and decrease in temperature. Zuo *et al.* (2000)²⁵ reported that GST plays an important role in plants under heat stress.

CONCLUSION: Plant growth in *Datura stramonium* was affected due to applied abiotic stress, *i.e.* temperature stress. Phytochemical contents were also influenced in stress-treated plants indicating that abiotic stress affects the physiological processes in the treated seedlings. There was an increase in enzymatic and non-enzymatic antioxidants indicating that the plant can scavenge or control the level of cellular ROS and can be grown successfully under stressful conditions. Alkaloidal content increased at high temperature as well as in low-temperature conditions. The study revealed the tolerance ability of *Datura stramonium* and strong antioxidant defense mechanism in temperature stress.

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CONFLICT OF INTEREST: Nil

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