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PHYSIOLOGICAL EFFECTS OF HYPERTHERMIA ON THREE PLANT SPECIES

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In the present study physiological responses of three plant species (*Boerhaavia diffusa* L., *Adhatoda vasica* Nees and *Parthenium hysterophorus* L.) to hyperthermia have been investigated. Supra-optimal temperature was found to exert profound influence on stability of photosynthetic pigments, soluble proteins, membrane permeability and enzymes. The least decay in photosynthetic pigments was observed in *Adhatoda* while *Parthenium* was characterized by the maximum degradation of photosynthetic pigments. Treatment of leaves at high temperature also led to reduction in extractable aqueous buffer soluble proteins in all the plants. The membrane permeability under hyperthermia also differed markedly.
Apparent differences were observed among these plants with respect to thermostability of the enzymes. Peroxidase was found to be the most thermo tolerant enzyme in all the three plants studied. The enzyme PR of *Adhatoda* exhibited comparatively better thermotolerance than those of *Boerhaavia* and *Parthenium*.

Keywords: Hyperthermia, Membrane permeability, Photosynthetic pigments, Soluble proteins, Thermo tolerant.

Introduction

Repercussions of greenhouse warming are discernible in all the natural resources of the Earth. The apparent initial consequences of which are raising oceans, inundation of coastal regions and melting ice caps, but reverberations of climate change are limitless. Weather and climate conditions have significant effects on health and the well being of life on planet Earth. Human beings, animals and plants respond physiologically to a number of atmospheric conditions, including those of temperature, precipitation, humidity, wind, solar radiation and air pollution. Although ecosystems have a great capacity to adapt to variations in climate and environmental conditions yet stress beyond tolerable limits can be very destructive.

Higher plants are subjected to a variety of supra- optimal environmental factors such as moisture, stress, salt stress, radiation stress and temperature stress. Plants react to environmental stress at the morphological, anatomical and gene level[1]. Major alterations occur in chloroplasts like altered structural organization of thylakoids, loss of grana stacking and swelling of grana under heat stress[2,3]. The decline in chlorophyll pigment is also a result of lipid peroxidation of chloroplast and thylakoid membranes as observed in sorghum due to heat stress (40/30 °C, day/night)[4] . In soybean, heat stress (38/28 °C) significantly decreased total chlorophyll content (18%), chlorophyll a content (7%), chlorophyll a/b ratio (3%). As a result, decrease in sucrose content (9%) and increased reducing sugar content (47%) and leaf soluble sugars content (36%) were observed[5]. Hyperthermia is known to adversely affect the various facets of growth and development of plants which eventually affect the productivity of ecosystem of deserts. Increasing global warming due to human activities like urbanization also impose serious threats on the productivity of crop as well as wild plants.

In major parts of Rajasthan, plants are subjected to environmental hazards which include scarcity of water, extreme of thermal load, intense illumination and at specific places, high salinity. A systematic evaluation of factors responsible for poor performance and reduced yield under these hostile environments has not been given priority. Apart from drought, plants growing in arid regions have to face higher than normal temperatures also. The successful growth and yield of plants in arid and semi-arid regions largely depend on the stability of the plants to either avoid or resist the ill effects of hyperthermia.

There is paucity of data on the plant responses to high temperature in Rajasthan. It was therefore decided to investigate various physiological and biochemical parameters under high temperature stress in certain plant species of Rajasthan.

Material and Methods

In the present study, effects of hyperthermia on three plant species (*Boerhaavia diffusa* L., *Adhatoda vasica* Nees and *Parthenium hysterophorus* L.) was studied. Regular observations were made on effect of high temperature on plant constituents, membrane permeability and enzymatic activities. All three are common weeds growing in mixed habitats throughout the state.

In order to study the effect of hyperthermia, thermal treatment was given[6]. Leaf discs of respective plants were placed in water in a vial, pre-incubated at desired temperature. While an identical set kept at room temperature served as control. Leaf discs were removed at specific time intervals for analysis. For determination of in vitro enzyme activities, the extracted enzyme was incubated at the desired temperature.

Analytical Methods for Constituents

1. Chlorophylls

Plant material was homogenized in 80% alcohol, centrifuged and the supernatant was used for the estimation of chlorophylls. The absorbance was measured at 663 and 645 nm using 80% acetone as blank. Chlorophylls were measured[7].

2. Total soluble proteins were measured[8]. Standard curve was prepared using Bovine Serum Albumin protein.

3. Membrane permeability was determined[9].

4. Enzyme assay

Extraction of enzyme

Desired sample (200 mg, fresh weight) was homogenized in 10 ml. of 0.2M Tris- HCl buffer pH 7.0. The extract was centrifuged for 30 minutes at 10,000 rpm. The supernatant was stored in ice bath and used as source of enzyme.

Peroxidase was assayed[10]. Nitrate reductase activity was estimated[11].

Results and Discussion

Supra- optimal temperatures and moisture stress are the two main environmental hazards encountered in deserts. High temperature (40-47°C) can bring about significant reduction in growth of plants[12,13]. Plants inhabiting arid and semi- arid regions have to resort to certain strategies in order to grow and survive under high thermal load.

The plants investigated in the present work were found to exhibit higher stability of photosynthetic pigments, although there were marked differences with respect to thermal stability of pigments. *Adhatoda* exhibited much more thermal stability of pigments than *Parthenium,* while the response of *Boerhaavia* was found to be intermediate (Fig. 1).

The potential of higher thermal stability of pigments has been found to be associated with heat tolerance of plants[14]. Greater stability of pigments at high temperature in *Adhatoda* might enable it to grow even during hottest months of the year.

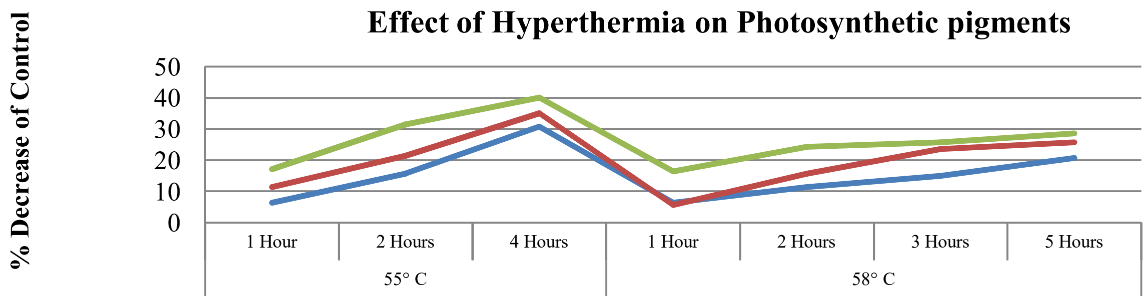
Exposure of plants to high temperature also led to a decline in soluble proteins. The reduction in proteins was comparatively less in *Adhatoda* than in *Boerhaavia* and *Parthenium* (Fig. 2). Loss in soluble proteins under high thermal load has been observed in *Atriplex sabulosa* Rouy and *Tidestromia oblongifolia* (S.Watson) Standl.[15]. These workers have also found a relation between the reduction in soluble proteins and a corresponding decline in photosynthetic rates.

Pasture plants of Indian desert also exhibited differences in the membrane stability at high temperature. The damage in the membranes due to high temperature was more drastic in case of *Parthenium*, while *Adhatoda* exhibited greater thermal stability of membrane. The response of *Boerhaavia* was found to be superior to *Parthenium* but inferior to *Adhatoda* (Fig. 3). In sorghum, increased membrane stability at high temperature has been found to be associated with heat tolerance. Damage to membrane permeability has been considered as the primary cause of heat injury. All other deleterious effects of thermal stress are supposed to be secondary effects arising as a consequence of membrane injuries, a number of physiological processes are mediated through membrane- bound systems. Thus, loss of membrane integrity would impair functioning of these physiological processes and eventually result in a reduction in growth and yield.

Wide variations were also recorded in the thermostability of different enzymes. NR was found to be most sensitive to elevated temperatures. *Boerhaavia* exhibited higher thermal tolerance as compared to *Parthenium* and *Adhatoda*. Thus, at 45°C for 1 hour treatment there was complete denaturation of this enzyme in *Adhatoda* while *Boerhaavia* and *Parthenium* retained 40% and 15% activity respectively. In vitro thermal tolerance of NR in *Adhatoda* was much inferior to that of *Boerhaavia* and *Parthenium* (Fig 4) Higher thermal tolerance of NR, of *Boerhaavia* was also evident in vivo (Fig 5) However, in *Adhatoda* which showed greater thermal decay of NR in vitro but in vivo exhibited more tolerance than *Parthenium*. Possibly, in this plant some endogenous thermal protections of NR may be present which are lost during in vitro study. Also, thermal tolerance of enzyme may be different in intact cell from that in extract due to pH and the presence of protectants.

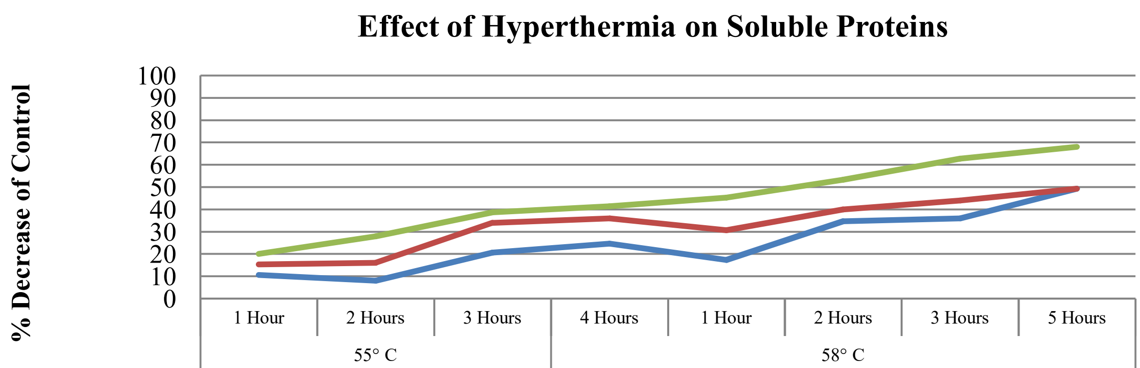
The most tolerant enzyme of plants in the present study was found to be PR. This enzyme exhibited higher thermal tolerance both in vivo as well as in vitro in all plants. In contrast to NR, complete inactivation of PR in vitro did not occur even after 1 hour at 55° C (Fig 6 and 7). Most of the heat injuries in plants are supposed to be mediated through inactivation of enzyme[16]. The ability of plants to grow, survive and reproduce successfully under supra- optimal temperatures would largely depend upon thermal tolerance of key enzymes. In fact, co-relation between thermal tolerance of enzymes and of organisms have been found to exist[17].

Higher thermal tolerance of C_4 plants has been attributed to higher thermal stability of their primary carboxylating enzyme[18]. Of the enzymes PR exhibited remarkable thermal tolerance irrespective of origin, while NR exhibited low thermal tolerance with quantitative differences in enzymes may reside in the difference in amino acids, presence of endogenous thermal protectants and the stability of membrane permeability in case of membrane bound enzymes. Thus, phenolase, RUDP- case, PEP-case and glucose-6p-dehydrogenase are thermal tolerant, while NR is very sensitive to heat stress. Rapid inactivation of NR at supra-optimal



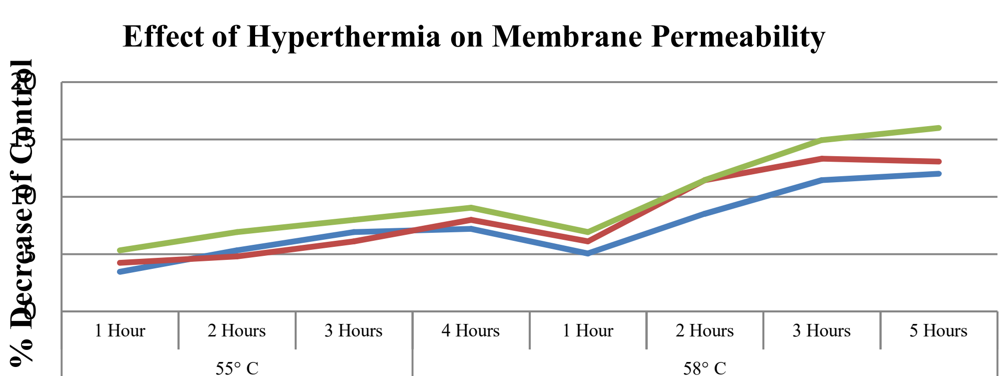
	1 Hour	2 Hours	4 Hours	1 Hour	2 Hours	3 Hours	5 Hours
	55° C			58° C			
Adhatoda sp.	6.43	15.71	30.71	6.43	11.43	15	20.71
Boerhaavia sp.	11.43	21.43	35	5.71	15.71	23.57	25.71
Parthenium sp.	17.14	31.43	40	16.43	24.29	25.71	28.57

Fig. 1: Effect of Hyperthermia on Photosynthetic pigments



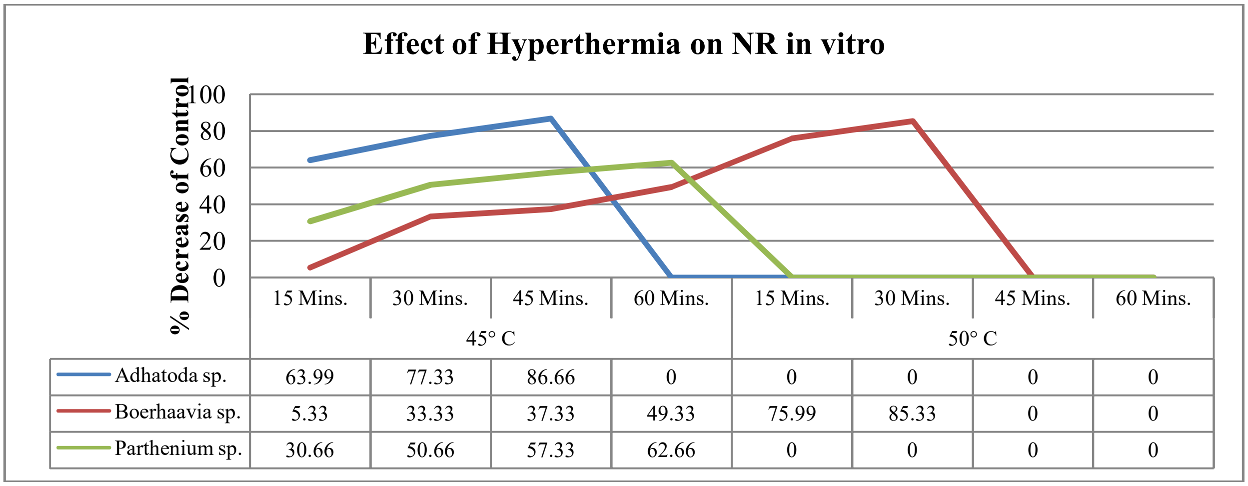
	1 Hour	2 Hours	3 Hours	4 Hours	1 Hour	2 Hours	3 Hours	5 Hours
	55° C				58° C			
Adhatoda sp.	10.67	8	20.67	24.67	17.33	34.67	36	49.33
Boerhaavia sp.	15.33	16	34	36	30.67	40	44	49.33
Parthenium sp.	20	28	38.67	41.33	45.33	53.33	62.66	68

Fig. 2: Effect of Hyperthermia on Soluble Proteins



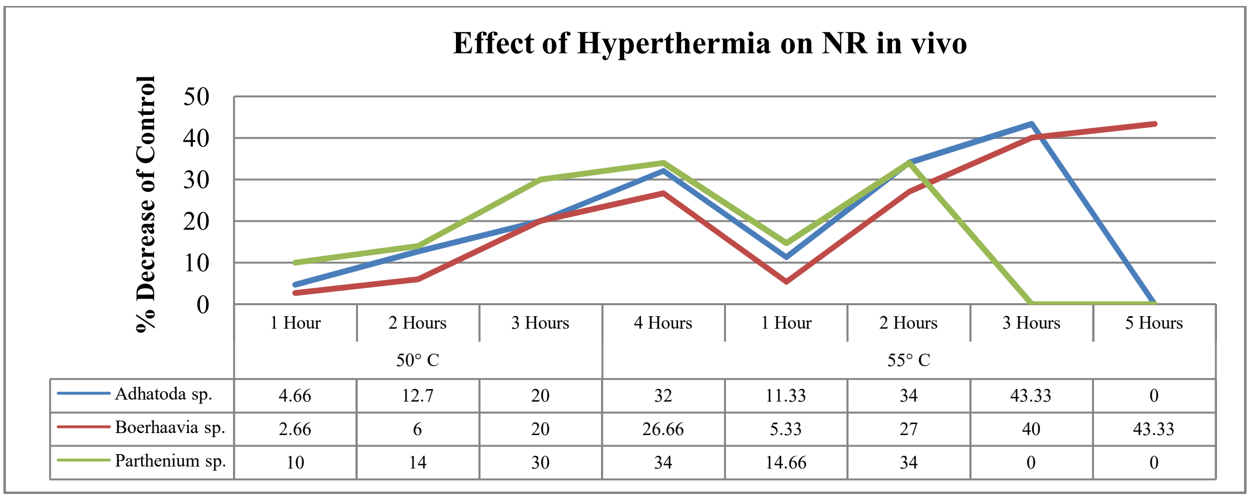
	1 Hour	2 Hours	3 Hours	4 Hours	1 Hour	2 Hours	3 Hours	5 Hours
	55° C				58° C			
Adhatoda sp.	3.46	5.33	6.93	7.2	5.06	8.53	11.46	12
Boerhaavia sp.	4.26	4.8	6.13	8	6.13	11.46	13.33	13.06
Parthenium sp.	5.33	6.93	8	9.06	6.93	11.46	14.93	16

Fig. 3: Effect of Hyperthermia on Membrane Permeability



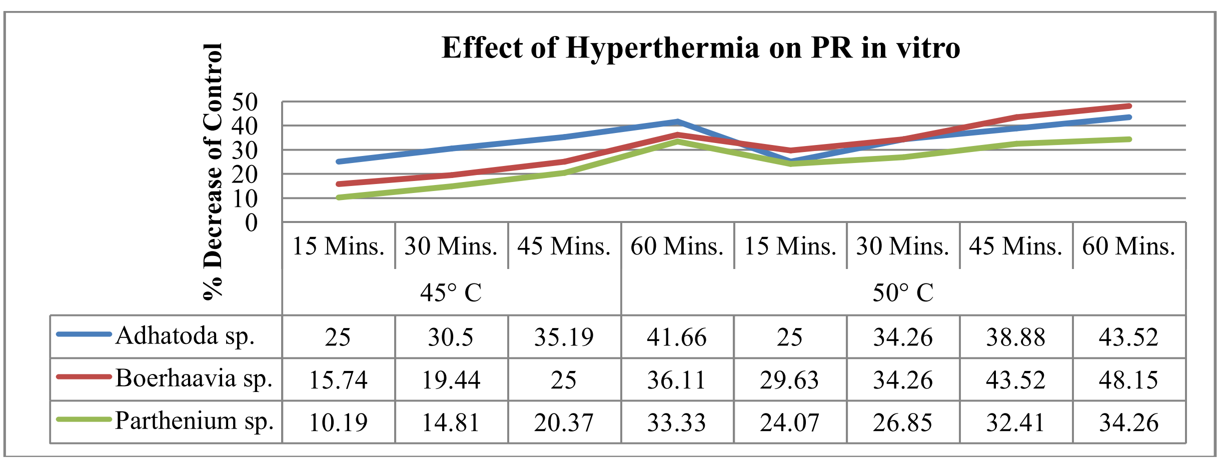
	45° C				50° C			
	15 Mins.	30 Mins.	45 Mins.	60 Mins.	15 Mins.	30 Mins.	45 Mins.	60 Mins.
Adhatoda sp.	63.99	77.33	86.66	0	0	0	0	0
Boerhaavia sp.	5.33	33.33	37.33	49.33	75.99	85.33	0	0
Parthenium sp.	30.66	50.66	57.33	62.66	0	0	0	0

Fig. 4: Effect of Hyperthermia on NR in vitro



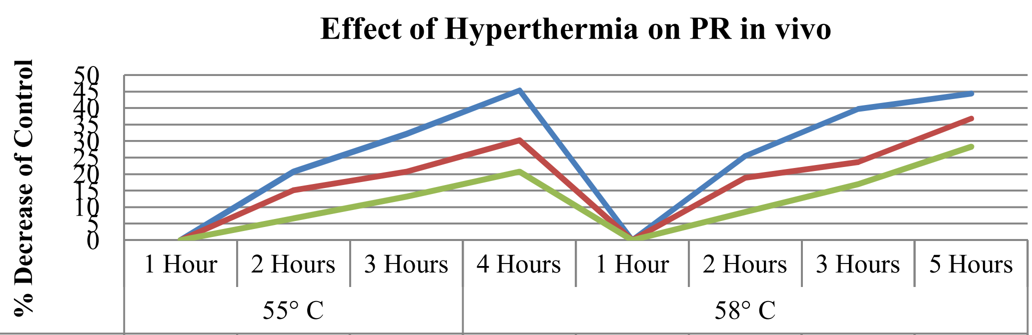
	50° C				55° C			
	1 Hour	2 Hours	3 Hours	4 Hours	1 Hour	2 Hours	3 Hours	5 Hours
Adhatoda sp.	4.66	12.7	20	32	11.33	34	43.33	0
Boerhaavia sp.	2.66	6	20	26.66	5.33	27	40	43.33
Parthenium sp.	10	14	30	34	14.66	34	0	0

Fig. 5: Effect of Hyperthermia on NR in vivo



	45° C				50° C			
	15 Mins.	30 Mins.	45 Mins.	60 Mins.	15 Mins.	30 Mins.	45 Mins.	60 Mins.
Adhatoda sp.	25	30.5	35.19	41.66	25	34.26	38.88	43.52
Boerhaavia sp.	15.74	19.44	25	36.11	29.63	34.26	43.52	48.15
Parthenium sp.	10.19	14.81	20.37	33.33	24.07	26.85	32.41	34.26

Fig. 6: Effect of Hyperthermia on PR in vitro



	55° C				58° C			
	1 Hour	2 Hours	3 Hours	4 Hours	1 Hour	2 Hours	3 Hours	5 Hours
Adhatoda sp.	0	20.75	32.08	45.28	0	25.47	39.62	44.34
Boerhaavia sp.	0	15.09	20.75	30.19	0	18.87	23.58	36.79
Parthenium sp.	0	6.6	13.21	20.75	0	8.49	16.98	28.3

Fig. 7: Effect of Hyperthermia on PR in vivo

temperature has been observed by different workers[13,19-20].

The property of thermal tolerance is not absolute as it varies with the plant age and season. Also, in addition to physiological parameters, cytological criteria such as protoplasmic viability, stability of nucleic acid plasmolysis etc. should also be considered while evaluating thermal tolerance of plants. Morphological and growth changes are manifested by plants as a response to hyperthermia. Various plants have different levels of adjustments to high temperature in the form of physiological effects like photosynthetic hindrance due to effects on photochemical reactions, interference of leaf water relations, anatomical changes at cellular and tissue level. Knowledge of response and tolerance mechanisms will pave the way for transgenic heat tolerant plants and could be the basis for production of crops with higher yields under heat stress conditions.

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