

Estimation of the Pigment Concentration in Seven Roadside Colonizing Plant Species at Three Different Suburban Areas of Jaipur District, Rajasthan, India

Anubhuti Tiwari^{1*} C. P. Pokharna²

^{1,2} S.R.K.P. Government College Kishangarh Ajmer, Rajasthan, India

Abstract – Green plants synthesize chlorophyll and carotenoids that give color to the leaves and fruits, especially in the unripe stage. Chlorophyll content determines the photosynthetic capacity of the plant per unit area of leaf, stress, environmental pollution and nutritional deficiencies. In the present study the chlorophyll a, chlorophyll b and carotenoid was extracted from the leaves of seven roadside colonizing plant species growing in three different localities of Jaipur district of Rajasthan. Concentration of pigments was calculated using Arnon method. Both Chlorophyll a and Chlorophyll b content was higher in *Datura stramonium* and Carotenoid content in *Tephrosia purpurea* among all the other plant species studied.

Keywords: Chlorophyll A, Chlorophyll B, Carotenoids, Roadside Plants, Colonisation.

-----X-----

INTRODUCTION

Plant pigments are the synthetic products of plants formed during metabolic activities of cell. Chlorophylls are the most important green pigments in plants for the photosynthetic process (Bhatia & Parashar 1997). Higher plants contain Chlorophyll a, Chlorophyll b, Carotenoids, accessory pigments and several additional forms of chlorophyll. Both Chl a and Chl b pigments are associated with light harvesting processes (Ferus & Arkosiova 2001), which are solely responsible for photosynthesis in higher plants. Carotenoids are a class of natural fat soluble pigments found mainly in plants algae and photosynthetic bacteria.

Chlorophyll concentration in leaves is an indicator of plant health (Porra 2002). The chlorophyll a:b ratio also indicates the developmental state of photosynthetic apparatus in plants. It has a determinative role in growth and development of higher plants. The chlorophyll content indicates the photosynthetic capacity per unit area of the leaf (Kozlowski et al. 1991) that determines the rate of photosynthesis in the plant (Dickman & Kozlowski 1968). The chlorophyll content increases with leaf development and then decreases with the senescence phenomenon (Pereyra et al. 2014). The rate of photosynthesis is also higher in flowering and fruiting branches of sub-tropical fruit species in comparison to non-fruiting branches (Avery 1977). However, the pigment is a factor that might also be

responsible for the colour variation of leaf in different species. The present study was undertaken for estimation of chlorophyll content in the mature leaf of roadside plants growing in Jaipur district, Rajasthan

MATERIALS AND METHODS

Seven roadside colonising plant species namely *Withania somnifera* (L.) Dunal, *Datura stramonium* L. *Croton bonplandianum* Baill, *Cassia obtusifolia* L., *Cassia occidentalis* L., *Cleome viscosa* L. and *Tephrosia purpurea*(L.) Pers. and. were selected for the present study. The matured leaves were collected from the selected species growing in three different areas of Jaipur during the rainy season and winters. For the estimation of chlorophyll 'a' chlorophyll 'b' and carotenoids the method given by Arnon(1949) Vernon(1960) was followed. Fresh leaf sample of each selected plant was collected separately. Fifty milligram of leaf tissue was taken for each plant, then grounded and homogenized with 50 ml of 80% acetone in a mortar with pestle. The extract was centrifuged at 2000 rpm for 10 minutes. The volume was made to 10 ml with 80% acetone. The clear supernatant solution was examined for chlorophyll 'a' and 'b' at wavelength 645 and 663nm respectively and carotenoids at 480 and 510nm in a spectrophotometer (model, Spekol,

Carl Zeiss Make). The value of pigment were expressed in mg/g fresh weight.

$$\text{Chlorophyll a} = \frac{12.7(A663)-2.69A(645)}{1000 \times W} \times V \text{ mg/g}$$

$$\text{Chlorophyll b} = \frac{12.9(A645)-4.68(A663)}{1000 \times W} \times V \text{ mg/g}$$

$$\text{Carotenoids} = \frac{7.6OD(A480)-1.49OD(A510)}{1000 \times W} \times V \text{ mg/g}$$

Where, A = absorbance at specific wavelength

V = final volume of chlorophyll extract in ml

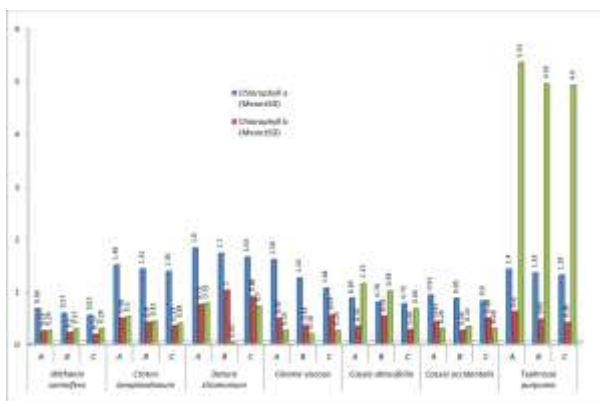
W = fresh weight of tissue extracted in gm

O.D=optical density (absorbance of light in 1 cm cell)

RESULT AND DISCUSSION

Table 1: Showing variation in pigment contents (mg/g fresh weight) in leaf of seven different plant species at three different sites

Plant name	Site	Chlorophyll a (Mean±SD)	Chlorophyll b (Mean±SD)	Carotenoid (Mean±SD)
<i>Withania somnifera</i>	A	0.66±0.015	0.23±0.028	0.24±0.015
	B	0.57±0.025	0.20±0.01	0.27±0.017
	C	0.53±0.049	0.17±0.02	0.28±0.015
<i>Croton bonplandianum</i>	A	1.48±0.017	0.48±0.015	0.50±0.005
	B	1.41±0.015	0.40±0.011	0.42±0.025
	C	1.36±0.02	0.33±0.026	0.38±0.011
<i>Datura stramonium</i>	A	1.80±0.035	0.73±0.020	0.76±0.032
	B	1.70±0.015	1.00±0.056	0.02±0.017
	C	1.63±0.026	0.88±0.026	0.70±0.01
<i>Cleome viscosa</i>	A	1.58±0.058	0.47±0.020	0.24±0.015
	B	1.24±0.020	0.34±0.045	0.18±0.026
	C	1.04±0.015	0.53±0.020	0.24±0.028
<i>Cassia obtusifolia</i>	A	0.85±0.03	0.32±0.07	1.13±0.062
	B	0.78±0.01	0.51±0.005	0.99±0.035
	C	0.75±0.04	0.26±0.037	0.66±0.028
<i>Cassia occidentalis</i>	A	0.91±0.032	0.41±0.045	0.28±0.020
	B	0.85±0.045	0.24±0.026	0.32±0.020
	C	0.80±0.011	0.48±0.032	0.28±0.011
<i>Tephrosia purpurea</i>	A	1.40±0.025	0.60±0.28	5.33±0.39
	B	1.33±0.023	0.45±0.07	4.93±0.24
	C	1.29±0.01	0.39±0.03	4.90±0.30



The study has revealed that the Chl a ranges from 0.66 to 1.80 mg g⁻¹ at site A, 0.57 to 1.70 at site B and 0.53 to 1.63 at site C and Chl b ranges from 0.23

to 0.73 mg g⁻¹ at site A, 0.20 to 1.00 mg g⁻¹ at site B and 0.17 to 0.88 at site C and the Carotenoids ranges from 0.24 to 5.33 mg g⁻¹ at site A, 0.18 to 4.93 at site B and 0.18 to 4.90 at site C in seven roadside plant species. From the result it is also seen that *Datura stramonium* L. has the highest concentration of Chl a, Chl b at all the three sites whereas *Withania somnifera*(L.)Dunal was found to have least quantity of Chl a, Chl b among all the three sites and among all the seven colonizing species (Table 1). Among the seven plant species the highest carotenoid content was found in *Tephrosia purpurea* (L.)Pers.at all the three sites and least in *Cleome viscosa* L. (Table 1). Hence *Datura stramonium* L. and *Tephrosia purpurea*(L.)Pers. plant species are found to be more resistant to hazardous condition and well adapted under local environmental condition. Our observations are supported by Singh et al. (2010) who stated that *Datura stramonium* L. and *Croton bonplandianum* Baill. shows maximum biomagnifications of heavy metals in their body parts and hence are considered as most tolerant towards heavy metal contamination. The quantitative variation of chlorophyll content in these spp. may be due to the health condition of the plants, habitat condition, air and dust pollution, leaf surface area, heavy metals and nutrients of the soil. According to Pandey & Sinha (1998) Chl a and Chl b occur together in the higher plants in the ratio of 2:1. Several literature and pieces of evidence suggest that the Chl a:b ratio plays an important role in higher plants to adapt to new light regions to make optimal use of ambient light intensities and quantities (Arnon 1949). Kamble et al. (2015) opined that the leaf chlorophyll concentration plays a vital role in maintenance of photosynthetic mechanism as well as plant metabolism. Apart from these the seasonal variation and maturity of the leaf also affect the concentration of chlorophyll content in leaf and chlorophyll a:b ratio remains substantially lower in plants growing in high CO₂ condition (Cave et al. 1981).

Observations on pigment contents revealed that chlorophyll a, chlorophyll b and Carotenoid contents of plant species varied at different sites and between different roadside plant species selected for study. Evaluated plants are from different taxonomic group exhibiting difference in growth patterns, leaf life span, texture, growth dynamics and phenological development.

A review of the literature revealed that productivity of higher plants is mediated by leaves and adaptations of plants to the environment involve leaf traits(Valladares et al.,2000). Kramer and Kaslowski(1979) explained that leaf chlorophyll levels are controlled through light received by them. In elevated radiation intensities, chlorophyll molecules are susceptible to photo-oxidation (Alvarenga et. al., 2003) hence, photosynthesis may be limited by temperature, stomatal control and light energy damage(Ottander et al.,1995and Castrillo et. al., 2001). On the contrary reduction of chlorophylls

do not result from severe photoinhibitory damage instead, it may be an adaptive response against the adverse conditions of the summer since water availability (Kyparissis et. al., 1995 and 2000; Oliveira and Penuelas, 2001) is the most limiting factor controlling plant growth, survival and distribution in dry climates (Kramer 1983 and Newton and Goodin, 1989). Nevertheless, in the present investigation, a variability of chlorophyll content in the roadside colonising plant species studied has a scope to consider as tools for identification of these plant species. From the (Table 1), an inference can be made that the Chl a, Chl b and Carotenoids content in mg/g leaf tissue of different plant species are an individual character of each such species studied. Variation of green colour of the leaf was observed as the same is dependent on the variability of chlorophyll and carotenoid content in the species. The alteration of pigment content may be due to the factors like water, soil, pollution, temperature stress etc. which indirectly affect the leaf area, morphology, thickness and chloroplast distribution.

CONCLUSIONS

The study provides a reliable data on chlorophyll content of seven roadside plant species growing in three different localities of Jaipur district. The quantitative analysis of photosynthetic pigment showed that the chlorophyll a and b is highest in *Datura stramonium* L. and Carotenoid content is highest in *Tephrosia purpurea*(L.)Pers. among the seven plant species. Further, the chlorophyll content can be used as indicators of plant health, stress and nutritional deficiencies. Our findings may be helpful in the further studies to monitor the effect of changing micro-climate on chlorophyll content in roadside plant species in terms of temperature, water, carbon dioxide concentration and soil condition of Jaipur district of Rajasthan.

REFERENCES

- Alvarenga, A.A., Castro, E.M., Lima, E. and Magalhaes, M.M. (2003). Effects of different light levels on the initial growth and photosynthesis of *Croton urucurana* Baill. in southeastern Brazil. *Rev. Arvore* 27: pp. 53-57.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant physiol.* 24: pp. 1-15.
- Avery, D.J. (1977). Maximum photosynthetic rate-A case study in Apple. *New Phytologist* 78: pp. 55-63.
- Bhatia, K.N. & Parashar, A.N. 1997 Plant physiology. Trueman Book Company, Jalandhar City, pp. 254-281.
- Castrillo, M., Vizcaino, D., Moreno, E., Latorraca, Z., 2001. Chlorophyll content in some cultivated and wild species of the family *Lamiaceae*. *Biologia Plantarum* 44: pp. 423-425
- Cave, G., Tolley, L.C. & Strain, B.R. 1981 Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. *Physiologia Plantarum* 51(2): pp. 171-174.
- Dickmann, D.I. & Kozlowski, T.T. 1968 Mobilization by *Pinus resinosa* cones and shoots of C-14 photosynthate from needles of different ages. *American Journal of Botany* 55: pp. 900-906.
- Ferus, P. & Arkosiova, M. 2001. Variability of chlorophyll content under fluctuating environment. *Acta Fytotechnica et Zootechnica* Vol. 4. (In: Proceedings of the International Scientific Conference on the occasion of the 55th Anniversary of the Slovak Agricultural University in Nitra, 123).
- Kamble, P.N., Giri, S.P., Mane, R.S. & Tiwana, A. (2015). Estimation of Chlorophyll content in young and adult leaves of some selected plants. *Universal journal of environmental research and technology* 5(6): pp. 306-310.
- Kozlowski, T.T., Kramer, J.P., & Pallardy, G.S. (1991). The physiological ecology of woody plants. Academic Press, Inc. San Diego. New York, pp. 37-44.
- Kramer, P.J. and Koslowski, T. (1979). *Physiology of Wood Plants*. Academic Press, New York.
- Kramer, P.J. (1983). *Water Relations of Plants*. Academic Press, Inc. San Diego, CA. p. 489
- Kyparissis, A., Drilias, P. and Manetas, Y. 2000. Seasonal fluctuations in photoprotective (xanthophylls cycle) and photoselective (chlorophylls) capacity in eight Mediterranean plant species belonging to two different growth forms. *Funct. Austral. Jour. Plant Physiol.* 27: pp. 265-272.
- Kyparissis, A., Petropoulou, Y. and Manetas, Y. (1995). Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. *Jour. Exptl. Bot.* 46: pp. 1825-1831.
- Newton, R.J. and Goodin, J.R. 1989. Moisture stress adaptation in shrubs. In: McKell, C.M.

(Ed.), The Biology and Utilization of Shrubs. Academic Press Inc. San Diego, CA. pp. 365–383

- Oliveira, G. and Peñuelas, J. 2001. Allocation of absorbed light energy into photochemistry and dissipation in a semideciduous and evergreen Mediterranean woody species during winter. *Austral. Jour. Plant Physiol.* 28: pp. 471–480.
- Ottander, C., Campbell, D., Öquist, G., 1995. Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. *Planta* 197: pp. 176–183.
- Pandey, S.N. & Sinha, B.K. 1998 *Plant physiology*, 3rd edition. Vikas Publishing House Pvt. Ltd., New Delhi, pp. 201–218.
- Pereyra, M.S., Davidenco, V., Nunez, S.B. & Argüello, J.A. 2014. Chlorophyll content estimation in oregano leaves using a portable chlorophyll meter: relationship with mesophyll thickness and leaf age. *Rev. Agronomía & Ambiente* 34(1–2): pp. 77–84.
- Porra, J.R. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophyll a and b. *Photosynth Res.* 73: pp. 149–156.
- Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E. and Manrique, E. 2000. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy. *New Phytol.* 148: pp. 79–91
- Vernon, L.P. 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. *Ann. Chem.* 32: pp. 1144–1150.

Corresponding Author

Anubhuti Tiwari*

S.R.K.P. Government College Kishangarh Ajmer, Rajasthan, India

anubhutipiwari0@gmail.com