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Pigments Contents in Cassia Plant Species: A comparative study

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Abstract

Establishment and colonization of herbaceous plants in semi-desert area is often a critical stage. Many plants grow along roadside areas of Jaipur during rainy season and make a green belt helping in reducing soil erosion , reducing dust pollution and air pollution caused by vehicular smoke and also gives asthetic value to the city. Hence a study was conducted on naturally growing herbaceous plant species of Cassia like *Cassia obtusifolia and Cassia occidentalis* as coloniser for the barren land. A field study was conducted around three selected sites of suburban area of Jaipur and data was collected. Pigment content like chlorophyll a, chlorophyll b and carotenoids was calculated by taking statistical one way ANOVA F test.

Keywords : establishment, pigment content, chlorophyll a, chlorophyll b, carotenoids, pollution

Introduction

Plant pigments are the synthetic products of plants formed during metabolic activities of cell. Chlorophyll and Carotenoids are essential pigments of higher plant assimilatory tissue and responsible for variations of color from dark-green to yellow. Moreover, they play important role in photosynthesis by capturing light energy which is converted into chemical energy. Both, chlorophyll 'a' and chlorophyll 'b' show maximum absorption in the blue-violet region with peaks of about 429nm and 453nm, respectively and with miner peaks at 410nm and 430nm. They also have a secondary absorption, maximum in red region with peaks of about 660nm and 642nm, respectively.Carotenoids are a class of natural fat-soluble pigments found mainly in plants, algae and photosynthetic bacteria, where they also play a critical role in the photosynthesis process. Moreover, carotenoids play important functions in plant reproduction, through their role is in attracting pollinators and in seed dispersal. No light is required for their formation. The absorption peak of carotenoids lie between 425nm and 490nm.They prevent photooxidation of chlorophylls

Industrialization and Urbanization of cities especially metropolitan cities are facing fast growth in automobile number which is the major cause of dust pollution. These dust when blown carries with it large amount of pollutant into our houses and are cause of many air born diseases. Hence, covering the roadsides by vegetation is the need of hour. Keeping in view the persistent dust pollution caused by automobiles, to reduce it some field and laboratory experiments were conducted to study the pigment contents of *Cassia obtusifolia L and Cassia occidentalis L*

A comparative study was made between sites. Three sub urban areas of Jaipur were selected as sites i.e. (Site A, Site B and Site C).

Site A- Vaishali Nagar Area

Site B- Mansarovar

Site C- Sanganer

Study Material

The two selected plant species for study were *Cassia occidentalis L* and *Cassia obtusifolia L*. Both these plant species belongs to family Fabaceae Sub family Caesalpinioidae.

1. Cassia occidentalis Its common names are Coffee Senna, coffeeweed, Negro coffee in English and Kasondi in hindi . *Cassia occidentalis* L is found as annual plant in North (including Haryana), North-West India but as a perennial plant in South India. It is native of South America, including the Amazon. It grows luxuriantly in all available spaces such as neglected gardens, roadsides, near lakes or streams and unused grounds of public buildings (Vashishtha *et al*., 2009).

Botanical Description- *Cassia occidentalis L* is an annual herb. The stem is furrowed with subglabrous branching. Leaves are 15to20 cm long, with a single sessile gland near its base. Stipules are lanceolate or ovate in shape and caducous. Leaflets are three to five pairs, membranous, glaucous, ovate to lanceolate in shape with short petiolules. Inflorescence is a raceme, corymbose or a terminal panicle. Flowers are pentamerous with short peduncles and yellow in colour. Calyx is nine mm long and divided to the base. Petals are five, bright yellow in colour oblong and obtuse in shape. Stamens are ten with upper three reduced to staminodes. Out of the remaining seven stamens three lower ones are longer than other four. Pods recurved, glabrous, compressed and transversely septate. Seeds are 20 to30 in number, ovoid in shape and acute at one end and rounded at the other. Their diameter is four to six mm, in diameter, hard, smooth, shining and dark olive green in colour.

2. ***Cassia obtusifoliais*** commonly known as *C*offee bean, Java bean, Sicklepod, Senna coffee, White fedegoso in English and Charota in Hindi. These are herbaceous plants growing in open land and roadside areas of Jaipur. They cover the barren land giving greenery, reducing dust pollution and soil erosion and gives aesthetic value to the city. *C. obtusifolia* is native to the American tropics. It is found throughout America, Asia, Africa and Oceania. In India it is distributed to North Western parts of India including Punjab, Rajasthan and Madhya Pradesh.

Botanical Description-*Cassia obtusifolia* is erect, bushy, annual, herb up to one meter high. Stems are erect, angular and glabrous. Leavespinnately compound 7.0-15 cm long and comprised of four to six leaflets that are obovate to oblong, rachis is grooved, more or less pubescent with a conical gland between the lowest pair of leaflets. Flowers are usually in subsessile pairs in the axil of leaves, pedicel is one to three cm long. Calyx has five free sepals, glabrous, long, ovate and acute. Petals five, yellow, subequal, oblong, obtuse, upper petal truncate. Stamens are ten out of which seven are fertile and three sterile (staminodes). The ovary has numerous ovules, stigma is oblique with an acute rim. Fruit is a brownish-green, subterete, obliquely septate, containing 30-35 seeds. Pods are slightly indented between the seeds. Seeds are rhombohedral, 5.0 mm long, shiny and yellowish brown to dark red.

Study Site and Methodology

This study site is natural population of plants growing along roadsides and open land in suburban areas of Jaipur at three sites of different localities were selected for experimental study for statistical analysis of pigment content chlorophyll a, chlorophyll b and carotenoids using Arnon (1949) and Vernon (1960) . 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’, Chlorophyll ‘b’ and Carotenoids contents at wavelength 645 and 663nm (for Chlorophyll) and 480 and 510nm (for Carotenoids) in a Spectrophotometer (Model, Spekol, Carl Zeiss Make). The values of pigments were expressed in terms of mg/g fresh weight. The pigment concentrations were calculated with the help of following formula.

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

$$\text{Chlorophyll b} = \frac{12.9A\ 645 - 4.68A\ 663}{1000 \times W} \times V\ \text{mg / g}$$

$$\text{Carotenoids} = \frac{7.6\ OD\ 480 - 1.49\ OD\ 510}{a \times 100 \times W} \times V\ \text{mg / g}$$

Here

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

V = volume of extract in ml

a = length of light path in cell (cm)

W = fresh weight of leaves in gm.

Observations and Results

Plant species selected for study are affected by climatic conditions(stress due to high temperature, drought conditions, high irradiance level etc.) and pollution (dust, air pollution and pollution due to heavy metals) are probably causing differences in the concentration of photosynthetic pigments (chlorophyll a, b and carotenoids) when considering effects at different sites.

The data comparing the variation in pigment contents of the plant species growing along roadsides in Rainy season

Cassia occidentalis: The data of pigment contents i.e. chlorophyll a, chlorophyll b and carotenoids are recorded in Table 1. The chlorophyll a content was 0.91±0.032 at site A(highest) and 0.28±0.020 at site C(lowest) with one way ANOVA F ratio 4.3979*. The chlorophyll b content obtained at site B was 0.85±0.045 and 0.24±0.026 at site C with one way ANOVA F ratio 34.9652. The carotenoid content was 0.80±0.011 at site A and 0.28±0.011 at site C with one way ANOVA F ratio 4.8

Cassia obtusifolia: The data of pigment contents i.e. chlorophyll a, chlorophyll b and carotenoids are recorded in Table 2The chlorophyll a content was0.85±0.03 at site A(highest) and 0.75±0.04 at site C(lowest) with one way ANOVA F ratio 4.4673*. The chlorophyll b content obtained at site Bwas 0.51±0.005 and 0.26±0.037 at site C with one way ANOVA F ratio 2.5989. The carotenoid content was 1.13±0.062 at site A and 0.66±0.028 at site C with one way ANOVA F ratio 96.1165NS

Observations on pigment contents revealed that Chlorophyll ‘a’, Chlorophyll ‘b’ and Carotenoids content of plant species varied at different sites and between the two different plant species.Evaluated plants are from same taxonomic group exhibiting difference in growth patterns, leaf life span, texture, growth dynamics and phenological development.Arthur *et al.* (1987) have explained that variation of pigments could be related to phenological phases of plant species such as flowering and fruiting.

Table- 1: Showing Variation in Pigment Contents (mg/g fresh weight) in the Leaf of *Cassia occidentalis* from all the Three Sites(values are mean of three replicates).

Sites	Chlorophyll a (mean ± SD)	Chlorophyll b (mean ± SD)	Carotenoids (mean ± SD)

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
B	0.80±0.011	0.48±0.032	0.28±0.011

NS=Not Significant *= Significant

Analysis of Variance:
F-ratio:
i. Chlorophyll a =4.3979*
ii. Chlorophyll b = 34.9652 ^{NS}
iii. Carotenoids = 4.8*

Table-2: Showing Variation in Pigment Contents (mg/g fresh weight) in the Leaf of *Cassia obtusifolia* from all the Three Sites (values are mean of three replicates).

Sites	Chlorophyll a (mean ± SD)	Chlorophyll b (mean ± SD)	Carotenoids (mean ± SD)
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

NS=Not Significant *= Significant

Analysis of Variance:
F-ratio:
i. Chlorophyll a = 4.4673*
ii. Chlorophyll b = 2.5989*
iii. Carotenoids = 96.1165 ^{NS}

Discussion and Result

The productivity of higher plants is mediated by leaves and adaptations of plants to the environment involve leaf traits (Valladares *et al*., 2000). Chlorophyll and carotenoids pigment absorb light energy and transfer it into the photosynthetic apparatus of leaves, therefore, determination of leaf pigment contents can provide a valuable tool to integrate and understand the physiological and biochemical function of leaves(Sims and Gamon, 2000). Kramer and Kaslowski (1979) explained that leaf chlorophyll levels are controlled through light received by them. Plants during summers have to deal with soil water deficits, high temperature and high irradiance levels (Gonzalez *et al*., 2004). Furthermore, these plants have low tissue water potential because they are exposed to low temperature in winters (Gonzalez *et al*., 2000).

The decline in net photosynthetic rate due to pollutants may be via damage to the electron transport system(Ishibashi *et al*. 1997) or decrease in PEP activity and concentration as a result of hydrolysis and mobilization from leaves (Joshi *et al*. 1993).Inhibition of net photosynthesis was reported when plants were exposed to automobile exhausts with SO_2 concentration greater than 0.2 ppm in *Medicago sativa* (White *et al*., 1974) in *Pisum sativum* (Bull and Mansfield, 1974) and *Vicia faba* (Black and Unsworth, 1979).The sensitivity of chlorophyll 'a' to air pollution is four and half times higher than carotenoids was observed in *Calendula officinalis* (Singh *et al*., 1985) and *Dahlia rosea* (Ahmad *et al*., 1988) under the stress of SO_2 caused by vehicular exhaust. Air Quality Index (AQI) Test was conducted at various sites of Jaipur to find out the ambient air quality. Moderate air pollution was recorded at Site A(59.67) and B(61.03) as both these sites come under residential areas of Jaipur(Kumar *et al*.,2011).

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 Peñuelas, 2001) is the most limiting factor controlling plant growth, survival and distribution in dry climates (Kramer, 1983 and Newton and Goodin, 1989).

A regular reduction of chlorophyll 'a', chlorophyll 'b' and carotenoids content at site C (Sanganer) of both the plants studied was due to presence of heavy metals (copper, lead, nickel, cadmium and iron etc.) in the soil.Results of the present study suggest that, even though, all plants differed in pigment content and followed a seasonal pattern, during adequate or adverse conditions such as extreme temperatures and water shortages, they still could play important roles in maintaining the productivity of dry rangeland ecosystems. However, studies on leaf tissue at morphological, anatomical, biophysical, biochemical, physiological and molecular level should be addressed to elucidate the underlying mechanisms employed by these plants to adapt to this ecosystem and to deal with prolonged drought periods, high temperatures and high irradiance levels with the purpose to identify fundamental mechanisms that increase or reduce pigment concentration and how they are related to photochemical efficiency, photoinhibition and tissue water relations

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