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The Comparative Study on Spectrophotometric Analysis of Chlorophyll and Carotenoids Pigments from Non-Leguminous Fodder Crops.

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Abstract:
The comparative extraction of chlorophylls (Chlorophyll-a, Chlorophyll-b and total chlorophyll) and carotenoids from cultivated some non-leguminous fodder crops by 80% Acetone as extraction method (Arnon, 1949).Was studied. The study relates to the amount of concentrations of chlorophyll and carotenoids between the control and treated non-leguminous fodder crops.

Keywords: carotenoids, chlorophylls, spectrophotometric analysis, non-leguminous fodder crops, extraction, Solvent,

Introduction:

Total pigment molecules present in the leaf, are chlorophyll-a, chlorophyll-b and carotenoids which are essential 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 crucial

Pigment 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). The concentration of chlorophylls and carotenoids vary from plant species to species and from season to season. The variations in foliar pigments may be due to internal factors as well as environmental conditions. 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).The quantification of chlorophyll provides important information about the effects of environments on plant growth (Schlemmer *et al*., 2005). 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 non-legumes fodder crops selected for the experiments were viz. *Zea mays* (L.), *Sorghum bicolor* (L.) Moench.,*Pennisetum typhoides* (Burm f.)S All the plants were cultivated in the field in Kharif season. The piece of land was measured about 100sq.ft. (10x10 ft) and was prepared before cultivation of plants. The land was ploughed and the farmyard manure was applied as per the recommendation. After ploughing and manuring, the plots were prepared by Randomized Block Design (RBD).Two separate plots were prepared, in one plot the control plants and in another plot the treated plants were sown. The crops were sown in rows and each plot having 10 rows, a single row measuring 10sq.ft.

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).

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.

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).

After cultivation all the crops were irrigated and weeds were removed whenever necessary. The pesticides and insecticides were not used. For experimentation, the crops were harvested at pre-flowering stage and were brought to laboratory form the cultivation site.

Sample Collection: For the experimentation viz. to find out the chlorophyll and carotenoids contents in the cultivated non-leguminous fodders, the leaf samples were collected from the field in fresh and clean polythene bags from the plot in the morning and were brought to the laboratory of Deogiri College, Aurangabad (M.S.) for spectrophotometric analysis of pigments. While bringing the leaf samples to the laboratory, precautions were taken so as to avoid the mechanical or other damage. All the samples were washed under tap water to remove dust particles and other unwanted particles from the surface of leaves and were then analyzed for the determination 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). 1g fresh leaf material was taken and homogenized with 80% acetone and centrifuged at 5000 rpm for 5 min. Supernatant was adjusted to 100 ml in the volumetric flask. The absorbance (O.D.) of this extracted solution was measured at 480, 510, 645 and 663λ. From these readings concentrations of chlorophylls and carotenoids pigment were determined by using following formula/equation:

Table-1

Solvent	Formula / Equation
80% Acetone	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 Spectrophotometric determination of absorbance for Chlorophylls and Carotenoids of treated Non-legumes.

Fodder Name	(A_{663} and A_{645})Chl-a Mg/g fresh.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.)	1.585	1.256	2.840	1.349
Sorghum bicolor (L.) Moench.	1.293	1.165	2.458	0.978
Zea mays (L.)	2.079	1.100	3.178	1.255

Table -3

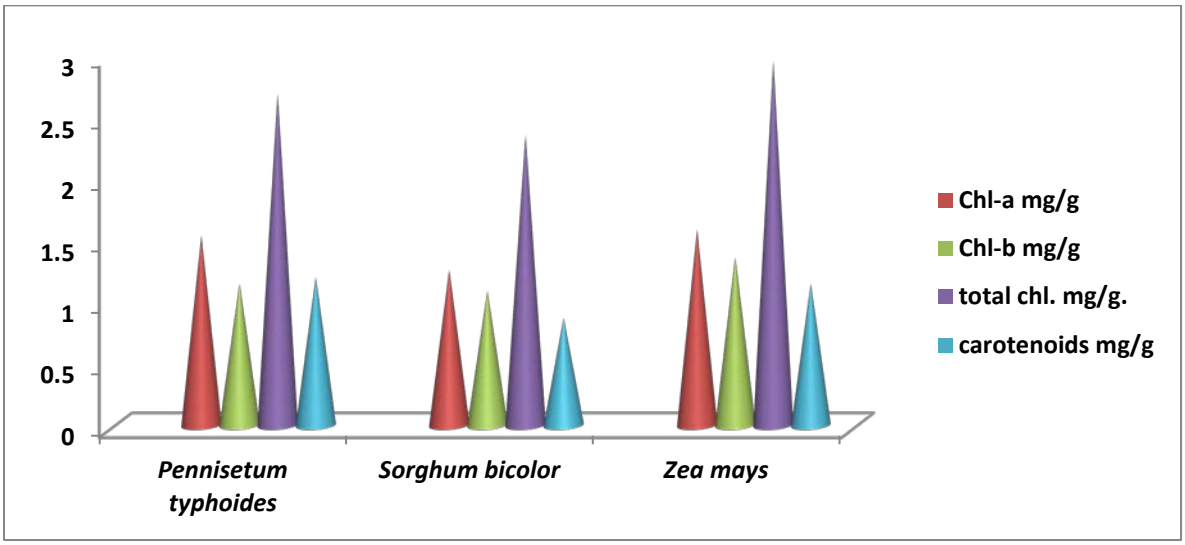
The Spectrophotometric determination of absorbance for Chlorophylls and Carotenoids of control Non-legumes.

Fodder Name	(A_{663} and A_{645})Chl-a Mg/g fresh.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.)	1.536	1.149	2.685	1.202
Sorghum bicolor (L.) Moench.	1.261	1.089	2.35	0.869
Zea mays (L.)	1.585	1.365	2.950	1.149

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

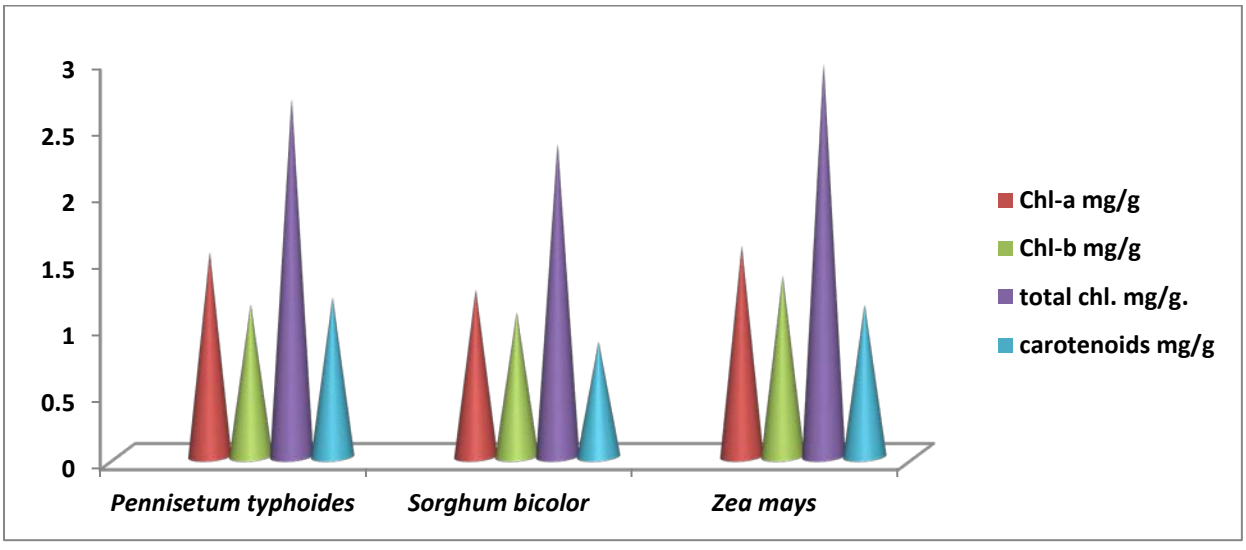
Result and Discussion: Leaf pigment content provides valuable information about the physiological status of plants. Chlorophyll-b as accessory pigments acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll-a (Costache et al., 2012).The content of foliar pigment varies depending on species. Variation in leaf pigments (chlorophyll and Carotenoid) and its relation can be due to internal factors and environmental conditions.

Graph No.-1



The Chlorophylls and Carotenoid content (mg/g fresh wt.) in treated Non-leguminous fodders.

Graph No. -2



The Chlorophylls and Carotenoid content (mg/g fresh wt.) in control Non-leguminous fodders.

The extractions of chlorophyll and carotenoids pigment molecules by 80% acetone method from the cultivated non-leguminous treated and control fodder crops were measured by spectrophotometer. In the treated fodder crops, chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of *Pennisetum typhoides* (Burm f.) S & H.) were high (1.585, 1.256, 2.840, 1.349 mg/g fresh wt respectively.) as compared to the chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of control plant (1.536, 1.149, 2.685, 1.202 mg/g fresh wt. respectively)

. The chlorophyll-a,chlorophyll-b,total chlorophyll and Carotenoid content of treated *Sorghum bicolor* (L.) Moench. were high (1.293, 1.165, 2.458, 0.978 mg/g fresh wt respectively.) as compared to the chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of control plant (1.261, 1.089, 2.350, 0.869 mg/g fresh wt. respectively)

The chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of treated *Zea mays* (L.) were high (2.079, 1.100, 3.178, 1.255 mg/g fresh wt respectively.) as compared to the chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of control plant (1.585, 1.365, 2.950, 1.149 mg/g fresh wt. respectively)

Conclusion:

The Results obtained during the analysis of cultivated Non-leguminous 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 photosynthetic pigment molecules 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 chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid in the treated plants in comparison with the control plants. By the application of recommended NPK fertilizers and farmyard manures to the treated fodders, little amount of differences were observed in the concentrations of pigments between treated and control plants selected for present study. 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 context.

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