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Original Article

Design of Spectral Absorbance-Based Electronic Reader for Chlorophyll Measurement

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Abstract *- Sensor-based Soil Plant Analysis Development (SPAD) meters are costly, and traditional chemical methods are complex and time-consuming for measuring the amount of chlorophyll in plant leaves. A simpler, less expensive handheld electronic chlorophyll reader is designed, built, and tested in various settings. Spectral absorption by chlorophyll in living leaves is the parameter for measuring chlorophyll. The highest absorption by leaf chlorophyll is shown in Red LEDs. This characteristic allows Red LED spectrum absorption measurements for various plant leaves. Then, the chlorophyll content is measured using Arnon's method in the Botany lab for the same leaves. To authenticate the Electronic Reader design, spectral absorbance readings for Red LED and chlorophyll content measurement by Arnon's method are compared for different leaves. Also, a comparison of CCI readings by the proposed reader and SPAD meter is done for cotton plant leaves. It is found that the Electronic Reader is working correctly.*

Keywords *- CCI, Leaf absorbance, SPAD, CCM, Spectral absorbance.*

1. Introduction

Measurement of chlorophyll concentration in plant leaves helps assess nutrient status, Fertilizer requirements, stress evaluation, and harvest optimization. The amount of chlorophyll present in the plant leaves reflects the overall condition of the plant itself; healthy plants have more chlorophyll content than less healthy ones.

Traditional methods of chlorophyll measurement are time-consuming and destructive [1]. In the spectrum of light, leaf chlorophyll has absorbance peaks in two distinct regions: the blue region (400nm to 500nm) and the red region (600nm to 700nm), with no transmission in the Near Infra-Red (NIR) region [3]. Scientists used this to design sensors emitting light in the red and NIR regions. Two commercially available electronic readers are widely used (Minolta, model SPAD-502 (Spectrum Technologies, Plainfield, Ill.) and Opti-Sciences model CCM-200 (Opti Sciences, Inc., Hudson, NH)) [3].

These readers are beyond the reach of most Indian farmers. A low-cost chlorophyll meter design method is based on light-to-voltage measurements of the remaining light after two LED light emissions through a leaf. Still, details of the photosensor design are not given [4]; similarly, many other papers have discussed chlorophyll measurement techniques and corresponding performance analysis considering different leaves. Still, photosensor design details are not mentioned. There are some other methods presented for chlorophyll measurement based on image processing [5-7], and deep learning predictions [8].

The main objective of the project work was to design an optimum electronic device that should be affordable to small-scale farmers. This reader will help the farmers make proper harvesting decisions, resulting in better yield and reduced production costs. Earlier, a paper written on this project work presents the performance analysis considering few samples. A patent [9] was also published in 2021 on the design concept of the proposed electronic reader. Other papers [10-25] presented different ways of chlorophyll measurement, standard operating procedures, and SPAD meter design & operation details.

This paper presents a design methodology for a low-cost handheld electronic reader. The performance analysis is given based on the spectral absorbance of chlorophyll for Red LED to estimate the chlorophyll content in plants. IR LED has zero absorbance by leaf chlorophyll, and Red LED has maximum absorbance by leaf chlorophyll. Using this property, first, we find out the % transmission through IR LED for reference; if it is nearly equal to 100 %, then we go for finding out the spectral absorbance of chlorophyll for Red LED.

Also, to calculate the Chlorophyll Content Index (CCI), IR LED transmittance & Red LED transmittance are needed. The first part of the paper gives details about the electronic reader design. Then, a process flow for spectral absorbance measurement for Red LED is shown. Next, Arnon's conventional botanical method for chlorophyll measurement is explained to calibrate the readings.

A comparison of spectral absorbance readings for Red LED by the proposed electronic reader & chlorophyll measurement readings by conventional Arnon's method is presented. A comparison of CCI readings by the proposed reader and SPAD meter is also done.

As the spectral absorbance by the Red LED is directly proportional to leaf chlorophyll content, in this paper, the spectral absorbance parameter is included for analysis to authenticate the photosensor design. Regression analysis is also carried out for performance analysis.

For RED LED spectral absorbance is the difference between the photodetector output without a leaf and the photodetector output with a leaf. The following two formulas are used for finding out % transmittance for each LED & the Chlorophyll Content Index (CCI).

$$\%\ \text{Transmission ThrougLED} = \frac{(\text{Photodiode Output with leaf})}{(\text{Photodiode Output without leaf})} \quad (1)$$

$$\text{CCI} = \frac{(\%\ \text{Transmission through IR LED})}{\%\ \text{Transmission through Red LED}} \quad (2)$$

The rest of the manuscript is organized as follows: Section 2 presents the experimental design, including the photosensor design in detail and an outline of its construction; the experimental results, together with discussion and comparison with previous works, are presented in Section 3; finally, Section 4 provides a future outlook and concludes the manuscript.

2. Materials and Methods

2.1. Experimental Design

An electronic reader for chlorophyll measurement was developed using the microcontroller chip Arduino Uno.

The components and devices used for the hardware implementation were Arduino UNO, Red LED-650 nm, IR LED - 940 nm, photodiodes, ESP8266 Wi-Fi module, GPS module-NE06M, OLED 128x64 display module, and 4x4 keypad. The software implementation is carried out using Arduino IDE software. Figure 1 illustrates the block diagram showing different input/output devices interfaced with the Microcontroller.

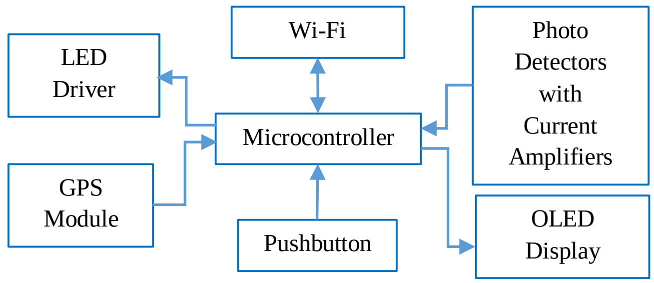


Fig. 1 Block diagram

The main functional blocks in the above block diagram are LED driver circuits and photodetectors with current amplifiers. Arduino output pins connected to base terminals of transistors (LED driver circuits) will switch on the Red LED & IR LED. Photodetectors are placed in front of the LEDs.

The first reading will be taken without inserting a leaf between LEDs and photodetectors; the second will be accepted after inserting a leaf between LEDs and photodetectors. Photodetector output will be connected to the Arduino Uno A/D converter. Then, this photodetector output in digital form is noted. Spectral absorbance for Red LED will be calculated and displayed on the OLED display. The GPS module will give information about the location of readings taken. GPS output is connected to the input pin of Arduino Uno.

The information regarding the location of the readings taken & chlorophyll measurement will be saved on the cloud (IoT) using a Wi-Fi module. The keypad is used here for the user interface & for displaying different messages on the OLED display.

Figure 2 illustrates the circuit diagram showing actual pin connections of the Arduino with input/output devices interfaced with it. Two output pins of Arduino Uno are connected to the inputs of LED driver circuits. Two outputs of photodetectors are connected to the two analogue inputs of Arduino. The figure shows the GPS module, Wi-Fi module, and keypad pin connections.

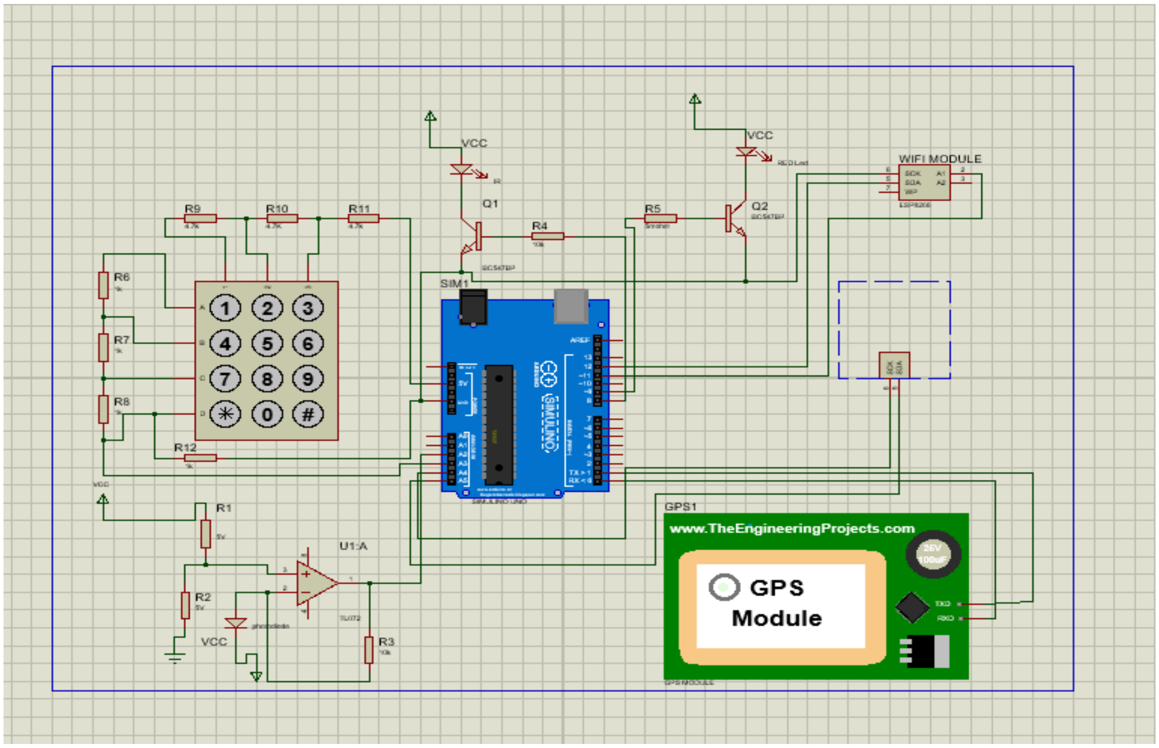


Fig. 2 Circuit diagram

2.2. Chlorophyll Detection Device

The design of the chlorophyll detection device consists of two parts,

- Transmitter Circuits (LED Driver Circuits)
- Receiver Circuits (Photodiode Detector Circuits)

The spectral absorbance of chlorophyll in living leaves is used here to determine the Chlorophyll Content. IR LED has zero absorbance by leaf chlorophyll, and RED LED has maximum absorbance by leaf chlorophyll; a Photosensor circuit is designed using this chlorophyll property. The main parameter considered here for chlorophyll measurement is the spectral absorbance of chlorophyll for Red LED. IR LEDs Spectral absorbance is used here for reference.

2.2.1. Transmitter Circuits

The transmitter circuits are designed using transistors. IR LED 940 nm & Red LED 650 nm are used to transmit the rays & driver circuits using transistors are used. Both the IR & RED LED driver circuits are connected to Arduino. Output pins of Arduino are connected to inputs (base terminals of the transistors) of two LED driver circuits to switch on or off IR and RED LED.

For calculating IR LED Driver Circuit parameters, Consider the IR LED specifications,

IR LED current specification – 25 mA
IR LED voltage specification – 1.6 V

For calculating Red LED driver circuit parameters, consider the RED LED specifications,

RED LED current specification – 25 mA
RED LED voltage specification – 3.6 V

2.2.2. Receiver Circuits

The receiver circuits are designed using photodiodes as detectors. This circuit uses a photodiode with a FET input current amplifier IC TL072; we may use LM 324 or other op-amps. The photodetector is connected in reverse-biased mode. Resistor R2 is connected between the inverting input terminal and the output pin of the IC.

The voltage divider circuit is connected at the non-inverting input terminal of the op-amp IC. The resistor values for the photodetector circuits for getting the proper output are selected accordingly. When the photodiode receives the LED rays, a small reverse current through the diode will get amplified by the current amplifier.

The output of the current amplifier IC will be given to the analogue input terminal of the Arduino. Photodetector testing is done for a plant leaf & output voltages measured, and percentage transmission calculated for red and IR LED are listed in Table 1 below. It is verified that after inserting the leaf, the Red LED photodetector output decreases as leaf chlorophyll absorbs the Red LED light. As leaf chlorophyll has zero absorbance for IR LED, the IR LED photodetector output is the same with & without leaf conditions.

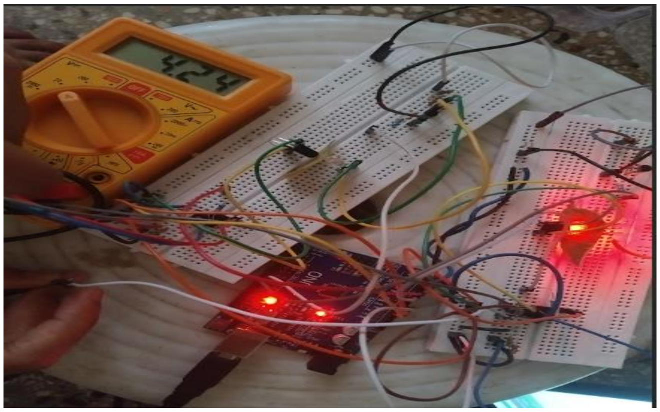


Fig. 3 Breadboard implementation of LED driver & photodetector circuit

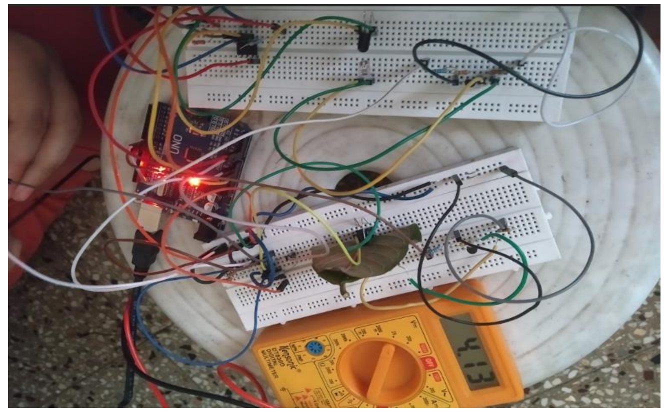


Fig. 4 Photodetector reading in volts (multimeter) for another leaf inserted between red LED and photodetector

Table 1. Photodetector testing & multimeter readings

LED	Conditions	Photodetector Output	% Transmission
Red	Without Leaf	4.26 V	97.88 %
	With Leaf	4.17 V	
IR	Without Leaf	3.72 V	100 %
	With Leaf	3.72 V	

Figures 3 & 4 show the breadboard implementation of the LED driver circuit and the implementation of the photodetector circuit. Multimeter readings show the photodetector output voltage for the Red LED. One can see the leaf inserted between the LED and the photodiode. The proper way to take the reading is to enclose the LED and photodetector in a cap so that no external light should enter the enclosure.

2.3. Methodology

Figure 5 illustrates the flow of the experimental procedure for finding spectral absorbance by leaf chlorophyll for the Red LED. An electronic reader for measuring the plant chlorophyll concentration is designed using the microcontroller chip. The working principle of the project can be explained with the help of the block diagram shown in Figure 5. Initially, an arrangement is to be made such that the respective photodetectors will receive the LED transmission.

At first, the microcontroller will give a signal to switch on the two LEDs [IR LED (940nm) & Red LED (650nm)] sequentially. The two Photodiodes will receive the LED transmitted rays, placed at proper positions. The output signal of photodiodes will be amplified with the help of two FET input current amplifiers (TL072). The output of current amplifiers is given to the analogue input (A/D converter) of the Microcontroller.

These readings are taken for two conditions, the first reading will be without inserting a leaf between LEDs and Photodiodes, and the second reading will be after inserting a leaf between LEDs and Photodiodes. After inserting a leaf, three readings will be taken for the leaf's top, middle, and bottom parts. The spectral absorbance for the Red LED will be displayed on the OLED display. The information about the reading's location, date, and time will be given by GPS module- NE06M.

The information collected from the GPS module and the readings will be stored in a cloud using a Wi-Fi module ESP 8266 connected, as shown in the figure. These readings can be downloaded in an Excel sheet from the cloud and accessed on mobile phones. The Breadboard implementation of the circuit using Arduino is shown in Figure 6.

Initial testing of the circuit is done using breadboard implementation. Later PCB implementation is done, and a 3D-printed model is designed for enclosing the circuit. In the website created, we will show each plant's GPS location, time and date, CCI Index, etc. The website is designed to inform farmers and government agencies about the crops. We are currently using XAMPP Server, i.e. php my admin, to store information/data of plants CCI Index. Inserting the leaf in our device will give the CCI Index value on the Serial monitor, which gets automatically stored in our database.

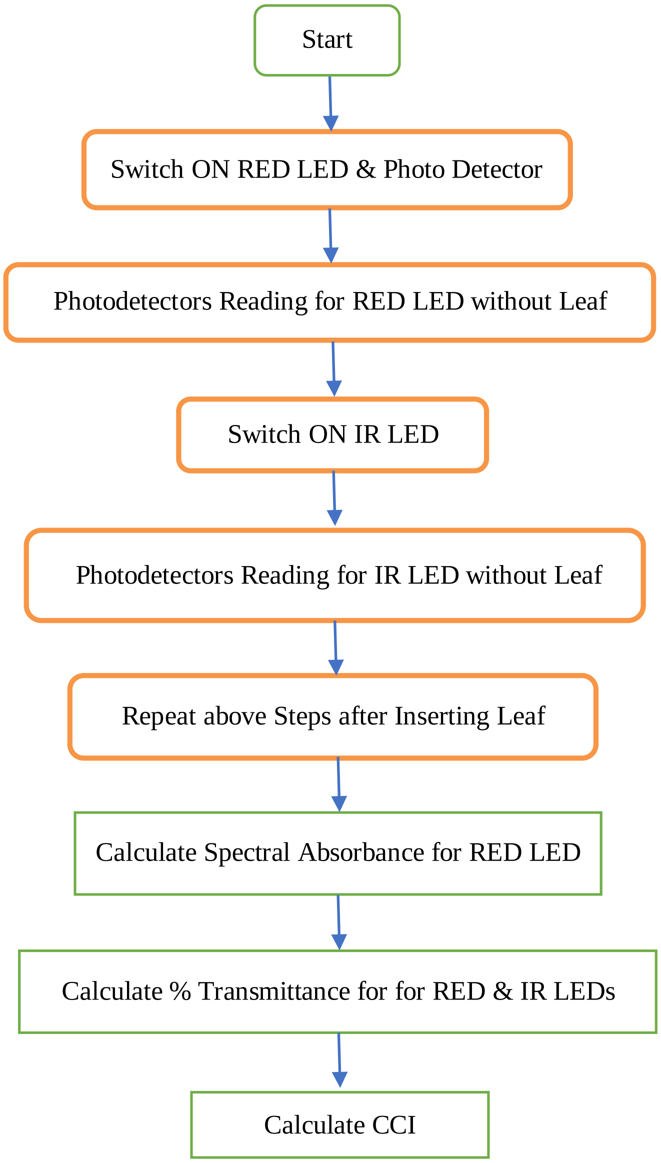


Fig. 5 Flowchart of the experimental procedure for CCI value for a plant

Fig. 6 Breadboard implementation of the electronic reader

Readings taken by the proposed electronic reader are compared with Arnon's method readings (Conventional Botanical process) explained below.

2.3.1. Botanical Process

Experiments are conducted in the RTMNU Nagpur University Botany lab to calibrate the electronic reader. To measure chlorophyll in the same leaves, the spectral absorbance readings are first taken using an electronic reader, and then the traditional Arnon method is used because the leaves must be crushed subsequently. The measurement of chlorophyll and spectral absorbance are then found to be correlated.

Fig. 7 Solution with crushed leaves

Figure 7 shows the solution of crushed leaves in the Botany lab during the experimentation. For the Botanical method, the requirements are acetone acid (20ml), a centrifuging machine, and UV – visible double spectrometer.

The process of chlorophyll measurement by the Arnon method is given below .

a) Take the leaves of the plant and crush the leaves by adding the acetone acid.
b) After crushing the leaves, the solution goes through the centrifuging machine for proper mixing.
c) Then, the solution is fixed in a spectrometer to check the absorption of the leaves by setting the wavelength.

While the process was going on, we checked the absorption on the Brahmi leaves with the four different wavelengths, as given below.

- The standard value of absorption is "1.2". Therefore, we got 630 as the proper wavelength.
- The standard value to check chlorophyll content is 645 and 663.

Table 2. Absorption on Brahmi leaves

S. No	Wavelength	Absorption
1.	450	3.000
2.	630	1.035
3.	650	2.538
4.	940	0.010

On the wavelength, we check the four plants' absorption levels, and the readings are shown in Table 3 . The following calculations are made to ascertain sample chlorophyll concentrations. Concentrations will be expressed on an area basis,

Chlorophyll a (mg/mL) = 12.7 A663 - 2.69 A645
Chlorophyll b (mg/mL) = 22.9 A645 - 4.68 A663

Where,
A645 = absorbance at a wavelength of 645 nm;
A663 = absorbance at a wavelength of 663 nm.

Total Chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b.

or

Total Chlorophyll (mg/g)=20.2 A645 + 8.02 A663.

Table 3. Arnon method of chlorophyll measurement

S.No.	Name of Plant	Absorption w.r.t. Wavelength		Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Total Chlorophyll (mg/g fresh weight)
		645 nm	663 nm			
1.	Mango Leaf	1.013	2.53	29.40	11.34	40.74
2.	Brahmi Leaf	2.680	3.000	30.89	47.33	78.22
3.	Papaya Leaf	2.80	3.000	30.57	50.08	80.65
4.	Ashoka Leaf	3.000	3.000	30.03	54.66	84.69

3. Results

The primary investigation was focused on the photosensor circuit, which uses two LEDs-Red LEDs at 650 nm and infraRed LEDs at 940 nm-as a phototransmitter and two photodetectors for each LED, respectively, to measure performance. Red LED has the highest level of leaf chlorophyll absorbance, whereas IR LED has the lowest level. The primary factor used to assess the efficacy of the suggested electronic reader is the Red LED spectrum absorbance by leaf chlorophyll.

Since leaf chlorophyll does not affect IR LEDs, IR transmittance readings are unaffected by variations in leaf chlorophyll. As a result, the primary attention was placed on how changes in plant leaves or leaf chlorophyll affected the Red LED's spectrum absorption. Here, Red LED spectral absorbance is the difference between the two readings of the photodetector output, i.e. Spectral absorbance = (Photodetector output without leaf) - (Photodetector output with leaf). Initially, for testing the proposed electronic reader, we selected a readily available vegetable with a more significant chlorophyll content, i.e. Spinach (Palak) & Jasminum sambac (Doodhi Mogara) . From Figure 8, as the spectral absorbance readings have large values, the leaf chlorophyll content for Spinach is higher ; similarly, 2nd set of readings was taken for the Jasminum sambac plant, and the different spectral absorbance readings for four other leaves are shown below.

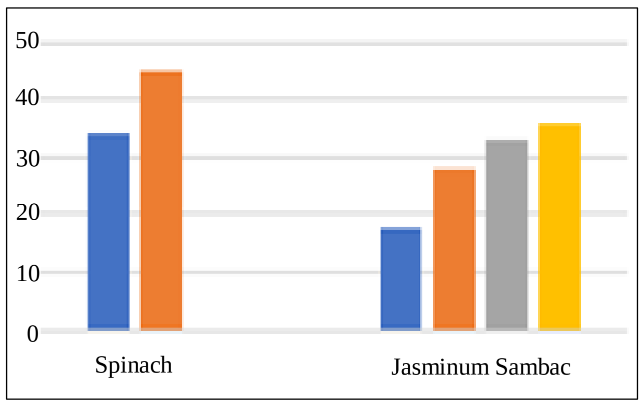
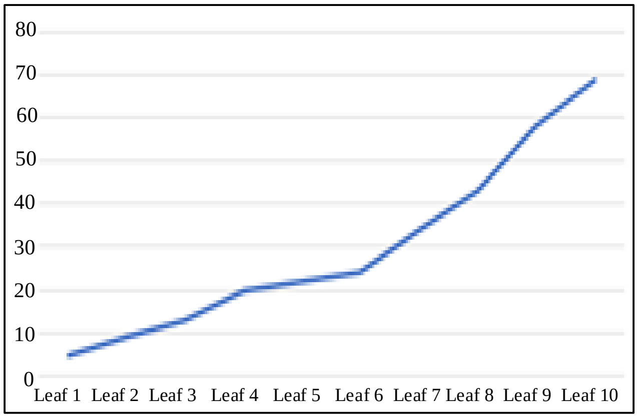


Fig. 8 Spectral absorbance testing for sample plants

Due to its all-encompassing impact on the central nervous system function as a memory enhancer, Brahmi (Bacopa Monnier) is a promising medicinal plant for the pharmaceutical and herbal industries. It is a crucial raw material for separating the active ingredients in numerous commercially significant medications.

The therapeutic properties of the Bacopa Monnier plant are impacted when the chlorophyll content of the plant's leaves decreases due to several conditions, and its commercial worth also declines. For these reasons, correct harvesting is crucial, which can be accomplished by measuring the chlorophyll content of leaves. Additionally, this plant's short development cycle-roughly three months-made it easy to acquire accurate readings.



Spectral absorbance readings at 650 nm (RED LED) were taken for the multiple leaves of the Bacopa Monnier plant (Brahmi) using the proposed electronic reader. Shree Shail Herbs Pvt. Ltd., Nagpur, helped to provide the different Bacopa Monnier plants (Brahmi) for experimentation.

These spectral absorbance readings were taken over a period of two months for other leaves of the Bacopa Monnier plant (Brahmi). In Figure 9, the spectral absorbance range reflects the corresponding range of leaf chlorophyll content for the same plant .

The botanical method is carried out to calibrate the proposed electronic reader to find the leaf chlorophyll content. The Botany Department & Pharmacy Department, Rashtrasant Tukdoji Maharaj Nagpur University, helped by permitting us to use their lab for experimentation.

Table 4 shows the readings taken by the Botanical procedure carried out in the Botany lab for finding the leaf chlorophyll content for three different leaves of the Bacopa Monnier plant (Brahmi). Total Chlorophyll (mg/g)=20.2 A645 + 8.02 A663.

Table 4. Chlorophyll content using botanical method for Bacopa Monnier (Brahmi)

Plant Name	**Absorbance w.r.t. Wavelength**		**Chlorophyll a (mg/ml)**	**Chlorophyll b (mg/ml)**	**Arnon Method**
Bacopa Monnier Plant (Brahmi)	**645 nm**	**663 nm**			**(Chlorophyll) (mg/g fresh weight)**
Leaf 1	0.206	0.462	5.31	2.55	7.86
Leaf 2	0.190	0.435	5.01	2.31	7.32
Leaf 3	0.121	0.268	3.07	1.51	4.58

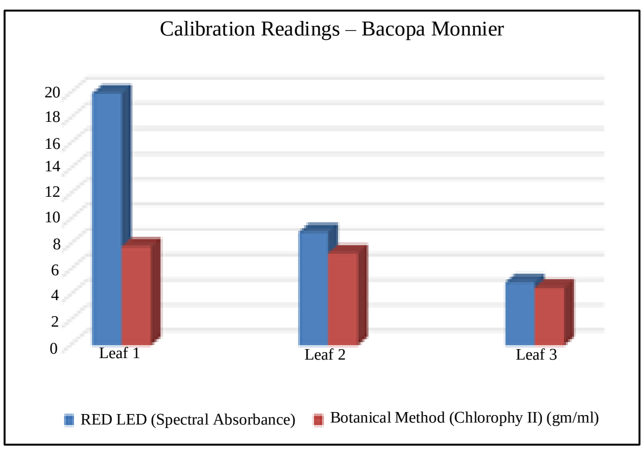


Fig. 10 Spectral absorbance and chlorophyll content correlation for Brahmi

Next, Table 5 shows the correlation between Electronic Reader readings and conventional Botanical method readings. If the leaf chlorophyll content is higher, the absorbance of Red LED (650nm wavelength) transmitted rays by the leaf will be higher. Consequently, the photodetector receives a reduced amount of transmitted rays and gives less output, which can be noted in the digital form on the IDE serial monitor.

On the other hand, if the leaf chlorophyll content is less, then the absorbance of Red LED (650nm wavelength) transmitted rays by the leaf will be less, and more portion of transmitted rays will reach the photodetector. Therefore, digital output on a serial monitor gives a higher reading. Figure 10 shows Spectra absorbance and chlorophyll content correlation for Brahmi for three leaves.

Figure 11 shows the digital readings of Red LED photodetector output on the serial monitor of Arduino IDE software. Table 6 compares CCI values measured by the SPAD meter and the proposed electronic chlorophyll reader for the cotton plant leaves.

The comparison shows that though CCI values are close, more readings are to be taken further for calibration. Figure 12 shows the screenshots of the website design process for monitoring data.

Table 5. Spectral absorbance and chlorophyll content correlation for Brahmi

Leaf	Plant Name	Electronic Reader (Spectral Absorbance)		RED LED (Spectral Absorbance)	Arnon's Method (Chlorophyll mg/g fresh weight)
	Brahmi	IR LED	RED LED	(Without Leaf - With Leaf)	
Leaf 1	Without Leaf	1017	951	20	7.86
	With Leaf	1017	931		
Leaf 2	Without Leaf	1017	951	09	7.32
	With Leaf	1017	942		
Leaf 3	Without Leaf	1017	948	05	4.58
	With Leaf	1017	943		

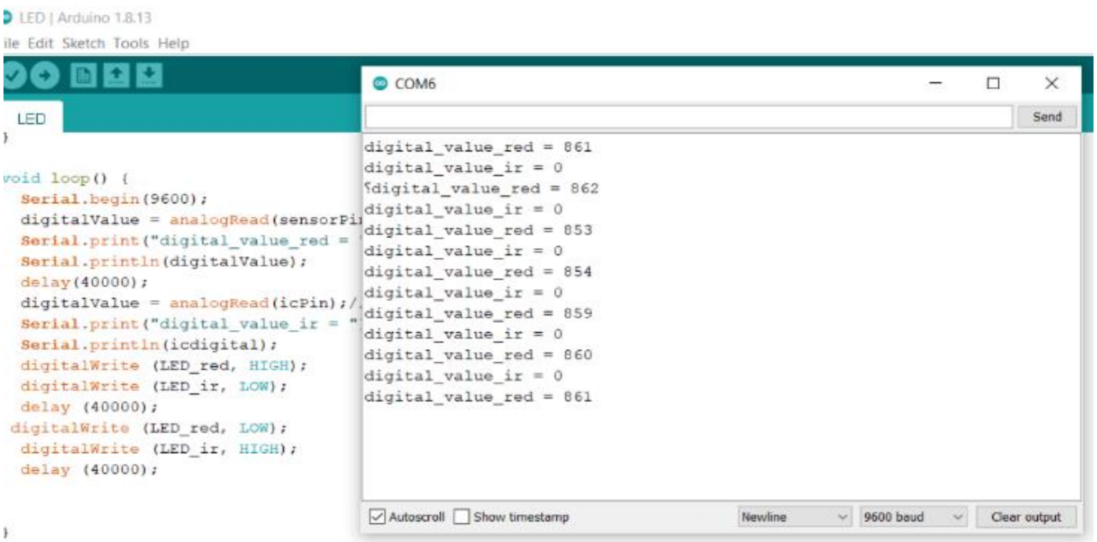


Fig. 11 Red LED photodetector reading on the serial monitor

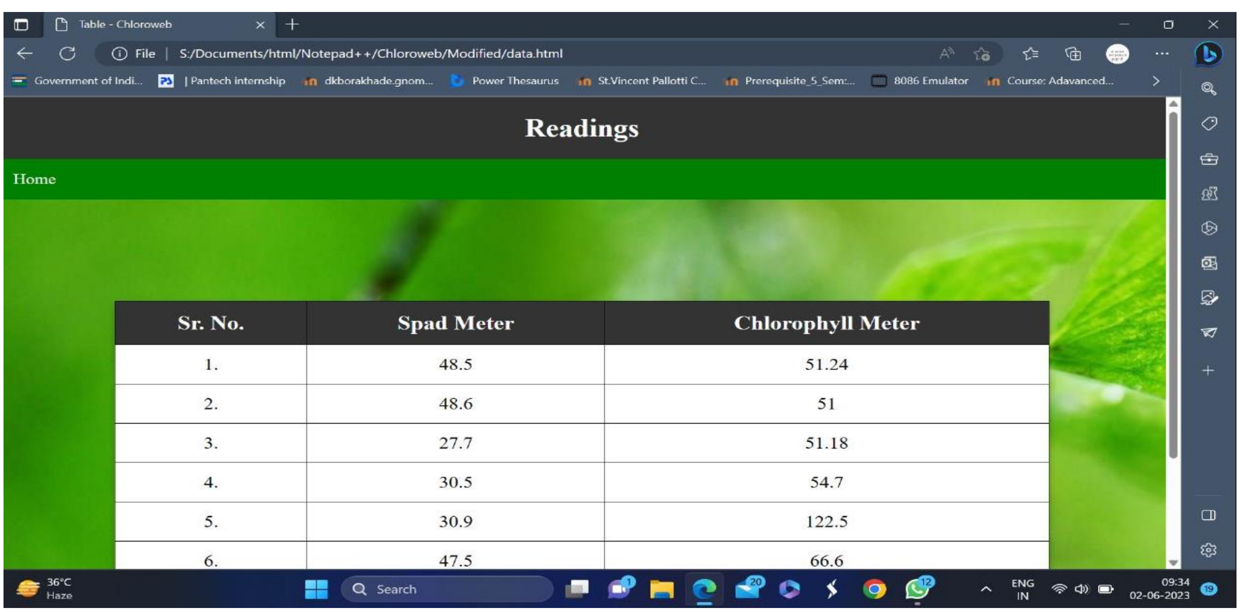


Fig. 12 Website design

Table 6. SPAD Meter readings for cotton plant leaves

S. No.	SPAD Meter	Electronic Chlorophyll Reader
1	48.5	51.24
2	48.6	51
3	42.7	49.8
4	48.1	59.5

4. Conclusion

According to this experimentation plan, a microcontroller-based device has the potential to determine the chlorophyll content of leaves. The values from the chlorophyll measurement device were calibrated for various plant leaves using the traditional Botanical procedure. The proposed system was found to function properly. Here, the performance analysis was done mainly based on spectral absorbance by the Red LED to ensure the proper functioning of the electronic reader design. Ultimately, a low-cost electronic reader design is implemented successfully, which can be converted to a commercial product affordable to farmers.

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