

American Journal of Environment Studies (AJES)



**Effect of Hyperthermia on Photosynthetic Pigments of
Some Selected Fern Taxa of Sitamata Wild Life
Sanctuary, Rajasthan**

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Abstract

Rajasthan, the state is situated in the north western part of India and covers an area of 3,42,274 sq.km. Despite the hostile climatic conditions of the state, pteridophytes which are otherwise known as shade and moisture loving plants. Sitamata wild life sanctuary is located in the southwest region of Rajasthan. Different species of pteridophytes in this region exhibit significant variation in thermal stability of their photosynthetic pigments of some pteridophytic flora of Sitamata wild life sanctuary, Rajasthan. Studies reveal that total chlorophyll contents are maximum in *Ceratopteris thalictroides* and *Selaginella repanda* and minimum in *Ampelopteris prolifera*. The other species range between these two. Total carotenoid contents were found maximum in *Cheilanthes bicolor* and minimum in *Selaginella repanda*. These observations suggest that species with greater resistance to drought possess higher quantity of carotenoids and show lesser degradation in their chlorophyll pigments during stress conditions. Thus, *Ampelopteris prolifera*, *Cheilanthes bicolor* and *Christella dentata* are most resistant to heat and drought conditions while *A. capillus-veneris*, *Ceratopteris thalictroides* and *Selaginella repanda* are the least resistant taxa to heat and drought conditions.

Keywords: *Thermal stability, photosynthetic pigments, carotenoids, chlorophyll content*

1.0 INTRODUCTION

The dry and hot climate of Rajasthan display abundant growth of pteridophytes from end of July to mid October after which these start to dry up, the photographs of selected fern taxa were shown in figure1. Sharma *et al.* (1977) have established the importance of chlorophyll stability index as an important factor for heat and drought resistance in plants. A higher chlorophyll stability index indicates higher capacity for heat and drought resistance. Studies on photosynthetic pigments shows that total chlorophyll contents are maximum in *Ceratopteris thalictroides* and *Selaginella repanda* and minimum in *Ampelopteris prolifera*. The other species range between these two. Total carotenoid contents were maximum in *Cheilanthes bicolor* and minimum in *Selaginella repanda*. These observations support the findings of Rathode and Sharma (1991) who concluded that aquatic and shade loving plants have more amounts of chlorophylls and less of carotenoids while reverse are true in drought resistant types. Analysis of photosynthetic pigments in some pteridophytes was studied in ratnagiri district of Maharashtra suggested that changes were found in amount of chlorophyll pigments during formation of sporangia (Masal *et al.*, 2010). Some Physiological studies including photosynthetic pigments like total chlorophylls, carotenoids, polyphenol, major organic and inorganic constituents of pteridophytic flora of Lohgad area of Maharashtra was studied by Gavade *et al.* (2015). The effect of arsenic (As) on photosynthesis of *Pteris cretica* studied by Zamanova *et al.* (2021) who reported that increasing doses of Arsenic decreases of net photosynthesis , Transpiration rate and photosynthetic pigments.





Figure 1: Showing photographs of *Ampelopteris prolifera*, *Christella dentata*, *Cheilanthes bicolor*, *Selaginella repanda*, *Adiantum capillus-veneris*, *Ceratopteris thalictroides*

2.0 MATERIAL AND METHODS

About 1gm of fresh leaves of mature plants was homogenized in 15 ml of 80% acetone. After centrifugation, the supernatant was used for quantitative analysis of photosynthetic pigments. Quantitative estimation of chlorophyll a, chl.b, total chlorophylls and total carotenoids was done following the method suggested by Robbelen (1957). Optical densities were recorded at 663 and 645 nm for chlorophylls and at 430 nm for total carotenoid contents using Systronics Spectrophotometer 106. Amount of chlorophylls and carotenoids were calculated as per the following formulae.

$$\begin{aligned} \text{Chl a (mg/gm fresh wt.)} &= \frac{12.3 \times \text{OD } 663 - 0.86 \times \text{OD } 645}{a \times 1000 \times w} \times V \\ \text{Chl b (mg/gm fresh wt.)} &= \frac{19.3 \times \text{OD } 645 - 3.6 \times \text{OD } 663}{a \times 1000 \times w} \times V \\ \text{Total chlorophylls (mg/gm fresh wt.)} &= \frac{20.2 \times \text{OD } 645 + 8.02 \times \text{OD } 663}{a \times 1000 \times w} \times V \\ \text{Total carotenoids (mg/gm fresh wt.)} &= \frac{4.75 \times \text{OD } 430 - (\text{Chl a} + \text{Chl b}) \times 0.226}{a \times 1000 \times w} \times V \end{aligned}$$

V = Volume of solvent (Acetone) in which the Fresh material was crushed.

a = Length of light path in the cell which is usually 1 cm.

W = Fresh weight of material in gms. This was crushed.

To determine heat and drought resistance of the selected taxa, thermal treatments of 40° and 45°C were given to 1 gm fresh leaves in 20 ml distilled water (Sullivan, 1967) in a water bath for 1h, 2h, 3h and 4h time duration along with controls at room temperature. The leaves were then homogenized in 10 ml of 80% Acetone. Photosynthetic pigments (total chlorophylls and total carotenoids) were measured according to Robbelen's method (1957) by Systronics spectrophotometer 106, set at 100% transmittance using pure acetone as a blank. Optical densities were recorded at 650 nm and 430 nm respectively. Total chlorophyll contents and percentage degradation of total chlorophylls and carotenoids were calculated as per the following formulae:

$$\text{Total chlorophyll (mg/gm fresh wt)} = \frac{\text{OD 650} \times \text{V}}{\text{a} \times 34.5 \times \text{W}}$$

$$\% \text{ degradation of total chlorophyll / carotenoids} = \frac{\text{A} - \text{B}}{\text{A}} \times 100$$

A = Total chlorophyll/carotenoids in control.

B = Total chlorophyll/carotenoids in treated plant.

A study of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids in leaves of mature plants of selected species of pteridophytes occurring in Sitamata wild life sanctuary, Rajasthan has been carried out. Photographs and data have been presented figure 1 and in table 1, respectively.

Table 1: Photosynthetic pigments (mg/gfw) in leaves of some pteridophytic taxa of Sitamata Wild Life Sanctuary, Rajasthan.

S.NO	Species	Chl a	Chl b	Total Chl	Total Carotenoids
1	<i>Selaginella repanda</i>	2.05	1.15	3.20	0.13
2	<i>Adiantum capillus-veneris</i>	0.98	0.46	1.44	0.38
3	<i>Ampelopteris prolifera</i>	1.21	0.19	1.40	0.43
4	<i>Ceratopteris thalictroides</i>	2.32	1.54	3.86	0.21
5	<i>Cheilanthes bicolor</i>	1.05	0.39	1.44	0.46
6	<i>Christella dentate</i>	1.20	0.59	1.79	0.39

3.0 RESULTS

Treatment of 1-4 hours at 40°C lead to higher total chlorophyll content degradation in *Ceratopteris thalictroides* followed by *A. capillus-veneris* and *Selaginella repanda* while in *Christella dentata*, *Cheilanthes bicolor* and *Ampelopteris prolifera* it was comparatively low. A temperature treatment of 45°C for 1-4 hours lead to further loss of total chlorophyll content which was comparatively more in *A. capillus-veneris*, *Ceratopteris thalictroides* and

Selaginella repanda. These species thus, show more thermal decay in total chlorophyll content compared to *Ampelopteris prolifera*, *Christella dentata* and *Cheilanthes bicolor*. *Ampelopteris prolifera*, *Cheilanthes bicolor* and *Christella dentata* show maximum degradation of total carotenoids after 4 hours treatment at 45°C. This percentage of total carotenoid content degradation was recorded to be minimum in *Adiantum capillus-veneris*, *Ceratopteris thalictroides*, *Ampelopteris prolifera* and *Selaginella repanda*. Maximum percentage of total carotenoid degradation at 40°C was observed in *Cheilanthes bicolor*, *Ampelopteris prolifera* and *Christella dentata* and it was to be minimum in *Adiantum capillus-veneris*, *Ceratopteris thalictroides*, and *Selaginella repanda*.

4.0 CONCLUSION

Inverse relationship in the degradation pattern of the two different types of photosynthetic pigments namely chlorophylls and carotenoids at higher temperatures is evident in ferns and fern allies of Sitamata forest of Rajasthan. Thermal decay in photosynthetic pigments is a limiting factor for plant growth and supra optimal conditions (Mothos, 1964). Bohra *et al.* (1979) and Yadav & Bhardwaja (1983) concluded that xerophytic species of ferns possess higher carotenoid contents and show lesser degradation of chlorophylls. This fits in well with the known functions of the carotenoids-protection of plants from photosensitised oxidation of chlorophyll pigments (Krinsky, 1966). The highest chlorophylls were found in gametophyte of *Adiantum flabellatum* and the highest carotenoids was obtained from the gametophyte of *Cheilanthes albomarginata* (Asia bibi *et al.*, 2019). These observations suggest that species with greater resistance to drought possess higher quantity of carotenoids and show lesser degradation in their chlorophyll pigments during stress conditions similar observations have been made by Kothari and Yadav (2003). Pareek *et al.* (2005) and Tripathi *et al.* (2005) on some ferns and fern allies of Rajasthan. Thus, *Ampelopteris prolifera*, *Cheilanthes bicolor* and *Christella dentata* with higher degradation in carotenoids and greater stability in chlorophyll pigments are most resistant to heat and drought conditions while *A. capillus-veneris*, *Ceratopteris thalictroides* and *Selaginella repanda* with higher degradation in chlorophyll pigments are the least resistant taxa to heat and drought conditions.

Total chlorophyll contents among ferns and fern allies were found to be highest in *Ceratopteris thalictroides* (3.86mg/gfw) and lowest in *Ampelopteris prolifera* (1.40 mg/gfw). Highest amount of total carotenoids has been observed in *Cheilanthes bicolor* (0.46 mg/gfw) and lowest in *Selaginella repanda* (0.13 mg/gfw). These observations suggest that species with greater resistance to drought possess higher quantity of carotenoids. Thus, *Cheilanthes bicolor* and *A. prolifera*, which withstand the unfavourable conditions for longer period, contain higher quantity of carotenoids.

5.0 DISCUSSION

Among various metabolic changes underlying the mechanism of heat and drought tolerance of these taxa, mention may now be made of the effect of hyperthermia on total chlorophylls and carotenoids. The data have been presented in Table 2-3. A perusal of Table 2 reveals that different species exhibit significant variations in thermal stability of their photosynthetic pigments. Thus, an hour's treatment at 40°C led to maximum chlorophyll degradation in *Ceratopteris thalictroides* (22.37%) followed by *A. capillus-veneris* with (18.63%), *S. repanda* (18.13%) and *Cheilanthes bicolor* it was (9.78%) while the minimum chlorophyll degradation was recorded in *Christella dentata* (3.62%). However, 4 hour's treatment at 40°C showed highest degradation of total chlorophyll contents in *Adiantum capillus-veneris* (61.99%) and *Ceratopteris thalictroides* (61.14%) and the lowest in *Ampelopteris prolifera*

(37.81%). Other species are ranging between these two. A temperature treatment of 45⁰C for four hours led to further loss of total chlorophyll content which was comparatively more in *Adiantum capillus-veneris* (75.19%) and *Ceratopteris thalictroides* (70.60 %). These two taxa show more thermal decay in total chlorophyll contents compared to *Cheilanthes bicolor* (47.00%), *Ampelopteris prolifera* (47.23%) and *Christella dentata* (51.11%). Data pertaining to carotenoid contents in response to specified periods of temperature treatment are presented in table-3. It may be observed from these data that *Cheilanthes bicolor* (13.51%), *Ampelopteris prolifera* (11.93%), *Christella dentata* (9.93%) and *Adiantum capillus-veneris* (9.92%) show higher degradation in carotenoid contents after 1-hour treatment at 40⁰C. This percentage of total carotenoid content degradation was recorded to be lower in species of *Ceratopteris* and *Selaginella*. Almost similar pattern of carotenoid degradation has been observed at different time duration at 40⁰C and 45⁰C temperature treatment.

ACKNOWLEDGEMENTS

Authors are thankful to botany department of MLV Govt. College, Bhilwara for providing infrastructure and research facilities. We also appreciate Dr. Shahdab Husssain for his guidance and moral support.

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APPENDIX

Table 2: Stability of photosynthetic pigments (total chlorophylls) in leaves of some pteridophytic taxa of Sitamata Wild Life Sanctuary, Rajasthan, at various temperature regimes for a specified period (values represented as % degradation)

S. No.	Species	40°C				45°C			
		1h	2h	3h	4h	1h	2h	3h	4h
1.	<i>Selaginella repanda</i>	18.13	24.46	40.67	56.27	22.56	33.93	46.78	63.46
2.	<i>Adiantum capillus-veneris</i>	18.63	39.72	52.06	61.99	24.62	43.67	69.11	75.19
3.	<i>Ampelopteris prolifera</i>	11.66	16.98	23.30	37.81	12.94	19.59	29.15	47.23
4.	<i>Ceratopteris thalictroides</i>	22.37	34.57	53.00	61.14	26.80	42.64	59.94	70.60
5.	<i>Cheilanthes bicolor</i>	9.78	14.09	28.15	41.43	13.73	17.71	33.34	47.00
6.	<i>Christella dentata</i>	3.62	9.10	19.35	39.18	6.72	13.91	42.29	51.11

Table 3: Stability of photosynthetic pigments (total carotenoids) in leaves of some pteridophytic taxa of Sitamata Wild Life Sanctuary, Rajasthan, at various temperature regimes for specified period. (Values represented as % degradation.)

S. No.	Species	40°C				45°C			
		1h	2h	3h	4h	1h	2h	3h	4h
1.	<i>Selaginella repanda</i>	8.67	12.82	21.33	30.25	11.14	16.71	33.14	44.58
2.	<i>Adiantum capillus-veneris</i>	9.92	15.58	22.86	26.18	12.22	18.75	26.08	36.17
3.	<i>Ampelopteris prolifera</i>	11.93	19.62	26.09	40.24	15.82	23.34	32.91	47.65
4.	<i>Ceratopteris thalictroides</i>	6.25	18.12	21.25	29.73	8.75	21.87	26.85	36.87
5.	<i>Cheilanthes bicolor</i>	13.51	20.90	30.63	38.73	18.91	29.72	44.14	56.75
6.	<i>Christella dentata</i>	9.93	17.57	27.48	36.64	13.74	22.90	33.58	47.20