

The Studies of the Ecophysiological Characteristics of Different Species of Weed from Northern Rajasthan



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Abstract

Ecological adaptations of *Amaranthus spinosus*, *Chenopodium album* and *Parthenium hysterophorus*, which are most widely distributed weed species of northern Rajasthan have been investigated to evaluate the underlying eco-physiological characteristics of these weed species. Calcium, potassium, foliar mineral content, moisture content, total bound water, proline, photosynthetic pigments and effect of hyperthermia (40°C, 42°C and 45°C) for specified time period on pigments stability and membrane permeability of some selected weed species have been studied. This study has revealed that these weed species have a wide range of thermal tolerance so far as chlorophyll and carotenoid stability is concerned. The leachates of sugars and proteins have shown that their membrane is also quite resistant to hyperthermia.

Keywords: Adaptation, Investigated, Estimation, Hyperthermia, Permeability, Tolerance and Resistant.

Introduction

In general, the vegetation of Rajasthan tolerates higher temperature and intense solar radiations for the most of the year. The aridity and light, temperature, pigments stability and membrane permeability and other factors influencing the vegetation of Rajasthan desert. A number of lists of plants and accounts on the vegetation of Rajasthan desert have been published during the last four decades (Das and Saurp, 1951; Biswas, 1952; Biswas and Rolla, 1953; Puri et. al., 1964; Bhandari, 1978; Dawre et al., 1979). These studies have further contributed to our knowledge of the flora of Rajasthan desert. The aspects of economic and harmful plants and the plant introduction, afforestation etc. have been covered in more than 50 papers (Jain, 1970). Shingi, (1981) has enumerated the grasses of Jhalawar district. Accounts of medicinal plants from tribal area of Rajasthan (Singh and Pandey, 1980) have been published. Poisonous and harmful plants of Rajasthan have been worked out by Singh and Parmer, (1980, 1981 and 1982). Economics due to weeds warrant urgent attention and point out the need for developing weed control programme in high yielding varieties as well as cash crops (Datta and Banerjee, 1975). Weeds utilize a good portion of moisture from the soil and transpire it into the atmosphere with the introduction of high yielding varieties of rice, wheat, maize etc. more fertilizers are being used. Weeds consume large proportion of fertilizers for their own needs. In tropical countries like India weed problems are more complex than those of temperate regions (Holm, 1971; Parker, 1972).

In this study three selected weed species are the most widely distributed weed species of northern Rajasthan have been investigated to evaluate the ecophysiological characteristics. Calcium potassium ratio, foliar mineral contents of dried leaves samples were investigated from these weeds species and also observed moisture contents and total bound water. In order to study the effect heat and drought tolerance fresh leaves of the selected weed species were subjected to various degree of temperature for specified time period. Quantitative estimation of proline and photosynthetic pigments (Total chlorophylls and total carotenoids) was carried out.

Materials and Methods

Determination of Moisture Content

For moisture content 5 mg of fresh leaves of each plant species were heated at 110°C for 24 hours. These leaves were allowed to cool at

E: ISSN NO.: 2455-0817

Remarking An Analisation

room temperature and weighed again. Percentage of moisture content was calculated as per the formula:

$$\% \text{ Moisture content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

2.2 Total bound water estimation

The total bound water was calculated by vacuum drying method. 5 gm of leaves were weighed and subsequently heating at 110°C for 24 hours. After heating leaves were weighed again. Percentage bound water was determined by the formula:

$$\% \text{ Bound water} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.3 Total free proline estimation

Free proline was estimated in the leaves according to Bates et al. (1973) method. Briefly, 100 mg of healthy leaves were homogenised in 10 mL of sulphosalicylic acid. After centrifugation the supernatant was used as aliquot. To the 1mL of aliquot, 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent was added. The test tubes were placed in boiling water for 45 minutes and then cooled. 4 mL of toluene was added to each test tube and thoroughly shaken. The upper pink coloured organic phase was separated, optical density was recorded at 540 nm using Spectroplus model D. Standard curve of proline was used for calculating the amount of proline.

Photosynthetic Pigment Estimation

Chlorophyll a, b and carotenoids were estimated for each plants species according to Robbelen, (1957). 100 mg of fresh leaves were homogenised in 10 mL of 80% acetone. Homogenate was separated and centrifuged. Optical density was recorded at 663 nm, 645nm and 430 nm for chl a, b and carotenoids, respectively. Calculations were done by the following formulae:

$$\text{Chlorophyll a (mg/gm)} = \frac{12.3 \times D_{663} - 0.860 \times D_{645} \times V}{a \times 1000 \times W} \times 100$$

$$\text{Chlorophyll b (mg/ gm)} = \frac{9.3 \times D_{645} - 3.6 \times D_{663} \times V}{a \times 1000 \times W} \times 100$$

$$\text{Carotenoids (mg/L)} = \frac{4.75 \times D_{430} - (a+b) \times 0.226 \times V}{W \times a \times 1000} \times 100$$

$$\text{Total chlorophylls} = \frac{20.2 \times D_{645} + 8.02 \times D_{663} \times V}{A \times 1000 \times W} \times 100$$

Whereas, V denotes volume in mL, a denotes length of path cell (1cm) and W denotes fresh weight in gm.

Calcium and Potassium Ratio Determination

For determination of calcium, potassium and chloride in leaf tissue, to prepare the extracts by grinding about 1g of dry leaves with distilled water (about 10 mL) at 25 °C for 10 min. The homogenate is centrifuged at 3000xg for 15 min, and the supernatant filtered through qualitative filter paper. An aliquot of filtrate is used for Ca⁺ and K⁺ determination by flame photometry and Cl⁻ by precipitation titration with silver nitrate by Mohr's method.

The calcium and potassium ratio of dry leaves sample of three selected weed species were analysed.

Foliar Mineral Content Estimation

The mineral contents of dried leaf samples of the selected weed species of weeds collected from various part of the northern Rajasthan were analysed. 100 gm fresh sample of each species and material were dried to 65 degree centigrade .The dried sample .5 gm add with 5ml concentrated nitric acid and 3ml of hydrogen peroxide at a maximum temperature . The used a CEM corporation closed vessel microwave to digest 0.5 gm. The resulting solution was diluted to 25ml in reverse osmosis water and sample were analysed near infrared reflectance spectroscopy (NIRS) as described by Corson et al.(1999) to determine the acid detergent fibre (ADF), ash and total crude protein content.

Effect of Hypothermia

To determine the heat and drought resistance of the selected species, thermal treatments of 40°C, 42°C and 45°C were given to 100 mg fresh leaves in 10 mL distilled water (Sulliyani, 1967) in a water bath for 1h, 2h, 3h and 4h time periods along with controls at room temperature. Effect was recorded in terms of consequent degradation of carotenoids content in response to these temperatures.

Cell Membrane Permeability

Cell membrane permeability of the selected weed species was determined. Efflux of the soluble sugars and proteins were taken as criteria of membrane permeability (Kaloyears, 1958). Briefly, leaves of mature plants of the selected weed species were subjected to different temperatures for specified time periods. Temperatures selected were 40°C, 42°C and 45°C. Total soluble sugars and protein leachates were recorded after 1h, 2h, 3h and 4h time period.

Statistical Analysis

One way ANOVA was calculated in excel 2007.

Result and Discussion

The present ecophysiological investigations of the selected weed species have been taken up to study the mechanism of stress – physiology of these taxa. Similar study was done by Sen, (1976). They carried out ecophysiological studies on weeds of cultivated fields with special references to Bajara (Pennisetum typhodium Rich) and till (Sesamum indicum Linn.) crops. Estimation of free proline, photosynthetic pigments and effect of hyperthermia on chlorophyll and carotenoids degradation and cell membrane permeability have been taken as criteria of physiological adaptations of these selected weed species.

Calcium and potassium ratio for the three selected weeds is summarized in Table 1. From the results obtained it is clear that higher calcium potassium ration was obtained for Parthenium hysterophorus and lowest for Chenopodium album. Amaranthus spinosus has been found to occupy an intermediate position in this respect.

Table 2 summarizes the foliar inorganic contents. Phosphorus, potasium, calcium, magnesium

and sodium contents have been recorded to be higher in *Amaranthus spinosus* as compared to the other investigated species. The amount of various minerals is variable in different species and their leaf litter upon decomposition liberates variable quantities of these minerals, which results in the growth of specific associates with these species under natural habitats.

The nitrogen content has been found to be maximum in *Amaranthus spinosus* while the least quantity of nitrogen has been recorded in *Parthenium hysterophorus*. Similar results were reported by Sharma, (1967), he studied carbon / nitrogen status of soil under some plants communities of Churu, Rajasthan. Calcium and sodium contents have also been observed to be lower in *Parthenium hysterophorus*. On the basis of proline contents, *Amaranthus spinosus* may be regarded as the most drought-tolerant species while *Parthenium hysterophorus* may be considered as the least drought tolerant species among the selected weed species.

Table 3 lists the moisture contents and total bound water in the three selected weed species. From the results obtained it is clear that the percent moisture content was higher in *Parthenium hysterophorus* (89.28%) and lower in that of *Amaranthus spinosus* (70.54%). Total bound water is found to be highest in *Amaranthus spinosus* (13.54%) and least quantity of bound water has been recorded in *Parthenium hysterophorus* (4.08%).

Quantitative estimation of proline was observed to be higher for the leaves as compared to roots and stem for each weed (Table 4). In root, *Amaranthus spinosus* (4.8 mg/g) possessed a higher quantity of proline than *Chenopodium album* (2.7 mg/g) and *Parthenium hysterophorus* (1.6 gm/g). Similarly in stem *Amaranthus spinosus* (4.5 gm/g) possessed more proline than the remaining two species. The amount of proline contents in leaves of *Amaranthus spinosus* (5.7 mg/g) again was more than of *Chenopodium album* (4.7 mg/g) and *Parthenium hysterophorus* (2.8 mg/g). On the basis of proline contents, *Amaranthus spinosus* may be regarded as the most drought tolerant species while *Parthenium hysterophorus* may be considered as the least drought tolerant species among the selected weed species.

Photosynthetic pigments (Total chlorophyll and total carotenoids) in mature leaves of the three selected weed species are summarized in Table 5. From the results it is depicted that *Parthenium hysterophorus* had highest total chlorophyll contents (1.249 mg/g) and *Amaranthus spinosus* (0.776 mg/g) had lower total chlorophyll contents. Total carotenoids contents were highest in *Amaranthus spinosus* (0.624 mg/g) and lowest in *Parthenium hysterophorus* (0.379 mg/g). Similar results were reported by Rigon et al., (2013), they studied indirect measurement of photosynthetic pigments in the leaves of *Jatropha curcas*. Likewise, Vittorio, (2009) studied enhancing a leaf radiative transfer model to estimate concentrations and in-vivo specific absorption coefficients of total carotenoids and chlorophylls a and b from single-needle reflectance and transmittance.

Effect of heat and drought tolerance on fresh leaves of the three weeds was carried out and results are summarized in Table 6 and Table 7. The weeds were subjected to various degrees of temperatures for a specified time period. An hour treatment at 40°C caused maximum total chlorophyll degradation (4.39%) in *Parthenium hysterophorus* while in *Amaranthus spinosus* 3.19% chlorophyll degradation was obtained. In similar way, in 4 hours treatment at the same temperature showed a further degradation in total chlorophyll contents, and highest values were obtained for *Parthenium hysterophorus* (30.82%) and the lowest values were obtained for *Amaranthus spinosus* (24.16%).

Likewise, in 4 hours treatment at the 42°C temperature resulted in loss of total chlorophyll in *Parthenium hysterophorus* revealed maximum total chlorophyll contents (40.15%) compared to *Amaranthus spinosus* (30.00%), showed minimum degradation.

Further degradation of chlorophyll content was observed at 4 hours treatment at 45°C, and values obtained for *Parthenium hysterophorus* (56.39%) and in *Amaranthus spinosus* (48.30%), respectively. Thus deterioration in the total chlorophyll contents between the three successive temperatures was found to occur after 4 hours treatment at 45°C in all the investigated weed species (Table 6). Similar effects were recorded in previous study by Gates, (1968) for transpiration and leaf temperatures.

At specified periods of time, temperature treatment was given to the selected weed species and the consequent degradation of carotenoids content in response to the temperature was recorded. Carotenoids content degradation (%) obtained for the weeds at different temperature and time period is summarized in Table 7. It was observed that an inverse relationship in the degradation pattern of the two different types of photosynthetic pigments i.e. chlorophylls and carotenoids were evident in these species. Thus, *Amaranthus spinosus* having minimum degradation of total chlorophyll content and being thereby more heat tolerant, showed maximum carotenoid content degradation of 57.01% at 45°C. The percentage of total carotenoid content degradation was lower in *Parthenium hysterophorus* (48.20%).

Cell membrane permeability in the three selected weed species was investigated. Results showed that in *Parthenium hysterophorus* the cell membrane permeability is less stable comparatively to *Amaranthus spinosus* and leaching of sugars and proteins were higher in *Parthenium hysterophorus* and lower in *Amaranthus spinosus* at all the temperatures. It can be observed that the *Amaranthus spinosus* exhibits maximum tolerance to higher temperatures in all selected weed species as is evident from Table 8 to 10. Similar work was done by Lichtenthaler, (1987), he studied Chlorophylls and carotenoids: pigments of photosynthetic biomembranes.

Table 1 Calcium, Potassium Contents and Their Ratio in The Leaves of Selected Weed Species

S.No.	Species name	Total calcium (Ca) (mg/g)	Total potassium (K) (mg/g)	Ratio (Ca:K)
1.	Amaranthus spinosus	1.12	2.66	1:2.38
2.	Chenopodium album	1.35	2.12	1:1.89
3.	Parthenium hysterophorus	1.50	4.15	1: 2.77

Values are significant at $p < 0.05$ **Table 2 Foliar Contents of Inorganic in The Selected Weed Species**

S.No.	Species	Elements (% of dry matter)					
		N	P	K	Ca	Mg	Na
1.	Amaranthus spinosus	1.95	0.72	2.22	1.56	0.56	0.03
2.	Chenopodium album	1.40	0.66	1.66	1.35	0.42	0.04
3.	Parthenium hysterophorus	1.15	0.56	1.27	1.22	0.33	0.01

Values are significant at $p < 0.05$ **Table 3 Percentage Moisture Content and Total Bound Water in Leaves of The Selected Weed Species**

S.No.	Name of species	Moisture content (%)	Total bound water (%)
1.	Amaranthus spinosus	68.50	12.54
2.	Chenopodium album	78.00	08.51
3.	Parthenium hysterophorus	87.24	03.08

Values are significant at $p < 0.05$ **Table 4 Total Free Proline Content in Different Parts of the Selected Weed Species**

S.No.	Name of species	Proline content (mg/g)		
		Root	Stem	Leaves
1.	Amaranthus spinosus	4.6	4.3	5.5
2.	Chenopodium album	2.5	3.4	4.5
3.	Parthenium hysterophorus	1.4	1.3	2.6

Values are significant at $p < 0.05$ **Table 5 Photosynthetic Pigments in The Mature Leaves of The Selected Weed Species Of Northern Rajasthan**

S.No.	Name of species	Chl a	Chl b	Total chlorophyll (mg/g)	Total carotenoids (mg/g)
1.	Amaranthus spinosus	0.472	0.296	0.774	0.620
2.	Chenopodium album	0.686	0.448	1.138	0.442
3.	Parthenium hysterophorus	0.823	0.418	1.245	0.375

Values are significant at $p < 0.05$ **Table 6 Effect of Hypothermia on Photosynthetic Pigment (Total Chlorophyll) Stability in Leaves of The Selected Weed Species At Various Temperature Regimes For A Specified Time Period**

S.No.	Species	40°C				42°C				45°C			
		1h	2h	3h	4h	1h	2h	3h	4h	1h	2h	3h	4h
1	Amaranthus spinosus	3.2	5.2	21.1	24.1	8.1	10.2	24.6	29.9	3.9	11.3	43.1	48.3
2	Chenopodium album	3.5	5.9	24.2	25.9	11.0	15.2	26.6	36.8	4.1	13.3	45.6	51.1
3	Parthenium hysterophorus	4.3	7.7	28.6	30.7	13.2	20.6	38.2	40.1	5.5	15.8	48.4	56.4

Values represented as % degradation

Values are significant at $p < 0.05$ **Table 7 Effect of Hypothermia on Photosynthetic Pigments (Carotenoids) Stability in Leaves of The Selected Weed Species At Various Temperature Regimes For A Specified Time Period**

S.No.	Species	40°C				42°C				45°C			
		1h	2h	3h	4h	1h	2h	3h	4h	1h	2h	3h	4h
1	Amaranthus spinosus	6.4	8.1	30.3	37.5	13.1	22.0	41.1	41.8	14.7	15.4	50.6	56.9
2	Chenopodium album	6.1	7.7	28.1	34.7	11.2	20.9	36.8	38.8	12.2	13.3	47.1	53.3
3	Parthenium hysterophorus	5.1	6.1	22.1	25.1	09.1	16.6	32.7	34.1	10.6	12.1	42.2	48.2

Values represented as % degradation

Values are significant at $p < 0.05$

Table – 8 Qualitative Estimation of Cell Membrane Permeability in Leaves of the Selected Weed Species at Various Temperature Regimes For A Specified Time Period

Cell membrane permeability										
S.N.	Species name	Temperature 40degree centigrade			Temperature 42degree centigrade			Temperature 45degree centigrade		
		1h	2h	3h	1h	2h	3h	1h	2h	3h
1.	Amaranthus spinosus	+	+	+	+	+	+	+	+	+
2.	Parthenium hysterophorus	-	-	+	-	+	+	-	+	+
3.	Chenopodium album	-	-	-	-	-	+	-	+	+

Conclusion

Weeds show wide range of tolerance towards the moisture content of the habitat and have exceptionally broad ecological range of adaptation. Effect of hyperthermia (40°C 42 °C and 45°C) for specified time period on the pigments stability and membrane permeability was carried out. Calcium and Potassium ratio has been found to be highest in Parthenium hysterophorus and lowest in Chenopodium album. These studies have revealed that these weed species have a wide range of thermal tolerance so far as chlorophyll and carotenoid stsbility is concerned. Similarly, the leachetes of sugars and proteins have shown that their membranes are also quite resistant to hypothermia. The present study of proline accumulation in the selected taxa has also revealed that free proline content is more in xerophytic species of the selected weed as compared to mesic or less xeric habitats. It can be concluded that Amaranthus spinosus is more heat tolerant than the remaining two species.

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