International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 2 | APR-JUN | 2018 | 570-578
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

COMPARATIVE ASSESSMENT OF LEAF CHLOROPHYLL CONTENT OF SEVEN SELECTED VEGETABLE CROP BY TWO ALTERNATIVE METHODS AT MURSHIDABAD, WEST BENGAL

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ABSTRACT

Judicious field application of nitrogen fertilizer and time fitted IPM management is required to minimize insect pest occurrence and to maximize sustainable vegetable production. Leaf chlorophyll content can be considered as a primary 'marker' of the physiological status of the green plant. The qualitative difference of chlorophyll content between young and adult leaves for seven selected vegetable crops were observed by both chemical method and by SPAD chlorophyll meter. As the portable estimating tool, SPAD chlorophyll meter is useful for instant chlorophyll measurement both in field and laboratory conditions and may be ideal for determining nitrogen requirement in contrast to destructive chemical methods. In gross, seven plant species namely jute (Corchorus capsularis), spinach (Spinacia oleracea), red amarantha (Amaranthus cruentus), brinjal (Solanum melongera), cabbage (Brassica oleracea), mustard (Brassica nigra), radish (Raphanus raphanistrum), were selected for the analysis of chlorophyll content. In all the cases the adult leaves showed higher chlorophyll content in comparison to young leaves. Maximum amount of chlorophyll was observed in red amaranth while the minimum was noted in brinjal. The age of leaves was found crucial factor for chlorophyll content. Present observation will help the cultivators to unearth the critical areas of insect pest occurrence and also to adopt to adopt based N fertilizer application in relation to insect pest seasonality in vegetable crop.

KEY WORDS

Nitrogen fertilizer, IPM management, SPAD.

INTRODUCTION

Chlorophyll is considered as the prime photosynthetic pigment of green plant. Chlorophyll is an antioxidant compound that is confined in the chloroplast of green leaves, stems, flowers and also in roots (Mirza *et al.,* 2013). Biochemical components of green pigment and nutrient of vegetables are among essential to check the physiological status of the plant (Chapke *et al*., 2006). Leaf chlorophyll content is an important factor that is frequently measured as an indicator of leaf chloroplast content, photosynthetic status of the plat and the extent of plant metabolism. Chlorophyll a and Chlorophyll b together are obligatory pigments in plant photosystem (Peterson *et al*., 1993). Chlorophyll A is the primary photosynthetic pigment in plants providing energy assimilation in plant (Schaper and Chaco 1991). In general, chlorophyll A concentration in plant is 2-3 times higher than chlorophyll b (Srichaikul *et al.,* 2011). The primary roles of this pigment are to react with the visible spectrum of light in photosynthesis. Chlorophyll content per unit leaf area reflects plant photosynthetic capacity, assimilation rate and growth status of the vegetable crop plants. Estimation of leaf chlorophyll content of green foliage can in turn explain the physiological status of the leaf for herbivory (Silva, 2011).

Leaf chlorophyll concentration ensures the degree of leaf greenness (Silva, 2012). Leaf greenness is principally influenced by the N assimilation in plant following field N application. Further, alteration of morpho-anatomical structure of leaf following plant growth and development corroborates with the extent of adaptation of leaf chlorophyll concentration and photosynthetic activity. Use of SPAD chlorophyll meter can indirectly measure N deficiency in leaf tissue. Chlorophyll meter thus can act as a tool for improving field N management. Alteration of measurement protocols are required for in relation to plant species and the existing growth condition of the particular plant species. Absorbance-based leaf chlorophyll assessment is widely accepted predominantly in small-scale eco-physiological experiments (Marenco *et al*.2009). Variation in leaf chlorophyll content can provide information about the physiological condition of plant. Apart from bio-chemical methods, quantification of chlorophyll content by most of the destructive methods relies on spectrophotometric or HPLC measurement, but the process are time consuming and relatively more expensive. Apart from that, the chlorophyll meter or SPAD meter is a simple, portable diagnostic equipment to measure the greenness or relative chlorophyll content of leaves **(**Singh *et al.*2011**)**. Chlorophyll meter readings are expressed in SPAD value. A delicate and definite linear relationship exists between SPAD value and leaf nitrogen concentration **(**Smeal and Zhang, 1994**)**. SPAD value reflects the plant N status and the amount to be required N in relation to plant growth and development (Silva, 2012)

In the present observation the chlorophyll content of seven different plant vegetable leaves was recorded. Relative difference between chlorophyll content in young and adult leaves of same plant species were also assessed (Marenco *et al*.2009)**.** The study will assist to understand the photosynthetic activity and the physiological changes of young and adult plant leaves in relation to the extent of insect herbivory.

MATERIALS AND METHODS:

Place of observation: The study was carried out at Murshidabad, West Bengal, India covering the calendar months of June 2017 to March 2018 in three replications for each of the vegetable crops.

The agro-ecological conditions:

The macro-climate: The climate of the experimental area, in gross, is hot with high humidity and well as dispersed during the monsoon. The total rainfall of the District is about 1144 mm. The rainfall during June-October accounts for about 80% of the total rainfall of the year. Summer lasts from March to mid-June. Cold weather beings from mid-November and persists up to the end-February. The crop season is broadly classified as *pre-kharif* (March to June), wet and warm or *kharif* season (July to October) and dry and cool or *rabi* season (November-February).

Micro-climate: The annual average day night temperature of the experimental area ranges between 20.7^{0}C and 29.9^{0}C with the mercury soaring even as high as 36^{o}C in April and cascading to a low of 10°C in January. The relative humidity at 8:30 hours is 58% and 88% in March and July respectively. The relative humidity in the afternoon at 17:30 hours is 51% and 85% in March and November respectively.

Pedological characteristics: Soil of the experimental fields showed pH value of 6.9 and EC value 0.28mmhs/cm. N, P_2O_5 and K_2O contents were 278, 26 and 265 kg/ha, respectively.

The plant sample and sample collection (Table 1): The plant species were selected randomly from the study area after supervision. Sampling was done for three times for each location. Both the young and adult leaves from the same plant as 'sample' were collected and subjected to standard chemical procedures for determination of chlorophyll content. Side by side a ready assessment by non-destructive method on the leaf chlorophyll content by SPAD meter was also done.

Table 1: Description of the varieties that are taken for the experimentation

Name of the crop	Variety under observation	Time of sowing (SMW)	Time of harvesting (SMW)	Total duration of the crop (days)	Yield (q/h)
Jute	Olitorius JRO 878	12-20	24-36	120-150	408-453
Spinach	Pusa palak	36-40	1-8	120	80-100
Red Amaranth	Amaranthus Paranensis	10	24	20-30	90-135
Brinjal	Arka neelkanth	16	26-32	140-150	250-500
Cabbage	Copenhagen market	36	40	120-150	220-235
Mustard	Pusa Agrani	36	40	110-140	4-10
Radish	Pusa Chetaki	12	32	25-30	80-100

Estimation of the quantity of leaf chlorophyll content:
By chemical estimation*:* Method as given in APHA (1989) was followed after suitable modification. Delicately cut one gram of each of the vegetable leaf sample was softly grinded and accordingly homogenized. 20ml. of 80% acetone and 0.5gm $MgCO_3$ powder was subsequently added to this mixture. The mixture was again grinded accordingly. The sample was then kept into a refrigerator at low temperature for about 4 hours. After that, the mixture was centrifuged at 500 rpm for about 10 minutes. The supernatant, so generated, was then transferred to volumetric flak of 100 liter capacity. 80% acetone is gently added to make the final volume of the solution 100 ml. The extent of colour absorbance of the solution was assessed by spectrophotometric method after using 645 and 663nm wavelength against the solvent. 80% Acetone was used as a standard to assess the light transmittance.

By SPAD chlorophyll meter:
Structure of SPAD chlorophyll meter: The SPAD chlorophyll meter assist in steady and non-destructive assessment of plant leaf chlorophyll depending on the quantity of light intensity. SPAD meter is a simple and moveable diagnostic equipment to assess the greenness or the relative chlorophyll concentration of leaves against SPAD reading.

Working principle: The SPAD chlorophyll meter has two parts- one flexible shaft and another rigid shaft. The leaves are held in between these two shafts. The flexible shaft has two diodes that emit light beams at 650 nm (red) and 940 nm (near infrared), through the leaf tissue, and two detectors, that are attached on the rigid shaft and measure light transmittance. The extent of light transmittance relies on the nature of greenness in the leaf. The light signals are subsequently converted into electrical signals; the signal is then amplified, and again converted into digital signals. The generated signal by microprocessor is then displayed in the monitor in SPAD value. The SPAD value is consonance to the amount of chlorophyll as the SPAD reading is based on two absorbance peaks of chlorophyll in vitro and assists to assess the physiological status of the plant.

Sampling protocol **(Fig 1):** Leaf samples were collected randomly at (i) tender age and (ii) maximum growth stage of the vegetable plant. Fully expanded leaf of 10 plants for each of seven varieties from 3 selected area were used for SPAD measurement. SPAD readings was in triplicate staking (i) on one side of the midrib of each single leaf blade, (ii) midway between the leaf base and (iii) tip of the leaf and then collected data was then averaged for further analysis.

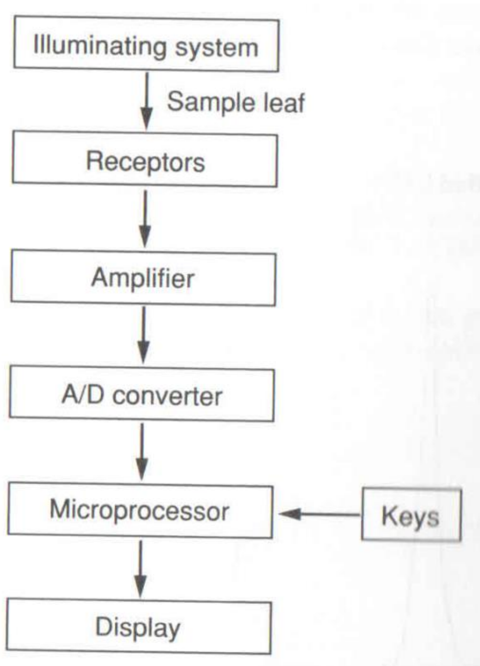

Fig 1: Flow diagram for the estimation of leaf chlorophyll content by SPAD chlorophyll meter

RESULT AND DISCUSSION:

In gross, seven plant species namely jute (*Corchorus capsularis*), spinach (*Spinacia oleracea),* red amarantha (*Amaranthus cruentus*), brinjal (*Solanum melongera),* cabbage (*Brassica oleracea*)*,* mustard (*Brassica nigra*)*,* radish (*Raphanus raphanistrum*)*,* were selected for the analysis of chlorophyll content. The results are delineated bellow:

Characterization of the leaf of the plant samples (Table 2, Fig 2):

1. *The Jute leaf:* The shape of jute leaf is lanceolate, oblong or obovate. The margin of the leaf is serrated. The tip of the leaf is accurate. The leaves are alternate, simple lanceolate,5-15 cm long, with an acuminate tip and finely serrated or lobed margin.
2. *The spinach leaf*: The shape of spinach leaf is obovate. The margin of the leaf is entire. The tip of the leaf is obtuse. The leaves are alternate simple ovate to triangular and very variable in size from 2-30 cm long and 1-15 cm broad with larger leaves at the base of the plant.
3. *The red amaranth*: The shape of red amaranth leaf is ovate. The margin of the leaf is entire. The tip of the leaf is refuse. Medium sized green leaves with burgundy red overlay.
4. *The brinjal leaf*: The shape of brinjal leaf is rhomboid. The margin of the leaf is lobed. The tip of the leaf is acuminate. It's simple leaves are oblong to oval. Slightly lobed with its underside a paler green than the upper side. Leaves are covered with fine hairs.
5. *The cabbage leaf*: The shape of cabbage leaf is reniform. The margin of the leaf is serrated. The tip of the leaf is emarginated. Cabbage heads generally range from 0.5 -4 kg leaves are smooth and integrated. Most cabbage has thick alternating leaves with margin that range from wavy or lobed to highly dissect.
6. *The mustard leaf*: The shape of mustard leaf is lyrate. The margin of the leaf is Incised or pinnatifid. The tip of the leaf is acute. Basal leaves pinnately divided, larger than upper leaves between 10-20 cm long. Upper leaves are alternate sessile or with short petiole and oval in shape. Margin of the leaf are mostly toothless.
7. *The radish leaf*: The shape of radish plant is spatulate. The margin of the leaf is crenate. The tip of the leaf is emarginate. Leaf of radish is characterized with either pinnate or entire leaf edge and both the type of leaf are simple.

Table 2. Characteristics of the plant leaf sample taken for experimentation

The plant species	Characteristics of the leaf		
	Leaf shapes	**Leaf margins**	**Leaf tips**
Jute	Lanceolate/Oblong/Obovate	Serrate	Acute
Spinach	Obovate	Entire	Obtuse
Red amaranth	Ovate	Entire	Retuse
Brinjal	Rhomboid	Lobed	Acuminate
Cabbage	Reniform	Serrated	Emarginate
Mustard	Lyrate	Incised/pinnatifid	Acute
Radish	Spatulate	Crenate	Emarginate

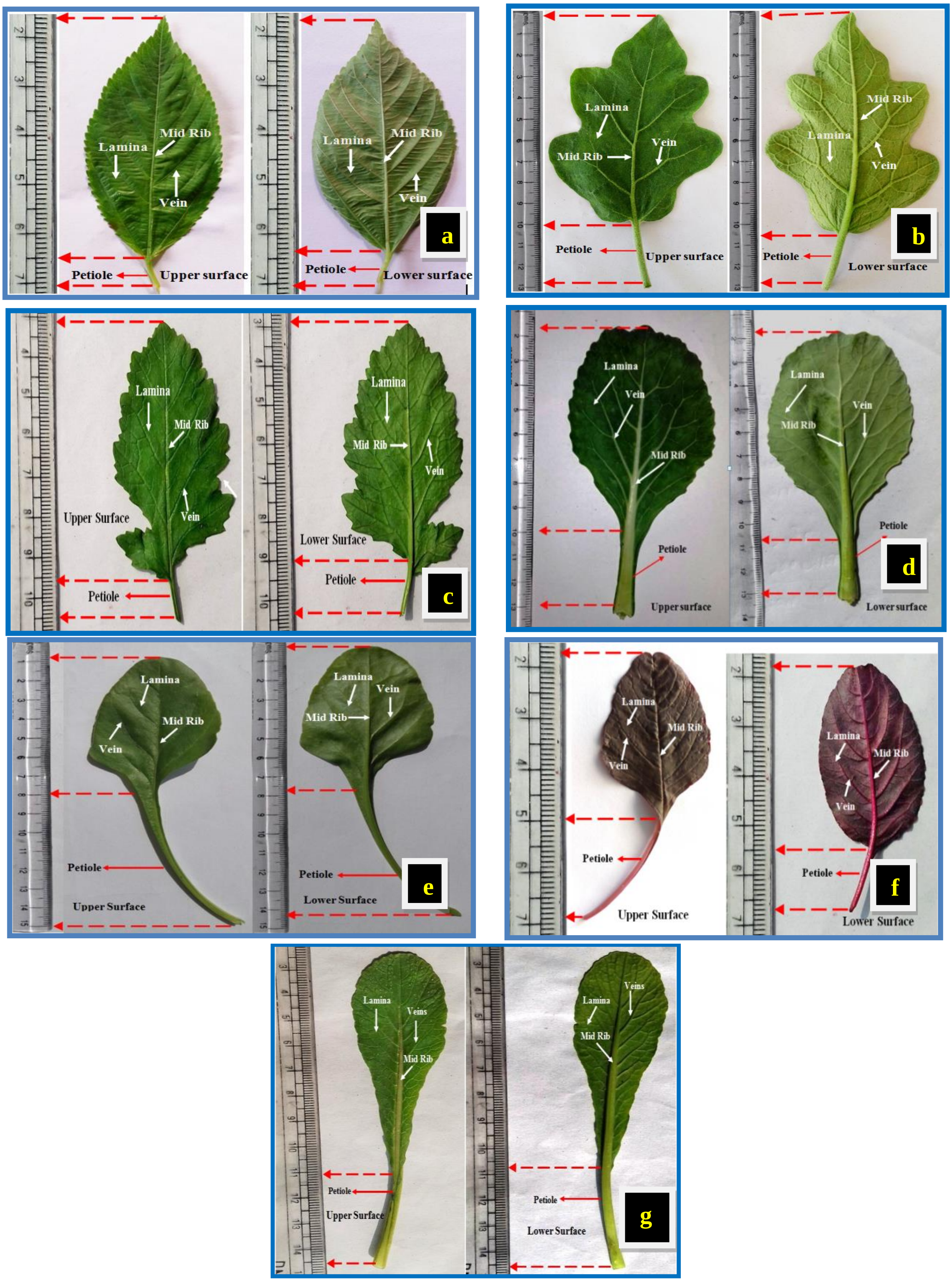

Fig. 2: Leaf samples of different crops that are considerd for experiment (a) jute (both upper and lower surface), (b) brinjal (both upper and lower surface), (c) mustard (both upper and lower surface), (d) Cabbage upper surface (both upper and lower surface), (e) Spinach (both upper and lower surface), (f) Red amaranth (both upper and lower surface), (g) Radish (both upper and lower surface).

In consideration of the leaf chlorophyll content (Table 3):

By chemical estimation methods: In mature leaf of red amaranth plants higher amount of chlorophyll was observed by chemical method. The amount of chlorophyll was comparatively low in the young leaves of the same plant. The values are 19.12 mg pigment/ m^3 and 32.70 respectively in tender and maturation stage. Likewise, in mustard leaves the chlorophyll content in young leaves were 18.98 mg pigment/m^3 while in adult leaves the value was 28.70 mg pigment/m^3. In tender and adult mature leaf, the content of chlorophyll for cabbage was 15.80 and 16.50 mg pigment/m^3 respectively. Chlorophyll content in young leaves of spinach was 15.46 mg pigment/m^3. However, in adult leaves the value was 21.70 mg per pigment/m^3. Tender leaves of radish contained comparatively higher amount of chlorophyll than the adult leaves. In jute the chlorophyll content in young leaves was 15.46 mg pigment/m^3 while in adult leaves it value was 29.81 mg pigment/m^3. In brinjal the chlorophyll in young leaves was 9.03 mg pigment/m^3 and in mature adult leaves the recorded amount was 9.50 mg pigment/$m^{3.}$. So, the tender leaves of brinjal plant had recorded higher chlorophyll than the adult leaves. In gross maximum chlorophyll content was noted in red amaranth this was respectively followed by mustard, cabbage, jute, spinach, radish, and brinjal in descending order.

By SPAD estimation methods (Fig:3): In mature leaf of red amaranth plants higher amount of chlorophyll was observed by SPAD method was 29.42 and 42.50 in tender and mature leaf respectively. The amount of chlorophyll was comparatively low in the young leaves of the same plant. Likewise, in mustard leaves the chlorophyll content in young leaves were 24.50 SPAD value while in adult leaves the value was 44.60 SPAD value. Tender leaves of radish contained comparatively higher amount of chlorophyll than the adult leaves 32.60 SPAD value in comparison to 27.50 SPAD value in tender leaves. In jute the chlorophyll content in young leaves was 18.50 SPAD value while in adult leaves it value was 38.61 SPAD value. Chlorophyll content in young leaves of spinach was 18.80 SPAD value. However, in adult leaves the value was 29.50 SPAD value. In tender and adult mature leaf of spinach the content of chlorophyll for cabbage was 18.80 and 29.50 SPAD value. In brinjal the chlorophyll in young leaves was 12.60 SPAD value and in mature adult leaves the recorded amount was 14.53 SPAD value. So, the tender leaves of brinjal plant had recorded higher chlorophyll than the adult leaves. In gross maximum chlorophyll content was noted in red amaranth this was respectively followed by mustard, cabbage, jute, spinach, radish and brinjal in descending order. In consideration two methods to assess leaf chlorophyll content, least variation was noted for jute while the maximum variation was noted for cabbage (Fig.4).

Table 3: Leaf chlorophyll content of different plant species (mg pigment/m^3)

Plant sample number	The plant species		Chlorophyll content				Average value of leaf chlorophyll (mg pigment/m^3)	
			Tender leaves		Mature leaves			
	Common English name	Scientific name	By chemical method (mg pigment /m^3)	By SPAD	By chemical method (mg pigment/m^3)	By SPAD	By chemical method (mg pigment/m^3)	SPAD
1	Jute	*Corchorus olitorius*	15.46	18.50	29.81	38.61	22.64	28.56
2	Spinach	*Spinacia oleracia*	15.46	18.80	21.70	29.50	18.58	24.15
3	Red Amaranth	*Amaranthus cruentus*	19.12	29.42	32.70	42.50	25.91	35.96
4	Brinjal	*Solanum melongera*	9.03	12.60	9.50	14.53	9.27	13.57
5	Cabbage	*Brassica oleracea*	15.80	18.50	16.50	27.50	16.15	23.00
6	Mustard	*Brassica negra*	18.98	24.50	28.70	44.60	23.84	34.55
7	Radish	*Raphanus raphanistum*	12.70	27.50	19.60	32.60	16.15	30.05

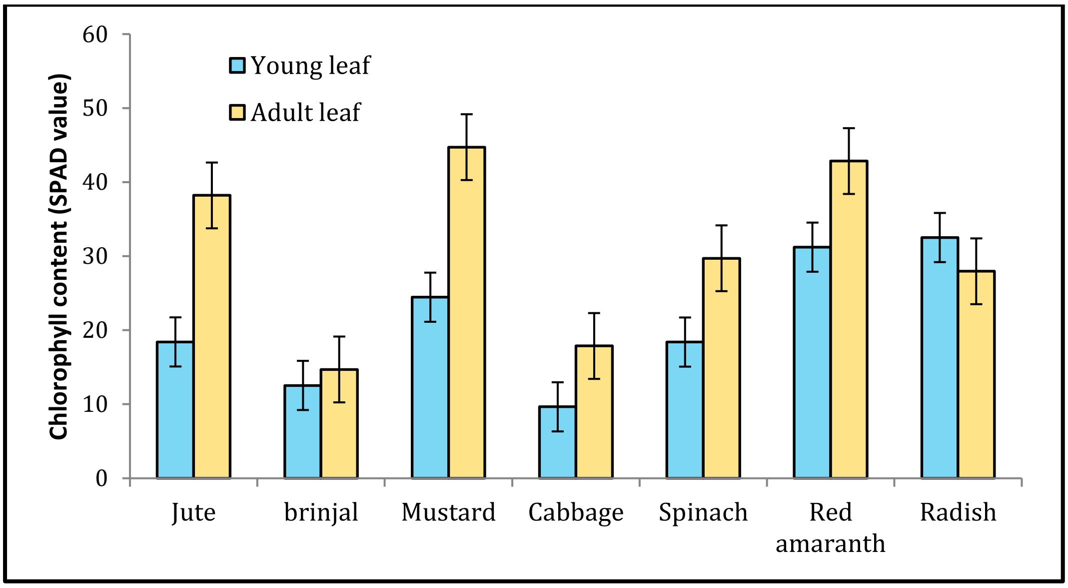

Fig.3: Variation of leaf chlorophyll content in relation to plant growth stages

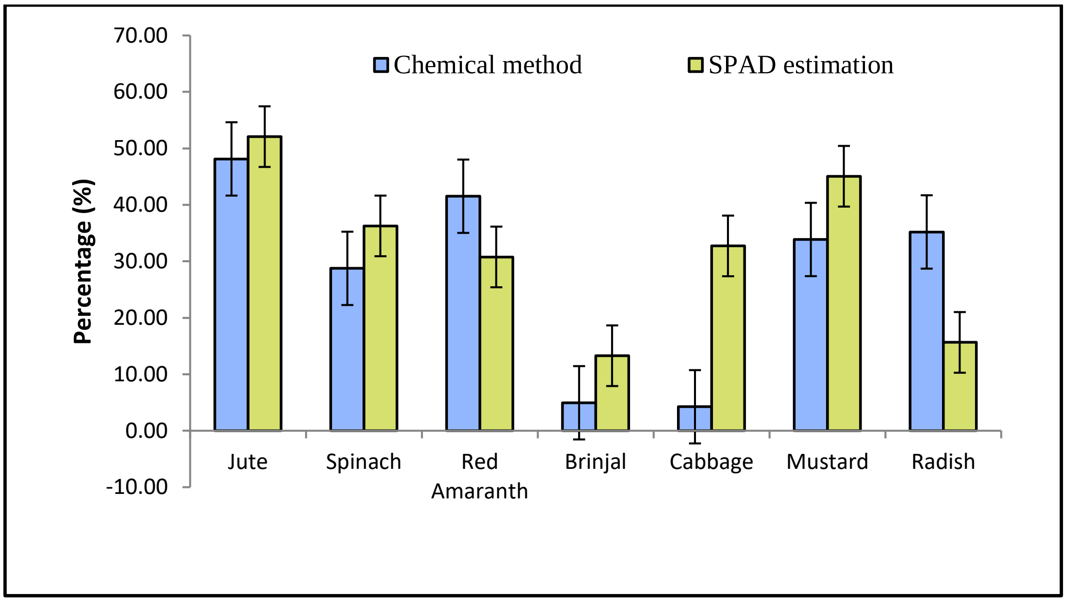

Fig 4. Relative variation during the estimation of leaf chlorophyll content by two methods

DISCUSSION:

Variation of leaf structure in relation to growth and development proportionately varies with leaf chlorophyll content. Grossly in consideration of all of the varieties, the content of chlorophyll is relatively low in young leaves and that gradually increases as the plant matures. In tender leaves, maximum chlorophyll content was noted in red amaranth and that was followed by mustard, cabbage, jute, spinach, radish and brinjal in descending order. In similarity to the tender leaf, mature leaf maximum chlorophyll content was assessed in mustard and that was followed by red amaranth followed by jute, spinach, radish, cabbage and brinjal in descending order. Chlorophyll content of the plant corroborated to the plant physiological stage and soil nutritional availability. However, the extent of proportionate increase of the chlorophyll content in the leaf tissue varied considerably in different cultivars. Maximum increase in chlorophyll content from young leaf to mature adult leaf was noted for radish while the least was noted for radish. Incidence of insect herbivores proportionately varies with the leaf chlorophyll content. Higher the content of leaf chlorophyll, greater was the incidence of the insect herbivores.

In addition to the chemical assessment, non-destructive methods have therefore been developed and inexpensive optical chlorophyll meters, such as the SPAD-502 (Konica Minolta, Osaka, Japan), are found effective for ready handed field N management. However, SPAD value depends not only on chlorophyll content but also on other aspects of leaf optics, which may be influenced by various environmental and biological factors. The establishment of reference curves relating SPAD-unit and total foliar chlorophyll under controlled environmental conditions is therefore a high priority. From non-destructive leaf N assessment presumption about the actual N requirement can be done **(**Singh *et al.*2011**)**. Chlorophyll meter helps to evaluate plant N status at 'real time' for 'precision agriculture'

The application of non-destructive methods of chlorophyll measurement in parallel to chemical measurement offers a consistent and supportive way for plant analysis agro-ecosystem analysis (Gitelson and Merzlyak 1997). Variation of leaf nutrient content has been related with both the survivorship and the quantity of defoliation by insect herbivores (Marenco *et al*.2009). Leaf chlorophyll content is considered as the 'community property' of agro-ecosystem to predict agricultural production. Leaf nutritional value relies principally on the nitrogen, moisture, chlorophyll and fibre content as observed by (Loh, *et al.* 2002.). Leaf chlorophyll content alters the growth rate of lepidopteran and coleopteran larvae (Godoy, 2002.). Extent of leaf damage due to herbivorous insects is found to correlated to leaf nitrogen and chlorophyll content (Evans, 1983). As there is spatial variation of chlorophyll within a leaf laminar, the extent of leaf herbivory was also varied considerably (Crusciol *et al*.2001). Damage to leaf due to insect pest attack is proportionally related to leaf chlorophyll content as observed by Santos *et al.,* (2011). Chlorophyll accounted for more than 98% of gross primary production variation in field crops (Sadat and. Chakraborty, 2017).

In comparison to young leaves, in almost all the selected plant vegetable plant, the chlorophyll content was high in mature leaves. This may due to the fact that the young leaves were not physiologically mature than the full grown mature leaves (Siwach and Gill, 2014; James *et al*., 1999). Leaf mesophyll plays a crucial role in the photosynthetic assimilation process (Siwach and Gill, 2014). James *et al*. (1999) have observed that the young leaves had comparatively low mesophyll tissue and appear in blue-gray in colour. Contrary to that the adult and mature leaves have comparatively higher concentration of mesophyll tissue and exibits green color (Rostami *et al*.2008; Siwach and Gill, 2014). For this reason, cholorophyll a/b ratio was higher in adult leaves than that of young leaves of same cultivar (Smith and Nobel, 1978). Apart from this, in almost in all cases the chlorophyll a concentration was higher than that of chlorophyll b. Chlorophyll a is considered as the primary pigment for photosynthesis in plant while other pigments including chlorophyll b are accessory pigments for other plant physiological metabolism purpose (Srichaikul *et al*., 2011).

Basically, variation of chlorophyll content in relation to leaf age is often considered as a comparatively late mechanism of photosynthetic adaptation. Chlorophyll content of leaves is a useful indicator of both 'potential photosynthetic' productivity and general plant vigour (Marquard and Tipton, 1987). Apart from this, variation and alteration in the amount of chlorophyll may be a part of adaptive responses specifically to the phyto-phagous pests. In view of this, total N content in the leaves has been estimated for fruit-bearing species like apple, peach, pear and also for grapevine by the non-destructive methods with the application of SPAD-502 for better crop management (James and Vogelmann, 1999).

From the present study it may be concluded that most of the plant showed higher chlorophyll content in adult leaves as compared to young leaves. Chlorophyll content can be used as measurement of healthiness of plants canopy and the rate of photosynthesis as well. This study will be helpful to do research in chlorophyll content analysis of various plants species and study the vegetation cover area.

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