



## On-Site Detection of Nitrogen Content in Maize using Optical Sensor

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### Abstract

Nutrients are essential for the growth of plants, and they also increase the yield. Among the major nutrients, nitrogen is the most important nutrient for crop growth. The current study aims to estimate chlorophyll content vis-a-vis nitrogen content using different techniques and attempts to establish comparative benefits among available techniques. Maize crop, being highly known for fertilizer responsiveness, was chosen to carry out experiments with different levels of nitrogenous fertilizers. A preliminary field survey was taken in the farmer's field in Coimbatore and Salem districts. Two field experiments and one pot culture experiment at different doses of nitrogen input were conducted in 2019, 2020, and 2021 in the Department of Nano science and Technology at Tamil Nadu Agricultural University. In this study conventional nitrogen was applied with various dosage viz., 0kg ha<sup>-1</sup>(T<sub>1</sub>), 50kg ha<sup>-1</sup>(T<sub>2</sub>), 100kg ha<sup>-1</sup>(T<sub>3</sub>), 150kg ha<sup>-1</sup>(T<sub>4</sub>), 200kg ha<sup>-1</sup>(T<sub>5</sub>), 250kg ha<sup>-1</sup>(T<sub>6</sub>), 300kg ha<sup>-1</sup>(T<sub>7</sub>). In the pot culture experiment, IFFCO Nano nitrogen was sprayed on the maize leaves with seven different doses, which were control (no spray) (T<sub>1</sub>), conventional nitrogen (T<sub>2</sub>), 0.5X RDF (Recommended Dose of Fertilizer) (T<sub>3</sub>), 1.0X RDF (T<sub>4</sub>), 1.5X RDF (T<sub>5</sub>), 2.0X RDF (T<sub>6</sub>), 2.5X RDF (T<sub>7</sub>). The chlorophyll content in all the trials was estimated using Dimethylsulfoxide (DMSO), a portable in-house developed nutrient meter, and Spectroradiometer method. Chlorophyll content was measured at two different growth stages of maize viz., 60 & 90 days after sowing. The total nitrogen was analyzed using Kjeldahl method in the maize leaf. Among the treatments, with different doses of nitrogen levels, 300kg ha<sup>-1</sup> RDN & 2.5X RDF (T<sub>7</sub>) recorded higher SPAD (Soil Plant Analysis Development) values, chlorophyll "a" and chlorophyll "b" content and total nitrogen content at 60 DAS (Days After Sowing) in all tested methods. Chlorophyll and total nitrogen content were significantly decreased in later days. In a stressed environment with no nitrogen (T<sub>1</sub>) applied condition, the chlorophyll and nitrogen content showed significantly lower levels.



**Key words:** *Nitrogen; Chlorophyll; Spectral values; SPAD values; DMSO method*

## **Introduction:**

In plants, all the metabolic activities, growth, and development are governed by 20 essential nutrients. Imbalance in any one of the nutrients results in significant growth reduction and yield losses. Such imbalance in nutrients can be visibly identified using various morphological cues. The plant shows its nutrient deficiency symptoms when it reaches the below minimum requirement level. The plant takes balanced nutrients from the soil minerals, inorganic fertilizers, and soil organic compounds. Alternatively higher levels of nutrient application also cause toxicity in the plant system, therefore, the application of a required amount of nutrients is crucial, which results in better growth and yield production.

Nitrogen is referred to as the kingpin among all other essential nutrients in crop growth. In chlorophyll, a major portion is made up of nitrogen. Chlorophyll is the most important component for photosynthesis. In plants all enzymatic reaction requires nitrogen. Nitrogen deficiency leads to earlier maturity which causes reduced yield production and grain quality level. Severe lower nitrogen condition causes death in plants. Pale green to light yellow color occurs on the older leaves due to chlorosis, a classical symptom of nitrogen translocating from older leaves to younger leaves. Hence, it is highly crucial to monitor the nitrogen levels vis-a-vis chlorophyll content in the plant system to enable improved yield.

Currently, SPAD chlorophyll meter is used for detecting N in plants. In this method, it is assumed that a major part of N gets accumulated in chlorophyll and the estimation of chlorophyll is an indirect measure of the N content of leaves (Simko and Veres, 2019; Yang *et al.*, 2018). Despite it being demonstrated well for many crops, the technique is highly sensitive to cloud cover. SPAD meter readings are reliable only for brighter sunny days. Traditional laboratory-based methods are laborious, time-consuming, and highly variable due to the time lag between sample collection and laboratory analysis. The study aims to compare the nitrogen vis-a-vis chlorophyll content in different treatments using various methods viz., chlorophyll estimation by DMSO method, total nitrogen Kjeldahl method, leaf nitrogen content by SPAD meter, and spectral reflectance using Spectral Radiometer signatures (Naik *et al.*, 2020). Therefore on-site detection is quite relevant. A spot detection of nutrient deficiencies in crops will enable the farmers to undertake an immediate corrective measure of proper fertilization and ensure the productivity of crops (Gabriel *et al.*, 2019). Diphylamine, phosphomolybdic acid, and cobalt nitrate are used for quick diagnosis of N, P, and K.

## **Materials and methods**

### ***Survey and data collection***

A field survey was conducted to ascertain the chlorophyll content prevailing in maize crops at farmers' fields located in Coimbatore and Salem districts. The field survey is representative of two geographical



locations. The data on chlorophyll content was recorded on 60 DAS at farmer's field using SPAD meter. The geo-coordinates of the farmers' field were plotted in ArcGIS software.

#### ***Establishing field crop with gradient nitrogen fertilizer application***

To assess the nitrogen content vis-a-vis chlorophyll content in plant systems using different methods viz., DMSO method (Total nitrogen), Chlorophyll estimation (SPAD meter), and Hyper Spectral Imaging. Two batches of maize crops were raised in the research plots of TNAU and operational details are tabulated (Table1). The field was prepared using a mould board plough and raised furrows were formed as per the stipulated spacing. Prior to land preparation, the land was broadcasted with 12.5tha<sup>-1</sup> of farm yard manure (FYM) as per the TNAU agriculture crop guidelines. The field was divided into 21 sub-plots, and each plot consisted of seven ridges.

#### ***Nutrient application***

The nutrients application and the package of practices were carried out as per TNAU crop production guidelines. For the treatments, one-quarter dose of nitrogen, full dose of phosphorus, and potassium was applied as the basal before sowing the seeds. The remaining splits of nutrients were applied as top dressing on 60 DAS and 75 DAS (Table2). In addition, phosphorus and potassium at a ratio of 75:75 kg ha<sup>-1</sup>; zinc sulphate at 37.5 kg ha<sup>-1</sup> was applied on 60 DAS as per the TNAU crop production guidelines.

#### ***Establishing pot culture experiment with gradient doses of IFFCO nano urea***

In addition to ascertaining the chlorophyll vis-à-vis nitrogen content in field conditions imposed by treatments of gradient nitrogen levels, attempts were made to study the nitrogen content in maize plants grown in pot cultures in controlled environmental conditions. IFFCO Nano urea is used as a sole source of nitrogen and it has been applied as a foliar spray. The parameters like chlorophyll content (a, b and total), total nitrogen content, SPAD values, and hyperspectral data were recorded in green house conditions and data was correlated to field data. The 21 pots were prepared with a mixture of soil and vermin compost. CRD (completely randomized designs) was adopted to impose treatments using IFFCO Nano Urea Sprays. Two sprays of IFFCO nano nitrogen were done neat 15 DAS and 30 DAS. The experiment setup and treatment details are tabulated (Table3&4). Samples were drawn for nitrogen and chlorophyll analysis using conventional methods on 60 and 90 DAS in addition. SPAD and spectral data were also recorded prior to sample collection.

#### ***Estimation of chlorophyll and nitrogen content***

Samples collected from two field experiments and pot culture trials were subjected to chlorophyll and nitrogen estimation. Data points representing chlorophyll and nitrogen content were assessed using 6 methodologies viz., chlorophyll a, chlorophyll b, total chlorophyll content (DMSO method), Total Nitrogen



content (Kjeldahl method), Soil Plant Analysis Development (SPAD), chlorophyll meter and hyper spectral imaging. Soil Plant Analysis Development (SPAD) chlorophyll meter (Yuan, 2016).

#### Chlorophyll estimation by DMSO method

0.1g of fresh maize leaf was placed in the 10mL of DMSO in a 100mL of volumetric flask for overnight incubation. After chlorophyll 'a', chlorophyll 'b' and total chlorophyll were quantified by reading the optical density at 663nm and 645nm. The chlorophyll content was calculated using the formula described in (1), (2), (3) (Yoshida *et al.*, 1971).

$$\text{Chlorophyll}'a' = \frac{(12.7 \times \text{O.D. at } 663) - (2.54 \times \text{O.D. at } 645)}{W} \times V \quad (1)$$

$$\text{Chlorophyll}'b' = \frac{(22.9 \times \text{O.D. at } 645) - (4.68 \times \text{O.D. at } 663)}{W} \times V \quad (2)$$

$$\text{Total Chlorophyll} = \frac{(20.2 \times \text{O.D. at } 645) - (8.02 \times \text{O.D. at } 663)}{W} \times V \quad (3)$$

Where, W - Weight of the leaf sample (g)

V - Volume of supernatant solution made-up

OD - Optical Density.

#### Total nitrogen estimation

In maize leaf, total nitrogen was estimated by Kjeldahl method (Jackson, 1883). It is a destructive method. Collected leaves were taken to the laboratory and samples were placed in to the diacid for digestion. After digestion, the solution was distilled and through that nitrogen, estimation has been done. The parameters for analysis were, volume of sample taken = 0.5g; volume of diacid extract make up = 100mL; volume of diacid extract pipette out for the distillation = 10mL and 1mL of 0.02 N H<sub>2</sub>SO<sub>4</sub> = 0.00028g of N. The total nitrogen content in plants are expressed as mg/g of a leaf.

#### Soil Plant Analysis Development (SPAD) Chlorophyll meter

SPAD readings are an indirect measure of chlorophyll content in plants and it is a non-destructive, handheld device used to measure chlorophyll content in *in situ* field conditions. SPAD values involve the recording of spectral data at two specific wavelengths of 650 nm and 940 nm and it is converted indices of chlorophyll content. SPAD-502 plus (Konica, Minolta, Germany), available at the Department of Crop Physiology, Tamil Nadu Agricultural University, was used for recording SPAD data (Bonneville and Fyles, 2006; Simet *et al.*, 2015). It is a non-destructive method. In each plot, five datasets were taken, and it was averaged to get mean values. The readings were recorded from the fully expanded leaves from the top of the plant at 60 and 90 DAS.



### **Spectroradiometer**

Spectral reflectance data at different wavebands of green, blue, and red region and NIR region ranging from 350 – 1050nm were recorded using GER 1500 Spectroradiometer (Geophysical and Environmental Research, UK) (Figure 1). Data was recorded at 60, and 90 DAS in addition to the SPAD readings, and the datasets were correlated (Alchanatis et al., 2005; Fu et al., 2021)

### **Results and discussion:**

#### **Field survey**

SPAD values were collected from the fully opened maize leaf in *in situ* field conditions. The geo-coordinates of the sampling points were recorded and the same has been plotted (Figure 2) where SPAD data were collected from different locations. In the Coimbatore district, the highest SPAD chlorophyll index is 63.6 and the lowest value is 48.8. In Salem highest chlorophyll index is 61.5 & lowest is 39.8.

#### **Chlorophyll index by SPAD meter**

In field experiment I, the highest SPAD chlorophyll index was recorded at 67.32, 61.71, and 65.35, 59.67 in T<sub>7</sub>(300kg ha<sup>-1</sup>) & T<sub>6</sub>(250kg ha<sup>-1</sup>) and lowest chlorophyll index was 34.16 & 32.02 in T<sub>1</sub> at 30 and 60 DAS was recorded. Similar observations were recorded in the SPAD data from field experiment II, with an highest SPAD chlorophyll index being 68.79 and 66.83 in T<sub>7</sub> and 63.19 and 61.13 in T<sub>6</sub> and the lowest is 35.64 and 33.5 in T<sub>1</sub> at 60 DAS and 90 DAS respectively. In the pot culture experiment, carried out under controlled environmental conditions, the SPAD chlorophyll index was high in the treatment (T<sub>7</sub>) at 64.69 & 62.66, and the lowest in control, which is 31.47 & 29.33 at 60 and 90 DAS.

#### **Chlorophyll estimation by DMSO method**

The chlorophyll 'a' & chlorophyll 'b' content of maize samples drawn from two field studies and pot culture experiments were estimated. In field experiment I, The highest chlorophyll content 'a' 1.72 & 1.55 mg g<sup>-1</sup> fl was observed in T<sub>7</sub>(300kg ha<sup>-1</sup>) and lower chlorophyll 'a' content 0.71 & 0.65 mg g<sup>-1</sup> fl in T<sub>1</sub> at 60 and 90 DAS. In T<sub>7</sub> the chlorophyll 'b' content is 0.578 and 0.467 mg g<sup>-1</sup> fl at 60 & 90 DAS. Similarly, in field experiment II, The higher chlorophyll 'a' content was registered 1.75 & 1.58 mg g<sup>-1</sup> fl in T<sub>7</sub> at 60 and 90 DAS, followed by 0.73 & 0.67 mg g<sup>-1</sup> fl in T<sub>1</sub> resulting in lower chlorophyll 'a' content at 60 and 90 DAS and significantly higher chlorophyll 'b' content of 0.608 & 0.487 mg g<sup>-1</sup> fl in T<sub>7</sub> with 300kg ha<sup>-1</sup> RDN and lower chlorophyll 'b' content of 0.297 & 0.265 mg g<sup>-1</sup> fl in T<sub>1</sub> with no nitrogen applied was recorded at 60 and 90 DAS. Whereas, In the pot culture experiment, higher chlorophyll "a" content of 1.68 and 1.51 mg g<sup>-1</sup> fl in T<sub>7</sub> respectively 60 & 90 DAS followed by T<sub>6</sub>. Lower chlorophyll "a" content was observed in T<sub>1</sub> (control) which was 0.67 and 0.61 mg g<sup>-1</sup> fl. A high chlorophyll "b" content of 0.54 & 0.43 mg g<sup>-1</sup> fl was observed in T<sub>7</sub> with 2.5X RDF at 60 and 90 DAS, respectively. This was followed by T<sub>6</sub> with 2.0 X RDF. Lower chlorophyll "b" content of 0.24 & 0.20 mg g<sup>-1</sup> fl was recorded in T<sub>1</sub> control with no nitrogen application at 60 and 90 DAS.

#### **Total Nitrogen by Kjeldahl method**



In addition to the indirect measure of chlorophyll content by SPAD meter, the actual total nitrogen present in the plant samples was analysed using Kjeldahl method. In field experiment I, The highest total nitrogen level of 1.466% and 1.576% were recorded in T<sub>7</sub> (300kg ha<sup>-1</sup>) followed by 1.309, 1.419% in T<sub>6</sub> and lowest total nitrogen of 0.651, 0.762% in T<sub>1</sub> at 60 and 90 DAS. Similarly, in field experiment II, the highest level of the total nitrogen content was 1.47 & 1.58% in T<sub>7</sub> with 300kg ha<sup>-1</sup> and the lowest total nitrogen content was 0.67 & 0.79% in T<sub>1</sub> with no nitrogen application (control) at 60 and 90 DAS. In the pot culture experiment, a higher nitrogen level of 300kg ha<sup>-1</sup> of RDN recorded significantly higher total nitrogen content of 1.406 & 1.516(%) in T<sub>7</sub> and this was followed by 1.286 & 1.396(%) of total nitrogen in T<sub>6</sub> at 60 and 90 DAS respectively. Significantly lower total nitrogen content of 0.611 & 0.742(%) was observed in T<sub>1</sub> with no nitrogen application at 60 and 90 DAS.

#### ***Chlorophyll estimation using spectral reflectance***

In field experiment I, the spectral reflectance data represents, in plots, subjected to lower levels of nitrogen application resulted from spectral reflectance in the visible spectrum region that had a higher percentage of reflectance. A higher dose of nitrogen applied conditions reflectance in the visible spectrum region has lower reflectance and higher reflectance in the near-infrared region.

The wavebands in blue and green of 460.1, 471.6, 488.0, 492.9, 549.8, 553.1, 557.9 and 588.6 nm in the visible spectrum resulted in a lower reflectance ranging from 4.26 to 7.04 and 5.24 to 6.87 per cent with 300kg ha<sup>-1</sup> RDN at 60 and 90 DAS. In nitrogen deprived treatments wavebands had higher reflectance of 6.68 to 9.88 and 8.63 to 14.27 per cent in the visible spectrum at both 60 and 90 DAS.

The effective wavebands in near infrared region from 820.3 to 927.4 nm resulted in higher reflectance of maize ranging from 43.45 to 45.44 and 41.47 to 42.29 per cent with 300kg ha<sup>-1</sup> RDN at 60 and 90 DAS. The effective wavebands were 820.3, 844.6, 850.7, 855.3, 864.3, 900.5 and 927.4 nm in the NIR region. Lower spectral reflectance of 30.74 to 32.77 and 29.61 to 31.70 per cent was recorded with no nitrogen application in NIR region under stressed conditions. It clearly showed that the NIR regions reflect more in plots applied with a recommended dose of nitrogen sources and less in nitrogen-deprived plots. A similar trend was observed in field experiment II, where the effective wavebands in near infrared region from 820.3 to 927.4 nm resulted in higher reflectance of maize ranging from 44.47 to 46.49 42.49 to 43.27 per cent with 300kg ha<sup>-1</sup> RDN at 60 and 90 DAS. The effective wavebands were 820.3, 844.6, 850.7, 855.3, 864.3, 900.5 and 927.4 nm in NIR region. Lower spectral reflectance of 31.76 to 33.72 and 30.61 to 32.28 per cent was recorded with no nitrogen application in NIR region under stressed condition.

#### ***Normalised Difference Vegetation Index (NDVI) establishing the relationship between data sets from nitrogen, chlorophyll content and SPAD datasets***

Nitrogen levels influences the relationship between the NDVI with SPAD, chlorophyll a & b. In field experiment I, the NDVI value is observed to be significantly higher than the SPAD, chlorophyll a & b values. The NDVI relationship with SPAD was 0.906\*\* and 0.972\*\*, NDVI with chlorophyll a 0.935\*\* and 0.908\*\*, and with chlorophyll b 0.935\*\* and 0.908\*\*.



chlorophyll b 0.948\*\* and 0.921\*\* at 60 and 90 DAS. Similarly, in field experiment II, relationship between NDVI with SPAD was 0.923\*\* and 0.976\*\*, NDVI relationship value with chlorophyll a was 0.927\*\* and 0.887\*\*, chlorophyll b value was 0.954\*\* and 0.935\*\* at 60 and 90 DAS. NDVI significantly increases with higher values of SPAD and chlorophyll ( $R^2$ ) values.

## Conclusion

Total Nitrogen content vis-à-vis chlorophyll content is an important factor that determines the growth and development of the plant system. Gaining insights in developing appropriate tools or yardsticks to capture the nitrogen content with high precision will empower farmers to carry out remedial measures or supplement nutrients and enable them to prevent drastic yield loss (Fomariet *al.*, 2020). In the current study, attempts were made to study the different methodologies in vogue to quantify the nitrogen content in plant system *viz.*, chlorophyll estimation (DMSO method), Total Nitrogen estimation (Kjeldhal method), SPAD meter, and spectral reflectance method. Estimation by DMSO method and Kjeldhal method are destructive and involve relatively cumbersome laboratory analysis, whereas the reflectance-based methods (SPAD and spectral imaging) are nondestructive methods and data can be obtained instantaneously (Habibullahet *al.*, 2020; Rongtinget *al.*, 2020). From the current study, a correlation has been established among the four methods. It is evident that SPAD and spectral signature based methods are highly versatile and with sufficient reference datasets, the method can be effectively employed in chlorophyll vis-à-vis nitrogen estimation in fields with larger areas in a relatively short time span (Fenget *al.*, 2021). Further, these imaging/spectral scanners are conducive to be integrated with AI and drone based technology pipelines (Cheema *et al.*, 2018) thereby providing increased efficiency and phenotyping applications.

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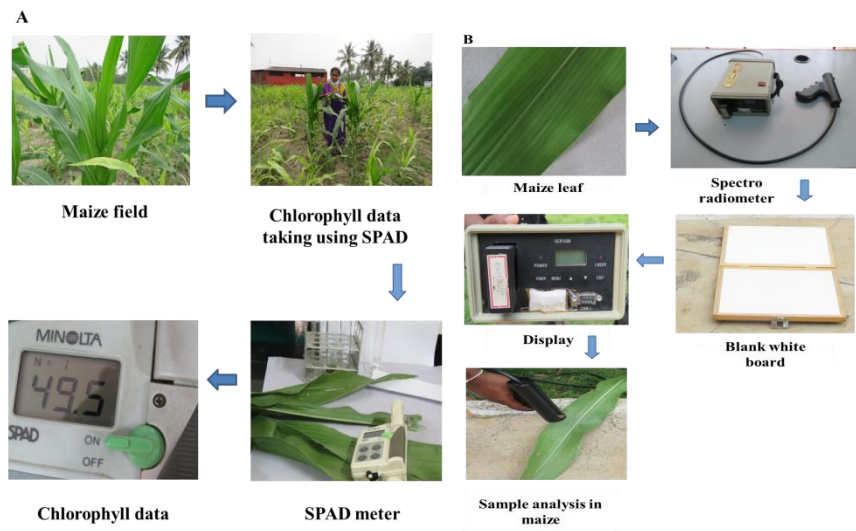


Figure.1 Represents the stages of recording data using SPAD meter (A) and Spectro radiometer (B)

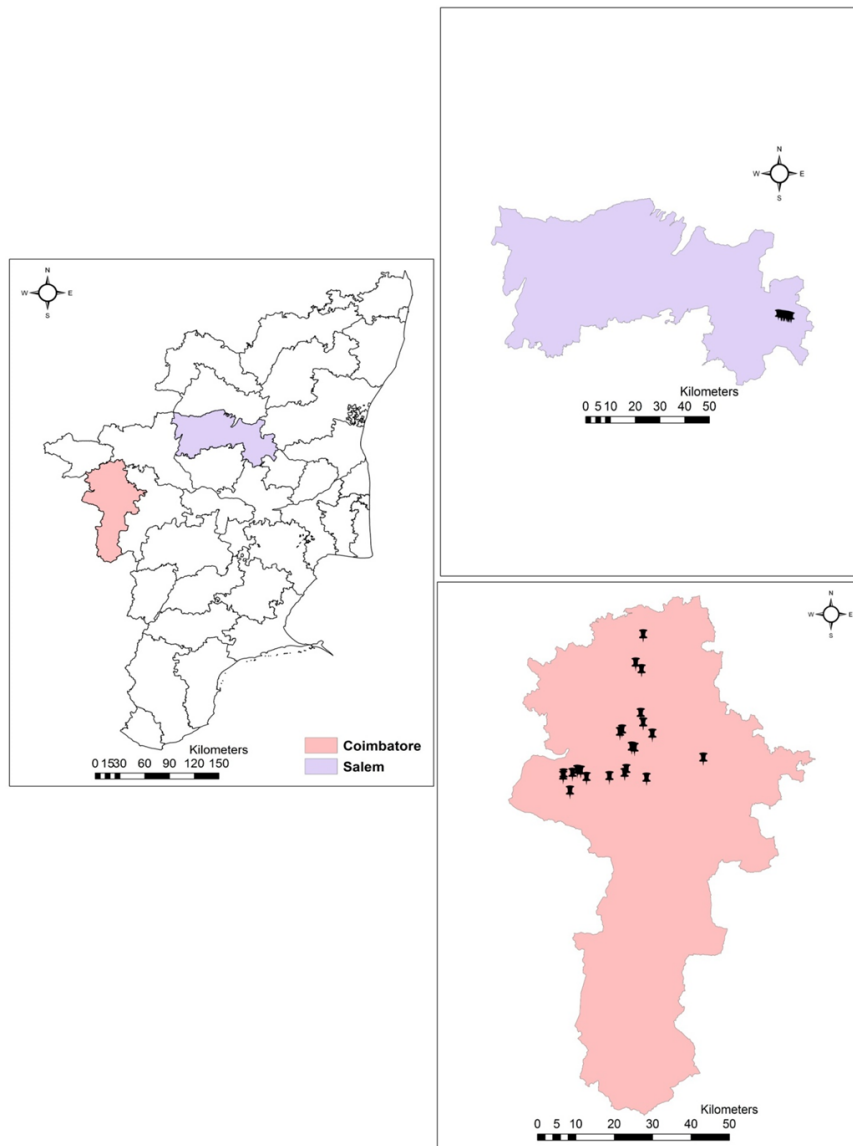


Figure2. Geo-coordinates of sampling points in two districts mapped using ArcGIS

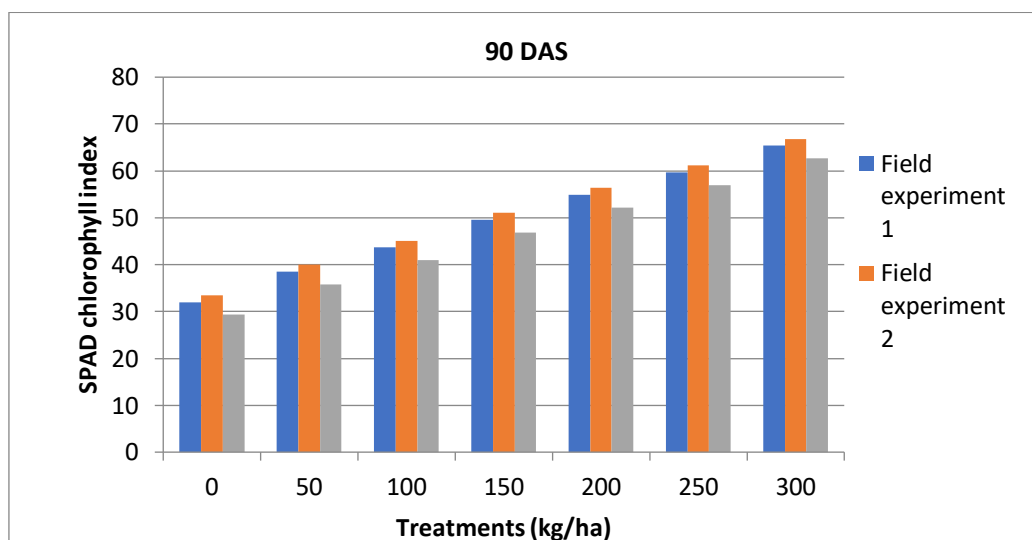
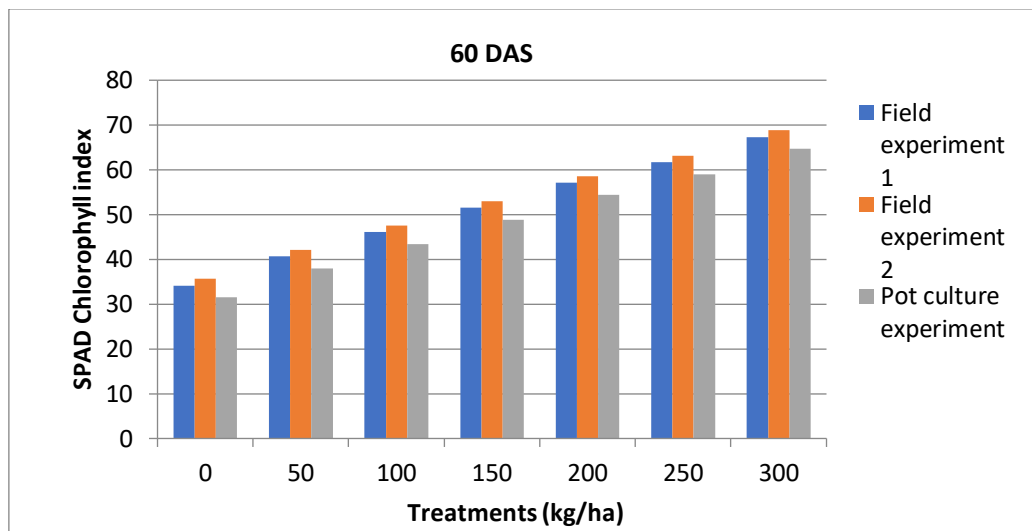
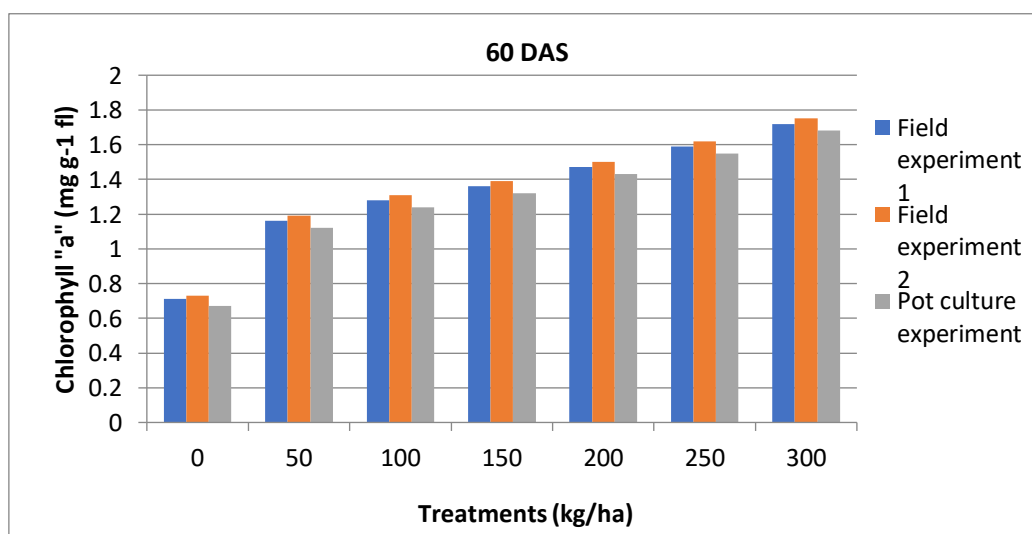


Figure3. Effect of nitrogen levels on SPAD value of maize



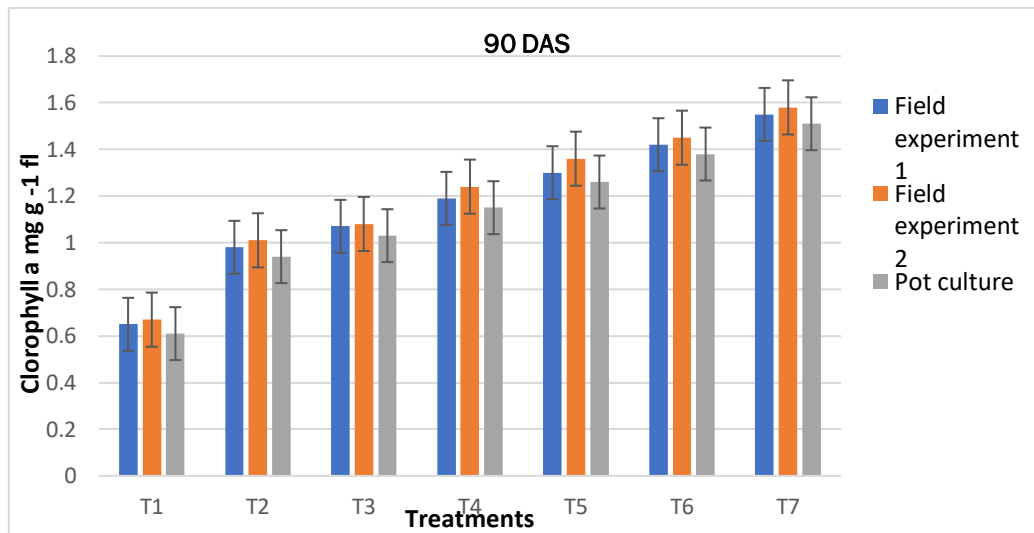
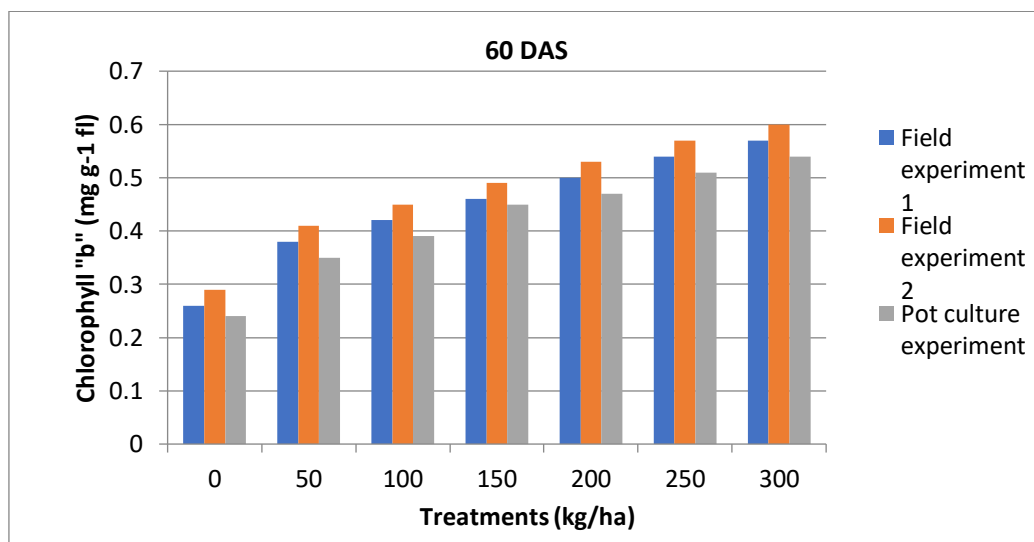


Figure 4. Effect of nitrogen levels on Chlorophyll "a" of maize



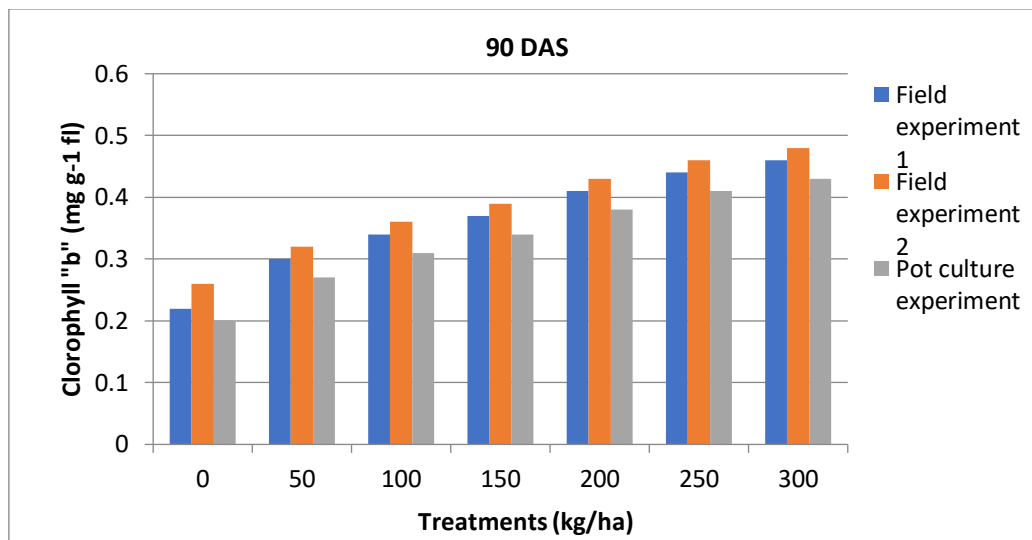


Figure5. Effect of nitrogen levels on Chlorophyll "b" of maize

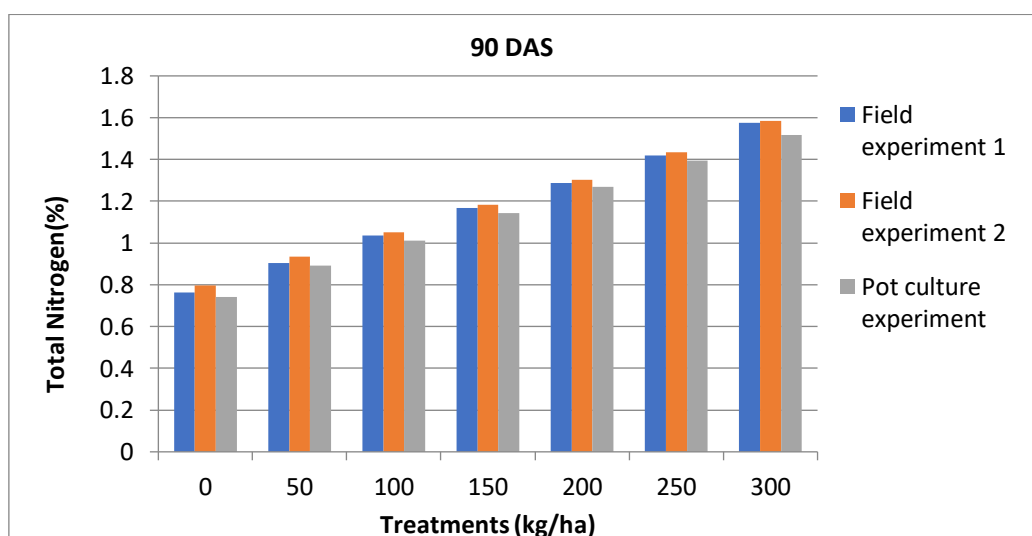
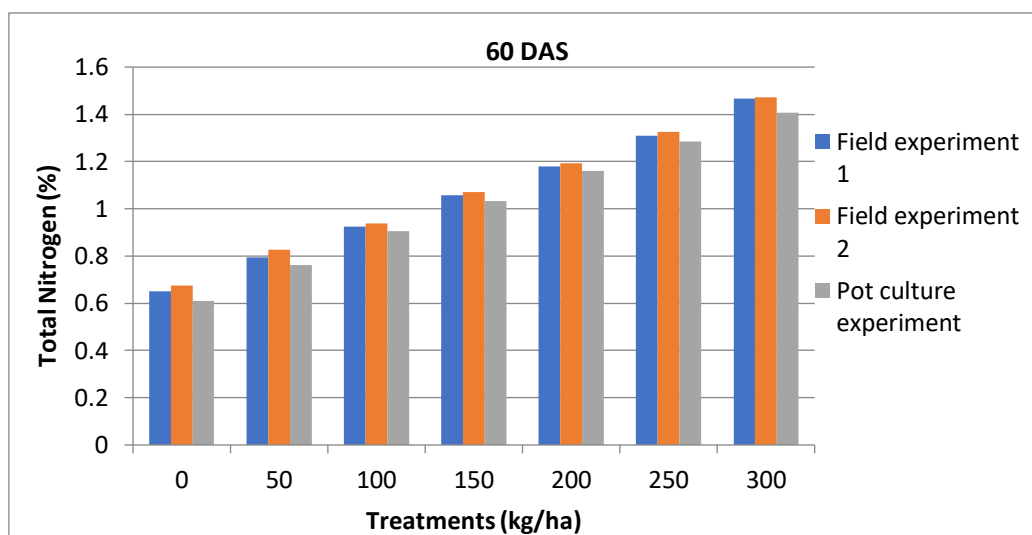




Figure6. Effect of nitrogen levels on Total nitrogen of maize

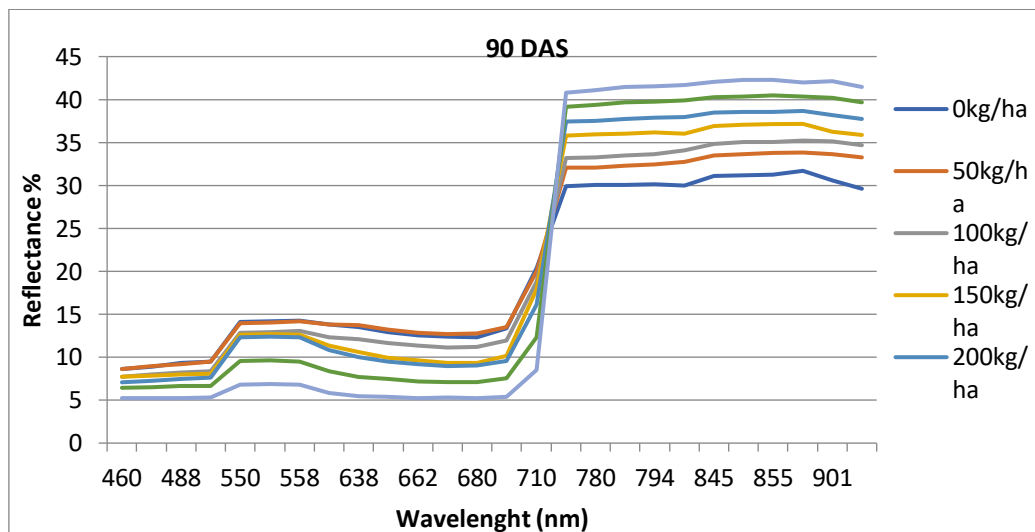
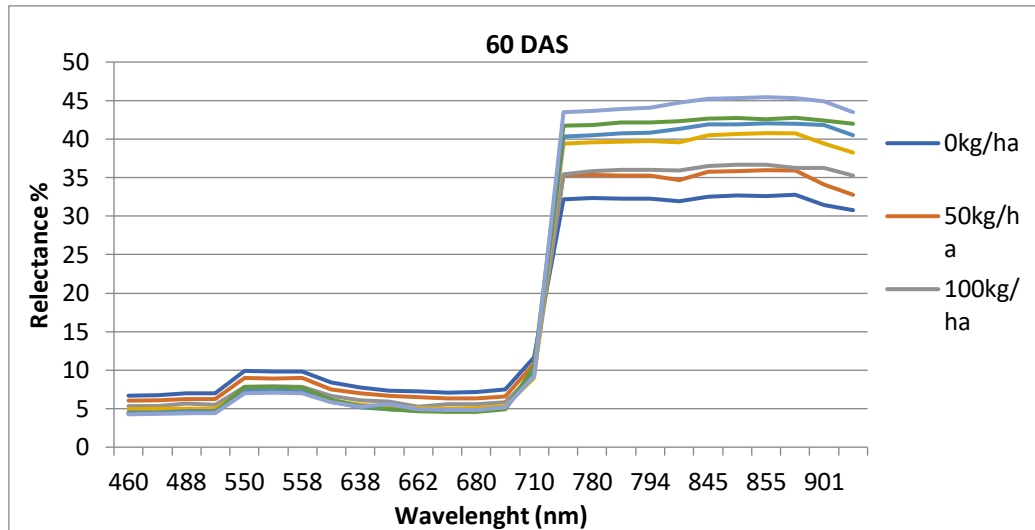


Figure7. Effect of nitrogen levels on Spectral reflectance (%) of maize in Field experiment I

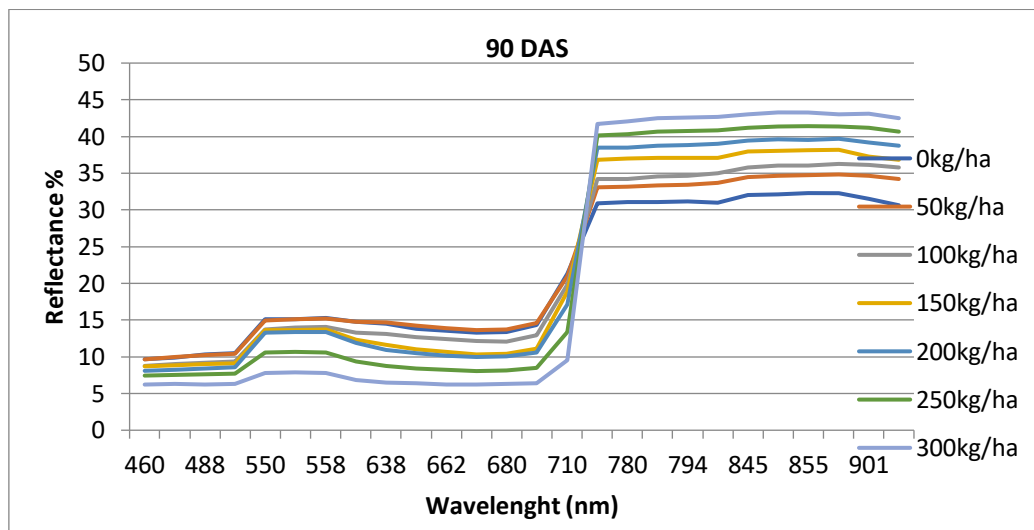
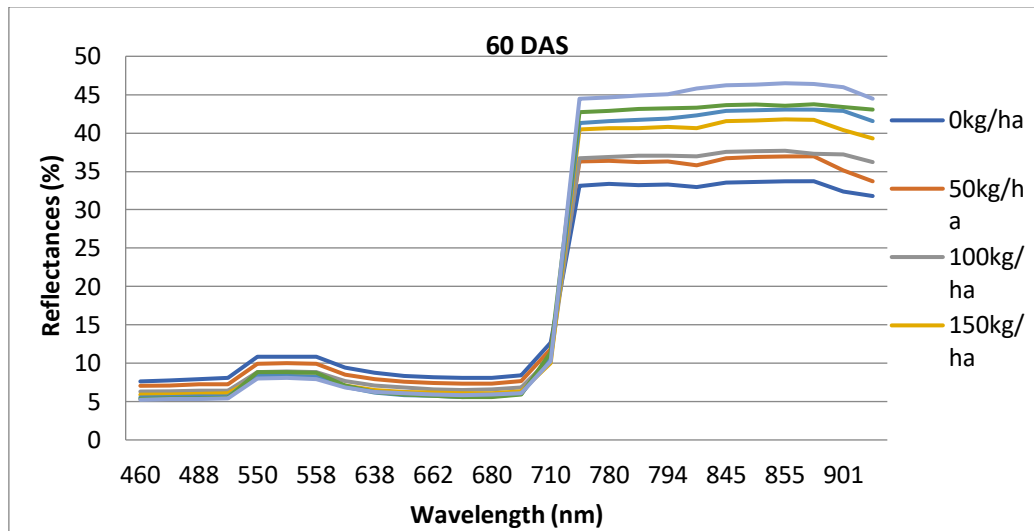
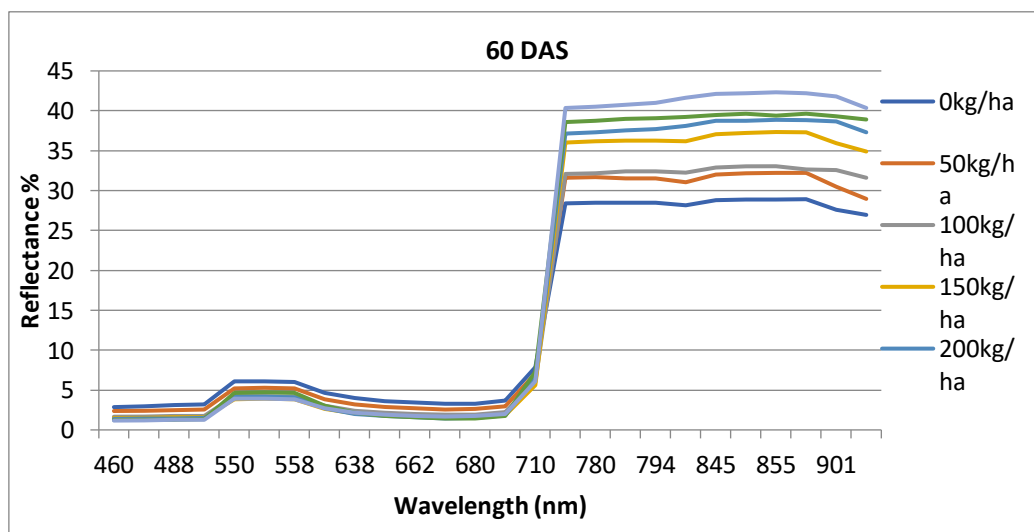


Figure8. Effect of nitrogen levels on Spectral reflectance (%) of maize in Field experiment II





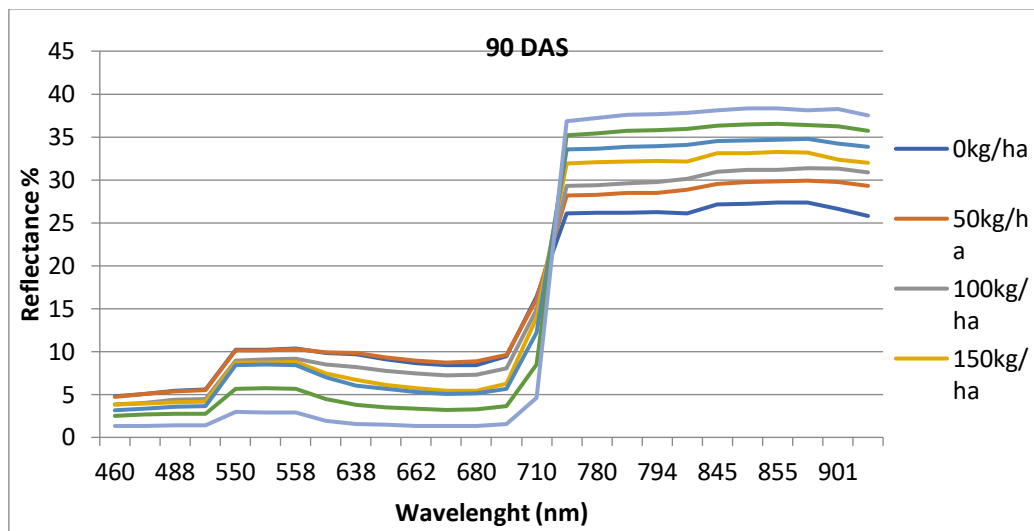


Figure9. Effect of nitrogen levels on Spectral reflectance(%)of maize in Potculture experiment II

Table1.Operational Details of Nitrogen Fertilizer application in maize crops

Description	Field experiment 1	Field experiment 2
Field number	37 A	37 A
Crop	Maize	Maize
Variety	Maize hybrid C06	Maize hybrid C06
Treatments	7	7
Replications	3	3
Design	RBD	RBD
Spacing	60X30 cm	60X30 cm
Date of sowing	08.03.2019	08.01.2020
Date of harvest	20.6.2019	23.04.2020

Table2.Details of split dose Nutrient application

Treatments	Nitrogen doses (kg ha <sup>-1</sup> )	Nitrogen doses (per plot or 23 m <sup>2</sup> )
T <sub>1</sub>	0	0
T <sub>2</sub>	50	250 g
T <sub>3</sub>	100	500 g
T <sub>4</sub>	150	750 g
T <sub>5</sub>	200	1 kg
T <sub>6</sub>	250	1.2 kg



T <sub>7</sub>	300	1.5 kg
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**Table 3.** Experimental details on gradient dose application of IFFCO Nano urea

Pot culture	Green house
Crop	Maize
Variety	Maize hybrid CO6
Treatments	7
Replications	3
Date of sowing	11.12.2021
Date of harvest	23.3.2021

**Table4:** Treatment details on gradient dose application of IFFCO Nano urea

Treatments	Nitrogen dose per pot
T <sub>1</sub>	Control
T <sub>2</sub>	Conventional N
T <sub>3</sub>	0.5X RDF
T <sub>4</sub>	1.0 X RDF
T <sub>5</sub>	1.5 X RDF
T <sub>6</sub>	2.0 X RDF
T <sub>7</sub>	2.5 X RDF

**Table5.** Relationship between NDVI vs SPAD and chlorophyll (R<sup>2</sup>) at Field experiment I

60 DAS	SPAD	Chlorophyll 'a'	Chlorophyll 'b'
NDVI	0.907**	0.917**	0.945**

90 DAS	SPAD	Chlorophyll 'a'	Chlorophyll 'b'
NDVI	0.975**	0.907**	0.924**

**Table6.** Relationship between NDVI vs SPAD and chlorophyll (R<sup>2</sup>) at Field experiment II

60 DAS	SPAD	Chlorophyll 'a'	Chlorophyll 'b'
NDVI	0.923**	0.922**	0.957**

90 DAS	SPAD	Chlorophyll 'a'	Chlorophyll 'b'
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NDVI	0.976**	0.915**	0.931**
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