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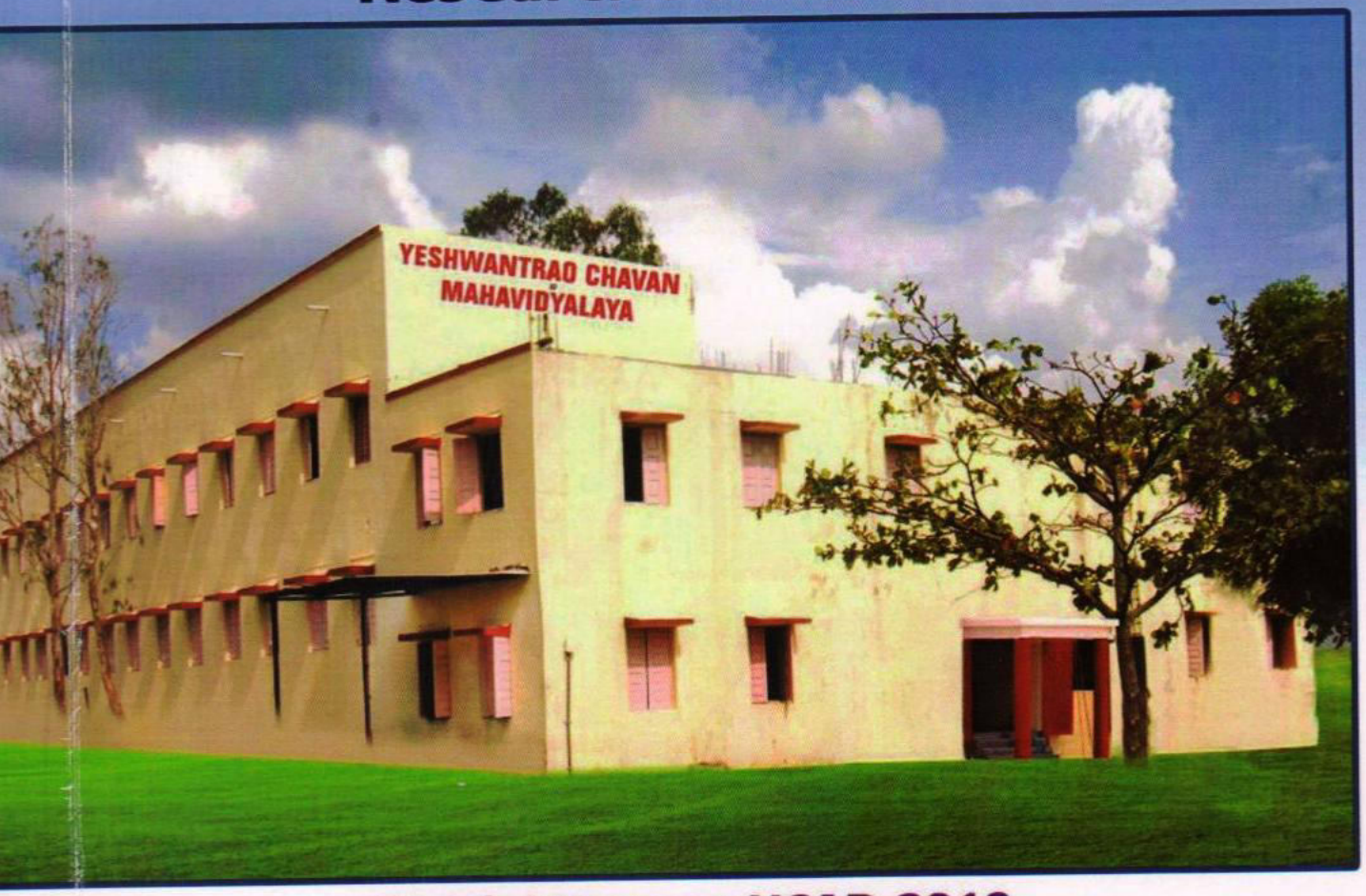

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THE SPECTROPHOTOMETRIC QUANTIFICATION OF CHLOROPHYLLS AND CAROTENOIDS FROM CULTIVATED SOME LEGUME AND NON-LEGUME FODDERS

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ABSTRACT

The present work is carried out on the comparative extraction of chlorophyll pigment molecules (Chlorophyll-a, Chlorophyll-b and total chlorophyll) and carotenoids from some cultivated legumes and non-legumes by using Acetone (80%) as extracting method (Arnon, 1949). The present investigation concerns with the total concentrations of chlorophylls and carotenoids between leguminous and non-leguminous fodder crops.

KEY WORDS: *Spectrophotometric analysis. Solvent, Chlorophylls, Carotenoids, Legumes, Non-legumes, Extraction, Fodders.*

INTRODUCTION:

Total pigment molecules present in the leaf, are chlorophyll-a, chlorophyll-b and carotenoids which are significant for photosynthesis. Most plants possess chlorophyll a and chlorophyll b which are the main photosynthetic pigments (Campbell and Reece, 2005). Chlorophyll content of leaf tissue is a good index of photosynthetic activity (Chowdhury and Kohri, 2003) and timing of fertilizer application (Haboudane *et al.* 2002; Wu *et al.* 2008) of crop. This crucialPigment also plays role as an index of plant growth and production of organic matter (Lahai *et al.* 2003). Chlorophylls and carotenoids are essential pigments of higher plant assimilatory tissues and responsible for variations of color from dark-green to yellow. Moreover, they play important roles in photosynthesis capturing light energy which is converted into chemical energy (Bauernfeind, 1981; Young and Britton, 1993). Carotenoids provide bright coloration, serve as antioxidants, and can be a source for vitamin A activity (Britton *et al.,* 1995). Carotenes contribute to light harvesting and also play a photo protecting role preventing damage to the photosynthetic systems (Gitelson, 2003; Merzlyak *et al*., 2003). The relation between higher photosynthetic activity and high yield has been reported in various crop plants (Wallace & Munger, 1966; Ishar & Wallace, 1967). Healthy plants with large amount of chlorophyll are expected to have maximum than unhealthy ones (Campbell and Reece, 2005). N is a key element in chlorophyll, therefore is usually a high correlation between them (Schlemmer, Francis, Shanahan, & Schepers, 2005). Positive correlation of nitrogen and chlorophyll is previously reported by some researchers (Ding *et al.*, 2005; DaMatta *et al.*, 2002). Chlorophyll concentration usually is a good indicator of plant nutrient stress, photosynthesis and growing periods, the content of chlorophyll in the plant leaves indicates the growth status of the crops, also it is the important condition for exchange of mass and energy from the outside world and therefore real-time monitoring of the content of chlorophyll is a key step to

complete crop monitoring and yield estimation (Canfield *et al*, 1993; Rao *et al*, 2007; Costache *et al*, 2012). Chlorophyll content has also been suggested as the most directly relevant to the prediction of productivity (Dawson *et al*., 2003). Chlorophyll content is an indicator for crop growth and development, therefore accurately determining and assessing of chlorophyll concentration is essential (Bannari *et al*., 2007).

MATERIAL AND METHODS:

Selection of Plants:

The fodders selected for the experiments were viz. *Zea mays* (L.), *Sorghum bicolor* (L.) Moench., *Pennisetum typhoides* (Burm f.)S & H.), *Vigna unguiculata* (L.)Walp), *Vigna radiata* (L.)Wilczek. and *Lablab purpureus* syn. *Dolichos lablab*-(L.) Sweet. All the plants were cultivated in the field in Kharif season and the dates of sowing the plants were recorded. The experimental plot was measured about 100sq.ft. (10x10 ft) and was prepared before cultivation. The selected field was ploughed and the farmyard manure was applied as per the recommendation of application of manures. After ploughing and manuring, the plots were prepared by Randomized Block Design (RBD) of the size of 10x 10ft. for sowing the crops. Two separate plots were prepared, in one plot leguminous crops viz. Cowpea, Mungbean and Hyacinth bean were sown and in another plot the non-leguminous crops viz. Maize, Jowar and Bajra were sown.

i) Cowpea: *Vigna unguiculata* (L.)Walp).: The cowpea plant was sown with the seed rate of 20-40kg/ha and with a row to row spacing of 30- 40cm and 6-15 cm between plants. The farmyard manure was recommended and applied 10tonnes per hectare and basal application of 25kg N, 60 kg P_2O_5 and 30kg/ha K_2O was given after sowing.

ii) Mung: *Vigna radiata*_(L.)Wilczek: Seeds were sown in the field plot in rows 20-30cm apart. The recommended manuring was applied 25-40 kg of P_2O_5 per hectare and 10 kg of Nitrogen per hectare.

iii) *Dolichos* Bean: *Lablab purpureus* syn. *Dolichos lablab*-(L.)Sweet: The crop was sown in the plot with the seed rate 8-10 kg per hectare. The spacing was 90x90cm between the rows.

iv) Bajra: *Pennisetum typhoides* (Burm f.)S & H.): The crop was sown in rows with distance of 30 cm apart and the plant to plant distance 10-12 cm and with the seed rate of 10kg per hectare. The recommended fertilizer applied was 25kg N, 25kgP_2O_5 and 25kgK_2O per hectare (Deore et al., 1982).

v) Jowar: *Sorghum bicolor* (L.) Moench: It was sown with the 30-45 cm distance in rows, with a seed rate of 30-40 kg /hectare and 80 kg N, 30 kg P_2O_5 and 50 kg K_2O per hectare fertilizer was applied after sowing.

vi) Maize: *Zea mays (L.)* The seeds were sown in the plot at the rate of 40-60 kg/ha (Desai and Deore, 1983) with spacing of 30 cm between the rows and 10-15 cm between the plants within a row. The farmyard manure 10tonnes per hectare was applied at the time of land preparation. The recommended dose of fertilizer applied was 45-60 kg Nitrogen, 20-45kg P_2O_5 and 20 kg K_2O hectare (Mungikar, 1974).

The use of pesticides and insecticides was avoided and selected crops were irrigated, weeds were removed from the experimental plot whenever necessary.

COLLECTION OF PLANT SAMPLES:

For the quantification of concentrations of chlorophyll and carotenoids in the cultivated legumes and non-legumes, the leaf samples after collecting from the field were brought to the laboratory of Deogiri College, Aurangabad (M.S.) for analysis of pigment molecules. The leaf samples were collected in fresh and clean polythene bags from the plot in the morning and certain Precautions were taken in order to avoid the mechanical or other injuries while carrying leaf samples in the laboratory and were washed under tap water to remove dust particles and other unwanted particles from the surface of leaves. The leaf samples were then analyzed for the estimation of Chlorophyll-a, Chlorophyll-b, total Chlorophyll and Carotenoids.

ANALYTICAL PROCEDURE:

The Quantitative estimation of chlorophyll-a, chlorophyll-b and total chlorophyll was carried out by the method of Arnon (1949), while carotenoids were determined by following Duxbury and Yentsch, 1956, Macalacham and Zalik, 1963). Fresh leaf material (1gram) was taken and homogenized with 80% acetone and centrifuged at 5000 rpm for 5 min. Then Supernatant was adjusted to 100 ml in the volumetric flask. The optical density of extracted solution was measured at 480, 510, 645 and 663nm and from readings concentrations of chlorophylls and carotenoids pigment were determined by using following formulae

Table-1

An equation for determining the concentrations (mg/g fresh wt) of chlorophyll –a, chlorophyll-b, total chlorophyll and carotenoids.

Solvent	Formula / Equation
Acetone (80%)	Chlorophyll -a mg/g tissue = $\frac{12.7\ (A_{663}) - 2.69\ (A_{645})\ \times V}{1000} \times W$
	Chlorophyll -b mg/g tissue = $\frac{22.9\ (A_{645}) - 4.68\ (A_{663})\ \times V}{1000} \times W$
	Total chlorophyll mg/g tissue = $\frac{20.2\ (A_{645}) + 8.02\ (A_{663})\ \times V}{1000} \times W$
	Carotenoid mg/g tissue = $\frac{7.6\ (A_{480}) - 1.49\ (A_{510})\ \times V}{1000} \times W$

where,

A = Absorbance at specific wavelengths

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of tissue extracted.

Table-2

The determination of absorbance (Spectrophotometric) for Chlorophyll -a, Chlorophyll- b, Total chlorophyll and Carotenoids with 80% Acetone as extracting solvent.

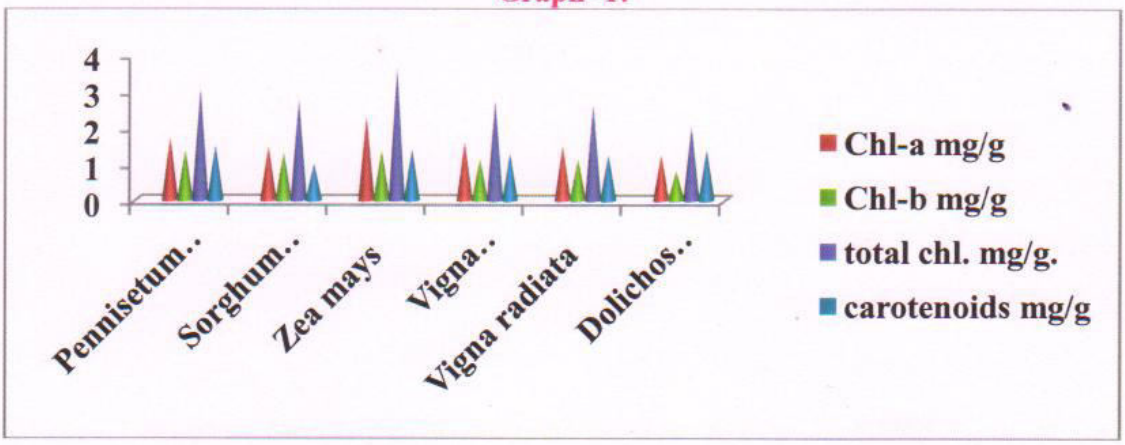
Fodder crop Name	($A_{663}$and A_{645}) Chl-a Mg/gfresh.wt.	($A_{645}$and A_{663})Chl-b Mg/g fresh.wt.	($A_{645}$and A_{663}) Totalchl. Mg/g fresh.wt.	($A_{480}$and A_{510}) Carotenoids Mg/g fresh.wt.
Pennisetum typhoides (Burm f.)S & H.)	1.693	1.358	3.051	1.487
Sorghum bicolor (L.) Moench.	1.451	1.295	2.741	0.989
Zea mays (L.)	2.269	1.356	3.625	1.386
Vigna unguiculata (L.)Walp).	1.582	1.128	2.71	1.268
Vigna *radiata* (L.)Wilczek.	1.485	1.126	2.611	1.239
Lablab purpureus syn. *Dolichos lablab*-(L.)Sweet.	1.235	0.795	2.030	1.384

A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll Total chl.= Total Chlorophyll.

RESULT AND DISCUSSION:

Chlorophyll is an integral component of plant pigments and plays a vital role in the process of photosynthesis. Chlorophyll-a is recognized as the main pigments which convert light energy into chemical energy. Chlorophyll-b as accessory pigments acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll-a (Costache et al., 2012). Leaf pigment content provides valuable information about the physiological status of plants. The content of foliar pigment varies depending on species. Variation in leaf pigments (chlorophyll and carotene) and its relation can be due to internal factors and environmental conditions.

Graph -1:



Leaf pigment composition of cultivated legumes and non-legumes in (mg/g fresh wt.):

The extractions of chlorophyll and carotenoids pigment molecules by 80% acetone method from the cultivated non-leguminous and leguminous fodder crops were measured with the help of spectrophotometer. In non-legumes, the total chlorophyll concentration of *Zea mays (L.)* was higher (3.625 mg/g fresh wt.) as compared to the total chlorophyll concentration of *Pennisetum typhoides* (Burm f.) S & H.) (3.051 mg/g fresh wt.) and *Sorghum bicolor* (L.) Moench (2.741mg/g fresh wt.). Carotenoid content measured in Bajra, Maize and Jowar was (1.487mg/g fresh wt, 1.386mg/g fresh wt., 0.989 mg/g fresh wt.) respectively.In legumes, the total chlorophyll concentration of *Vigna unguiculata* (L.)Walp) was higher (2.71mg/g. fresh wt.) as compared to the total chlorophyll concentration of Vigna *radiata*_(L.)Wilczek (2.611mg/g.fresh.wt) and *Lablab purpureus* syn. *Dolichos lablab*-(L.) Sweet (2.030mg/g fresh wt.). Carotenoid content measured in Dolichos bean, Cowpea and Mungbean was (1.384mg/g fresh wt., 1.268mg/g fresh wt. 1.239mg/g fresh wt.) respectively.

CONCLUSION:

The Results obtained during the analysis of cultivated fodder crops in the field indicate that the extraction of photosynthetic pigments by 80% Acetone used as solvent depends upon the chemical nature of bio-molecules or pigment molecules (chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids). Investigation revealed that method of Arnon (1949), is simpler method for extracting the pigment molecules along with other methods used for extraction and results showed high content of total chlorophyll in Maize plant than Bajra and Jowar. Likewise high total chlorophyll content observed in Cowpea crop than Mung and Dolichos bean. The differences observed in the trend for pigment extraction in the legume and non-legume fodder crops selected for present study were not significant. Though variations may persist among the experimented fodder crops due to physiological and environmental conditions along with this temporal and seasonal changes and local geological condition may also be the reason for variations in pigment concentrations in legumes and non-legumes, therefore further analysis is recommended in this reference.

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