RELATIONSHIP BETWEEN CHLOROPHYLL AND OTHER PIGMENTS AND SPECTRAL REFLECTANCE IN VARIOUS PLANT SPECIES

BY

Miss.ASHWINI ASHOKRAO BODKHE

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DISSERTATION

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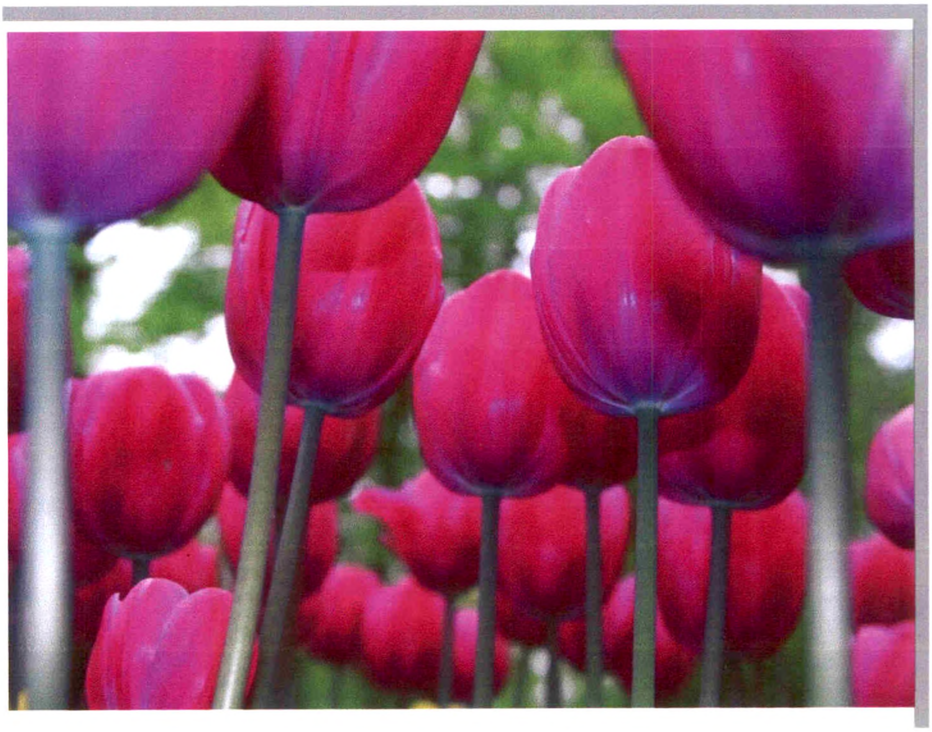
Dedicated to
Beloved

Aai and Baba



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I, hereby declare that the entire work embodied in this dissertation or any part thereof has not been previously submitted by me for a degree of any University or Institution

Place : PARBHANI
Date : 04 June, 2008

(A.A. Bodkhe)

Dr. V.D. Patil
M.Sc. (Agri.), Ph.D. (Soils)
FMASHAV (Israel), FINSA (Germany)
Associate Professor
Department of Soil Science and Agricultural Chemistry
Marathwada Agricultural University,
Parbhani - 431 402 (M.S.)

CERTIFICATE-I

This is to certify that **Ms..ASHWINI ASHOKRAO BODKHE** has satisfactorily prosecuted her course credits and research for a period of not less than two years and the dissertation entitled "RELATIONSHIP BETWEEN CHLOROPHYLL AND OTHER PIGMENTS AND SPECTRAL REFLECTANCE IN VARIOUS PLANT SPECIES" submitted by her is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination for the award of the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**.

I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

PARBHANI
Date : 04 June, 2008

(V.D. Patil)
Research Guide
&
Chairman, Advisory Committee

CERTIFICATE-II

This is to certify that the dissertation entitled **"RELATIONSHIP BETWEEN CHLOROPHYLL AND OTHER PIGMENTS AND SPECTRAL REFLECTANCE IN VARIOUS PLANT SPECIES"** submitted by **Ms.ASHWINI ASHOKRAO BODKHE** to the Marathwada Agricultural University, Parbhani in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **SOIL SCIENCE AND AGRICULTURAL CHEMISTRY** has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

(V. G. Takankhar)
External Examiner

(V.D. Patil)
Research Guide

Advisory Committee:

(A.S. Dhawan)

(Syed Ismail)

(S.S. Shinde)

(S.D. Jature)

Associate Dean (P.G.),
College of Agriculture,
M.A.U., Parbhani.

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“There always are in the world a few inspired men whose acquaintance beyond price”

Plato...

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ABBREVIATIONS

um - Micrometer
B - Band
BSS - Bright sunshine hours
cm - Centimeter
CASI-2 - Compact Airborne Spectrographic Imager-2
Chl - Chlorophyll
DAS - Days after sowing
DAP - Days after planting
DAE - Days after emergence
DMSO - Dimethyl sulfoxide
E/O - Earth observation
EMR - Electro magnetic radiation
E-W - East-West
FRF - Far Red Fluorescence
Fe - Iron
g - Gram
g ha^{-1} - Gram per hectare
HPLC - High performance liquid chromatography.
IPVI - Infrared Percentage Vegetation Index
IRS - Indian Remote Sensing Satellite
IST - Indian Standard Time
K - Potassium
kg ha^{-1} - kilogram per hectare
LAI - Leaf area index
LIF - Laser induced fluorescence
LAD - Leaf angle distribution
LIDAR - Light Detection and Ranging
mg ha^{-1} - Milligram per hectare
N - Nitrogen
Nm - Nanometer
NDVI - Normalized Difference Vegetation Index
NIR - Near Infra Red
N-S - North-South
P - Phosphorus
PAR - Photo synthetically active radiation
PRI - Physiological Reflectance Index
PRI - Photochemical Reflectance Index
PVI - Perpendicular vegetation index
R - Red
REIP - Red Edge Inflection Point
RVI - Relative Vegetation Index

S	-	Sulfur
SRRPS	-	Spectral reflectance response patterns
SAVI	-	Soil Adjusted Vegetation Index
SRI	-	Solar Radiation Intercepted
SVI	-	Simple Vegetation Index
UV	-	Ultraviolet
VIS	-	Visible
VI	-	Vegetation indices
WBI	-	Water Bank Index
WDVI	-	Weighted Difference Vegetation Index
Zn	-	Zinc

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Introduction

CHAPTER 1

INTRODUCTION

The remote sensing technique is being used extensively in natural and physical resource inventorying, mapping and monitoring. Effective crop management and yield prediction over a large area would be possible by remote sensing techniques through operational models of crop growth and development. Spectral reflectance properties in different wavelength bands are utilized in crop identification, yield forecasting and crop condition assessment (Baver, 1985). Crop growth, which is usually affected by climatic, and soil conditions can be monitored by spectral observations carried on crop canopy. Remote sensing instrumentation makes it possible to observe the environment with electromagnetic radiation. Sun is the source of all energy requirements of earth objects for growth and sustenance. The electromagnetic radiation (EMR) from Sun comes in all wavelengths, but major portion in the visible spectrum of the electromagnetic radiation reaches earth after going through the ionosphere. This in turn interacts with matter and part of it is absorbed, some reflected and some transmitted. In remote sensing the reflected radiations from the objects are of utmost importance. The various sensors record the reflected radiation information in all the wavelengths or in selective windows of the visible and near infrared wavelengths. The system that measures such information is known as multi-spectral radiometers. The information obtained on the basis of frequency or wavelength, reflective or emissivity properties is known as spectral characteristics. This spectral characteristic is the way to identify, monitor or predict the behaviour of target to which EMR interacts. The knowledge of interaction of electromagnetic energy with individual leaves contributes to the understanding of the processes involved and thus will help in the development of various spectral indices. These indices provide the information on various stresses observed by crop plant. Vegetation indices derived from remote sensing imagery are currently being used to monitor drought conditions, plant disease out break and growth of crop on a real time basis; often helping the decision makers to initiate appropriate strategies to achieve sustained food production through changing cropping patterns and agricultural practices.

Every object on the earth's surface yield distinctive "Finger Prints" called "spectral signature". Scientific remote sensing is based on the recording of the spectral signatures. Spectral reflectance of crops is a basic prerequisite for monitoring crop growth and evaluating its productivity. Therefore knowledge of leaf canopy spectral response, affected by the environmental and cultural factors, which differ due to crop growth and development, is important one. The spectral quality and intensity of crop reflectance and emittance depends on different factors such as leaf morphology and pigmentation, canopy geometry, crop maturity, soil background, canopy cover, management and cultural practices and weather. Leaf reflectance is important stress indicator of water, nutrient and disease and pests (Patil *et al.,* 2007). Nutrient and moisture availability affect the optical and radiation properties of plant. The limitation of water supply and nutrient deficiencies in plants affects the colour, moisture content, total metabolites and internal structure of leaves and as a result of which their reflecting power also changes (Ajay *et al.*, 1983, Das *et al.*, 1992 and Patil *et al.,* 2007). Difference in leaf area index are useful for spectrally discriminating healthy crop canopies from stressed crop canopies (Knipling, 1970) . Spectral parameters have been used to estimate important crop canopy variables such as LAI (Leaf Area Index), biomass and chlorophyll content of a crop species (Ajay *et al.*, 1983; Kamat *et al.*, 1983; Duncan *et al.,* 1993; Verma *et al.*, 2002; Kolte, 2002). Plant pigments are of tremendous significance in the biosphere. Indeed, it is argued that the chlorophylls are earth's most important organic molecules as they are necessary for photosynthesis, the carotenoids are essential for plant and mammal survival through their photosynthetic and nutritional functions, while other pigment groups are key to the physiology of plants and the organisms with which they interact (Davies, 2004).

There is relationship between plant pigments with spectral reflectance. Primarily plant pigments, like chlorophyll and carotenoids, influence spectral reflectance of leaves. Such reflectance can be used to study the changes in chlorophyll content, which is the primary pigment of leaves and stimulates photosynthesis. The primary pigments chlorophyll, carotene and xanthophylls absorb incident light for photosynthesis. The chlorophyll content of leaf tissue is different for different crop species, at various growth stages of the crop plant and crop grown under varied fertility level soils. The spectral index NDVI (Normalized Difference Vegetation Index) is linearly related to total chlorophyll content and growth stages of a crop up to flowering /grand growth stage. Nutrients, specially N, S, Fe and Zn are involved in chlorophyll synthesis. Further these nutrients are widely deficient in the soil of Maharashtra, in general and Marathwada in particular. Hence, in the presence study these nutrients are included to find out its effect on plant pigments. Light reflected from soil or plant surfaces that have a characteristic frequency and energy. The yellow green colour that associate with nitrogen and other nutrient deficiencies is characterized by having more violet light absorbed by the plant material or alternatively. The intensity of green in plant is characterized by the amount of red light absorbed. Plant leaf greenness can be related to plant leaf chlorophyll content and chlorophyll is directly related to plant nitrogen, sulfur, and iron content. What is actually being measured in sensor-based system is spectral radiance reflected from plant surfaces. This is outcome of stress and strain of the soil and climate observed by plant species.

As stated above soils of Marathwada, which are largely deficient in N, S, Zn and Fe nutrients are mainly responsible for greenness of crop. Amongst all nutrients, Nitrogen is a major nutrient responsible for greenness as its deficiency is wide spread. Most of the spectral studies on nutrient deficiency have therefore been concentrated on spectral response in relation to nitrogen supply. In nitrogen deficient crop because of low chlorophyll content, the red reflectance is much higher, while it is lower in the infrared region (Ajay *et al.,* 1984 and Patil *et al.*, 2004). Vegetation indices based on Red and IR reflectance have been developed as spectral parameters for distinguishing crop canopy under fertilized and nutrient stressed condition. The frequently used spectral indices for monitoring the plant health and vigor are Relative Vegetation Index (Jorden.1969), Normalized Difference Vegetation Index (Rouse *et al.,* 1973), Soil Adjusted Vegetation Index (Huete, 1988) and Infrared Percentage Vegetation Index (Crippen, 1990). Much lower values of NIR/ Red reflectance ratio and the Normalized Difference Vegetation Indices for N deficient wheat crop were reported as compared to that of nitrogen fertilized by Ajay *et al.,* 1984; Das *et al.,* 1985; Rao *et al.,* 1997. Further Patil *et al.,* 2003 also observed that physiological changes in cotton crop canopies like Reduced LAI, Chlorophyll content and biomass caused by N deficiency resulted in detectable spectral reflectance variation. Use of spectral data in nutrient deficiency detection not only help in delineating the nutrient stress areas but also helpful in recommending the corrective measures in time.

Solar radiation that striking the surface of a plant is reflected in wavelengths that have a characteristic frequently and energy. Only a portion of the incident light energy is reflected from the leaf. The remainder is either absorbed or transmitted. In the visible spectrum region, radiation is absorbed by leaf pigments, primarily chlorophyll, carotenoids, including xanthophyils and anthocyanins also affect absorption (Gates *et al.,* 1965). Variations in pigment levels within the plant cause different amounts of radiant energy absorption and reflection. It is these differences in reflectivity that are determined by optical sensing to provide indirect, real time measurements of plant status.

The most widely used vegetation index applied in optical sensor data has been the NDVI (Rouse *et al.,* 1973), in addition to SAVI, RVI and IPVI. These indices rely on the spectral contrast between the NIR and R region of the spectrum. Leaf reflectance is believed to have a strong relationship with leaf pigment concentration (Mass and Dunlap, 1989: Patil and Kolte 2003, Patil *et al.*, 2007, Patil *et al.*, 2008). Several studies have attempted to optically sense chlorophyll levels to determine plant stress (Litchtenthaler, 1996 and Patil *et al.*, 2007). During stressful periods, plant exhibits a measurable decrease in chlorophyll known as chlorosis (Litchtenthaler *et al.,* 1996). It is evident, however, that carotenoids including xanthophylls, also have very important functions in the process of photosynthesis. The ability to sense all pigments simultaneously may eventually provide a better plant health indicator than chlorophylls alone.

Going through the above paragraphs it is observed from strewed literature that spectral reflectance in plant is not only influenced by chlorophyll content but in addition to this other pigments like carotenoids, anthocyanins, lycopene, Xanthophylls also contribute (nearly 30 to 33%) to spectral reflectance. Therefore, the main goal in this study was to find out "Relationship between chlorophyll and other pigments and spectral reflectance in various plant species under different fertility levels", using maize as a test crop with the following coined objectives.

1. To study the effect of plant nutrients on growth attributes and pigment synthesis in maize.
2. To find out the relationship between plant pigments and spectral reflectance in different spectral bands and ratios in selected plant species.
3. To study the relative contribution of plant pigments towards the spectral reflectance.

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

The canopy reflectance is a function of leaf structure and pigments concentration of leaves. Radiation is one of the most important physical parameter which can be studied to predict the yield of the crop and other natural resources of earth surface. The chlorophyll concentration contributes 70 percent towards spectral reflectance. The rest of the spectral reflectance is occupied by other plant pigments like anthocyanin, lycopene, B-carotene etc. However strewed literature is available on this aspect. Therefore the observations documented on this aspect and associated areas of spectral parameters are reviewed from various sources like CAB Abstracts, Internet, Periodical, Books, Journals, Personal Communications, etc.

The reviewed literature is presented in this chapter under following sub heads.

2.1 Effect of plant nutrients on LAI and biomass production and spectral parameter of crop species

2.2 Effect of plant nutrients on plant pigments and spectral reflectance

2.3 Spectral reflectance studies in relation to plant pigments

2.1 Effect of plant nutrients on LAI and biomass production of crop species

2.1.1 Effect of plant nutrients on Leaf Area Index

Effect of nitrogen application on plant height was studied by Sekhon (1969) and revealed that the height of soybean was increased due to nitrogen application. Similarly, Sharma and Dixit (1987) observed that application of nitrogen caused significant increase in growth and yield of soybean. Shivay and Singh (2000) further reported that plant height of maize increased significantly with increasing doses of N applied.

Weigand *et al.,* (1974) for the first time related spectral data to LAI (Leaf Area Index). They concluded that LANDSAT (MSS-7-MSS-5) and (MSS-5/MSS-7) in combination with MSS-7 and MSS-6 bands were suitable, in practice, as indicators of soil cover and plant density, simulations by Bunnik (1978,1981) show this kind of Index are suitable for estimating the degree of cover, but slightly sensitive to variations of LAI after the soil is completely covered.

Daughtry *et al.,* (1982) developed method for combining spectral and meteorological data in crop yield models that capable of providing accurate estimates of crop condition and yield. Reflectance data were acquired with a Landsat and radiometer throughout two growing seasons for corn (*Zea mays* L.) canopies differing in planting dates, populations and soil types (Typic agriaquoll and Udolic orchraqualf). The spectral variable greenness was associated with 76 per cent of variation in LAI over all treatments. Single observations of LAI and greenness were found to have limited value in predicting corn yields. The proportions of Solar Radiation Intercepted (SRI) by these canopies were estimated using either measured LAI or greenness. Both estimates, when accumulated over the growing season, accounted for approximately 65 per cent of the variations in yields. They concluded that solar radiation using spectral data represents a viable approach for merging spectral and meteorological data in crop yield models.

Field experiment conducted during 1978-79 under four N treatment levels revealed that red reflectance was increased and NIR reflectance was decreased from N deprived canopies. The results confirm the potential of remote sensing for monitoring the growth and development of crops (Walburg *et al.,* 1982).

Leaf area index (LAI) and leaf angle distribution (LAD) are considered to be the most useful parameters for the characterization of canopy structure, defined as the spatial arrangement of the above ground organs of plants in a plant community. LAI is the total one sided area of leaves above unit area of soil (Campbell and Norman, 1989).

Costa (1991) studied at the Department of Plant Sciences University of Passo Fundo and observed that increase in N nutrient in growing media, increased leaf chlorophyll content, dry matter yield and leaf area. The N effect on chlorophyll content was evident only after 28 days old plants. Leaf area was highly correlated with both chlorophyll content and dry matter.

The total chlorophyll is found to be positively correlated with leaf area index (LAI), leaf nitrogen and grain yield and the ratio analysis of reflectance spectra have shown a very good agreement between measured pigments and the calculated pigments using reflectance values (Chappelle and Kim, 1992).

Sharma and Thakur (1995) reported that average height of maize was found to be increased with the rate of nitrogen application and was highest when applied in the 3 splits. Further they observed that nitrogen deficiencies delayed both vegetative and reproductive phonological development and slightly reduced, leaf emergence rate, and strongly diminished leaf expansion rate and leaf area. Nitrogen deficiencies reduced radiation interception as much as radiation use efficiency and their effects on the ear dry matter /total dry matter ratio at harvest were associated with crop growth rate reduction at flowering.

Carisan and Riply (1997) used a simple radioactive transfer model with vegetation, soil and atmospheric components to show how the Normalized Difference Vegetation Index (NDVI), Leaf Area Index (LAI) and fractional vegetation cover are dependent.

Martorana *et al.,* (1997) studied the relationship between N rate and chlorophyll 'a' and 'b' content in leaves of maize in pots in green house. Nitrogen fertilizer increased above ground dry matter production, total chlorophyll content and leaf area, throughout the growing season. Light absorption increased and transmittance decreased throughout the growing season and effect were magnified by N fertilizer. Light absorption was positively correlated with increase in LAI and total chlorophyll content. Light use efficiency increased throughout the growing season and increased with increasing N rate.

Reiad *et al.,* (1997) studied the responses of maize, to different nitrogen rates at Al-Hassa, Saudi Arabia during 1995-1996 seasons. The effect of nitrogen levels showed that maize plants surpassed other two species in plant height, number of leaves and its area : Plant, leaf: stem ratio on dry weight basis, fresh and dry yield of leaves, stem and whole plant. The same response for the measured characters was obtained as nitrogen rates increased with higher measures at 300 Kg N/ha.

Spectral indices and cumulative stress degree days were found to be significantly correlated with leaf Area Index (LAI), dry matter production and grain yield. The results indicated that nitrogen levels manifested changes in growth behaviour of wheat crop, which could be detected through spectral indices and canopy temperature (Das *et al.,* 2000).

Patil and Mali (2000) surveyed the oilseed dominating area of Parbhani and Latur district and reported that Entisol of Parbhani and Latur districts were low in sulfur. On an average 27 % soils were found sulfur deficient. Further the field trials conducted on farmers field using soybean, summer groundnut , sunflower and safflower with four levels of sulfur that are 0,15,30,45,S kg ha^{-1} observed the response to sulfur application. Application of 30 and 45 kg S ha^{-1} were at par and significantly superior over 0 and 15 kg S ha^{-1}.

Application of nitrogen up to 120 Kg/ha significantly increased plant height, dry matter, LAI, chlorophyll content, cobs/plant, grains/rows/cob, weight/cob, shelling percent, test weight and biological yields of the maize. The significant improvement in growth characters might be attributed to the fact that nitrogen helps in maintaining higher N contain level which might have resulted in better plant height, LAI and presumably chlorophyll content of the leaves of maize (Vyas and Singh, 2000).

Patil *et al.,* (2001) studied the leaf area index under the different moisture, plant protection and fertility levels and reported that cotton with irrigation sowed higher LAI as compared to rainfed upto 120 DAS. There after the variation was narrowed down. Protecting the cotton crop against pest and diseases resulted in higher LAI over unprotected cotton crops. The average LAI in protected plant was ranged between 0.80 to 2.06, where as in case of unprotected plant, it was between 0.5 to 1.13. The cotton crop with recommended N and P (100 Kg N and 50 Kg $P_2 0_5 ha^{-1}$) increased LAI. The next best treatment was application of only N followed by application of only P.

Andersen *et al.,* (2002) showed that remotely sensed precipitation from METEOSAT data and leaf area index (LAI) from NOAA, AVHRR data is used as input data to the distributed hydrological modeling of three sub catchments (82,000 km) in the Senegal River Basin. Further, root depths of annual vegetation are related to the temporal and spatial variation of LAI. The modeling results are compared with results based on conventional input of precipitation and vegetation characteristics. The introduction of remotely sensed LAI shows improvements in the simulated hydrographs, a marked change in the relative proportions of actual evapotranspiration comprising canopy evaporation, soil evaporation and transpiration, while no clear trend in the spatial pattern could be found. The remotely sensed precipitation resulted in similar model performance with respect to the simulated hydrographs as with the conventional rain gauge input. A simple merging of the two inputs did not result in any improvement.

Hong *et al.,* (2003) studied that the leaf area index (LAI) was estimated as a function of image-derived vegetation indices, and derived data was used to improve site-specific model – based estimation of crop growth and yield across an entire field in Centralia, Missouri, USA. The first research phase, reported herein, addressed the estimation of LAI based on vegetation indices and discusses the various methods for integration of the derived values with crop growth models. Remote sensing data were used to quantify biophysical variability at several within-field monitoring sites for maize (*Zea mays*) and soybean (*Glycine max*). Hyper and multi-spectral images at varying spatial and spectral resolutions were acquired using airborne and satellite platforms to investigate the relationship of spectral signatures and their ratio to LAI for two central Missouri experimental fields under a maize – soybean rotation. Measured LAI could be expressed as a function of image derived vegetation indices such as NDVI, RVI and SAVI both for maize and soybean. Vegetation indices overestimated LAI in early growth stages and underestimated LAI after the grain or pod filling stage. CERES- Maize and CROPGRO-Soybean models were used to simulate site-specific crop growth and development. The CERES-Maize Model over predicted LAI at all monitoring sites. The CROPGRO-Soybean model generally showed good agreement between simulated and observed LAI. Various methodologies for integrating remote sensing into crop models for estimating in field variation were discussed.

Patil (2003) found from the project on spectral and physiological measurements of cotton, wheat and Soybean canopies under normal and stress conditions protecting the crop against the pest and diseases and irrigating the crop as and when required showed higher values of biometric and spectral parameters than unprotected and rainfed crop.

Adequate levels of N fertilizer during early growth appear essential by the maize plant and may affect the ability of maize plants to resist insect predation (Bruns and Abel, 2003).

Petkar (2004) in her post graduate research work deficiency and growth assessment of maize (*Zea myas* L.) under varying levels of nitrogen by remote sensing observed that the various growth parameters of maize viz. height, LAI (Leaf area index) and total biomass and physiological parameters like total chlorophyll, chlorophyll "a", chlorophyll "b" and leaf N concentration were improved significantly due to application of graded doses of nitrogen. The spectral indices viz., RVI, NDVI, IPVI, SAVI found suitable for assessment of growth and vegetation vigour. However, SAVI had shown limitation indiscrimination of growth of crop due to the N levels. RVI and NDVI established significant correlation with growth parameters like LAI, biomass, chlorophyll and leaf N concentration. RVI found better index for assessment of chlorophyll and leaf N concentration.

Raffaele *et al.,* (2005) reported that a non conventional approach for the estimation of leaf area index (LAI) and leaf angle distribution (LAD), based on the use of information contained in multi angular images and the inversion of a canopy ray tracing model is proposed in this work. As an alternative to the use of overall image reflectance data, the image fraction components i.e. sunlight and shaded leaves and soil, are obtained by supervised clarification of ground based multi-angular images acquired using an inexpensive colour infrared camera, the Dycam ADC, these data are used for the inversion of a developed using the pov-ray tracer.

Ashtikar *et al.,* (2006) in her post graduate research on monitoring on growth profile and yield estimation of soybean under various fertilization levels by remote sensing reported that the maximum growth of soybean was noticed during 45 to 65 DAS . Total chlorophyll content was highest at 65 DAS with the application of N60P120S60treatment. The effect of application of N,P and S on growth parameters was identified by using RVI with increase in LAI, Chlorophyll and leaf N,P and S concentration. RVI NDVI and SAVI found significantly correlated with chlorophyll, LAI, biomass and yield

The results emerged out of the studies on spectral reflectance under normal and nitrogen and phosphorus stress condition in soybean (*Glycine max* L.) conducted at Marathwada Agricultural University experimental farm, Parbhani during *Kharif* 2004-05 showed that crop growth and bio-physiological parameters viz. Height, chlorophyll, leaf area index and total biomass influenced by pest and disease and nutrient stress resulted in detectable spectral reflectance variation. Poor crop growth, reduced canopy cover, chlorophyll content and biomass production are the effects observed in nutrient deficient crops. These above changes in soybean crop were related to spectral indices (RVI and NDVI) that are resulted in to discrimination of stressed and normal (nor stressed) soybean crop (Patil *et al.,* 2007).

2.1.2 Effect of plant nutrients on biomass production

Ajay *et al.,* (1983) studied the spectral assessment of LAI, chlorophyll content and biomass of the chickpea in the field experiments conducted at Indian Agricultural Research Institute, New Delhi and observed that spectral parameters can be used to estimate the chlorophyll content, LAI and biomass of wheat and chickpea crop.

Spectral reflectance data from the field of wheat (*Triticum aestivun* Linn. emend. Thell) collected during the growing seasons were correlated with its yield. Infra red/red reflectance ratio and the normalized difference vegetation index were found highly and linearly correlated with total dry matter production and grain yield. The correlation was maximum during a period of 40 to 55 days after sowing (Kamat *et al.,* 1983).

Das *et al.,* (1989) developed prediction models for wheat to assess crop growth in terms of leaf area index, dry matter production and grain yield from remotely sensed temperature and spectral indices. The cumulative stress degree days for the period of flowering to grain formation stage showed significant correlation with dry matter ($r = 0.940$) and grain yield ($r = 0.939$).

Casanova *et al.,* (1998) calculated vegetation indices, perpendicular vegetation index (PVI), normalized difference vegetation index (NDVI), weighted difference vegetation index (WDVI) and relative vegetation index (RVI) from rice crop reflectance. They observed that estimation of biomass from remote sensing data appear more reliable than estimation of LAI.

Semebiring *et al.,* (1998) identified the optimum wavelength for detection of N and P status in winter wheat and observed that NDVI (NIR-R/NIR+R) and RVI (NIR/R) ratio were good indices to predict biomass and N and P uptake by wheat crop.

Krishnan *et al.,* (1998) used ground - based remote sensing techniques for discriminating *kharif* (Monsoon) rice cultivars. The crop biometrics like LAI, chlorophyll content, leaf nitrogen content and biomass production were responsible for the cultivar discrimination. The discrimination was well pronounced in red and infrared bands and also in vegetation Indices like IR/R and IR-R/IR+R.

Serrano *et al.,* (2000) observed that vegetation indices derived from reflectance data are related to canopy variables such as above ground biomass, leaf area index (LAI), and the fraction of intercepted symptoms. They also derived the indices from spectra obtained from high spectral and spatial resolution Compact Airborne Spectrographic Imager 2 (CASI-2) imagery to determine if reasonable estimates at a scale of < 1 m can be achieved. One of the indices (R 850/R 710 index, where R is reflectance) derived from hand-held spectro-radiometer data showed a moderate correlation with relative leaf chlorophyll content ($r = 0.59$, $P < 0.05$) for all dominant eucalypt species in the study area. The R 850/R 710 index derived from CASI-2 imagery yielded slightly lower correlations over the entire data set ($r = 0.42$, $P < 0.05$), but correlations for individual species were high ($r = 0.77$, $P < 0.05$). A scaling analysis indicated that the R 850/R 710 index was strongly affected by soil and water cover types when pixels were mixed, but appeared to be invariant to changes in proportions of understudy, which may limit its application.

Senay *et al.*, (2000) analyzed digital images of a maize and soybean site in Ohio acquired several times during the growing season of 1994 using a multi-spectral scanner mounted on an aircraft. The goal of this study was to evaluate the use of these high spatial resolution (1 m) data to identify maize and soybean crops at various growth stages. Maximum distinction between maize and soybeans was achieved using the near- infrared bands when the crops were mature, while the visible bands were more useful when the soybeans were senescing. Spectral class differences were related to leaf nitrogen, soil water content, soil organic matter and plant biomass. An approach is presented for identifying maize and soybean crops where little or no reference data are available. The approach is based on the red and near-infrared bands and using the Simple Vegetation Index or the Normalized Difference Vegetation Index

Gole (2003) in his post graduate research work on studies on spectral reflectance under normal nitrogen- Phosphorus stress condition in soybean (*Glycine max* L.) of Marathwada Agriculture University, Parbhani crop observed that the growth parameters and physiological parameters viz. height, chlorophyll, leaf area index and total biomass influenced by pest and disease stress and nutrient stress resulted in detectable reflectance variation through RVI and NDVI. Poor crop growth reduced canopy cover, chlorophyll content and biomass production are the effects observed in nutrient deficient crop canopies. These changes in soybean crop were related to spectral indices (RVI and NDVI) that are resulted into discrimination of stressed and normal soybean crop.

Dhoble *et al.*, (2004) reported the spectral behaviour of wheat under varying levels of nitrogen at MAU, Parbhani Maharashtra. Growth of wheat was monitored by collecting data on LAI, chlorophyll content and total biomass. It was observed that protecting the crop against, pest and diseases and irrigating the crop as and when required, showed higher biometric and spectral parameters than unprotected and rainfed crop. Physiological change in wheat crop canopies caused by nitrogen deficiency resulted in detectable spectral reflectance variation.

2.2 Effect of plant nutrients on plant pigments and spectral reflectance

Gausman *et al.,* (1973) studied the chlorophyll and carotenoids interaction was highly significant. Nitrogen deficiency reduced the chlorophyll concentration of leaves and unmasked the carotenoids pigments. The carotenoids content of the leaves also decreased as N became limiting, but apparently at a slower rate than chlorophyll.

Elvidge (1990) showed that the ranking orders of reflectance or absorption spectra at wavelength band 680 nm coincided with the ranking of N fertilization set up on the calibration plots, which suggest that differences in N fertilizer rates, and thus the chlorophyll concentration, were responsible for these ranking orders.

Palani and Shanthi (1992) stated that chlorophyll concentration was generally increased by recommended N rates, while in 40 days old plants a high rate of urea decreased plant chlorophyll. While Sharma *et al.,* (1993) studied the *Mung bean* cv. ML-5 were intercropped with maize cv. Pratap. Four sowing patterns were tested under two row directions (E-W and N-S) and 3 rate (80, 120 or 160 Kg Nha^{-1}). *Mung bean* chlorophyll a, b and total chlorophyll content were greatest with 120 Kg N, whereas LAI and seed yield were greatest with 160 and 120 Kg N in 1982 and 1983, respectively. PAR interception and protein content found to be increased with increase N rate. Further, Choubey and Choubey (1999) showed that the period between 66 to 70 days after planting was most suitable for assessment of rice crop yield, based on chlorophyll 'a' concentration.

Ercoli *et al.,* (1993) concluded that N and chlorophyll content of field grown maize leaves can be quickly estimated from measurements of absorbance / transmittance, but not of reflectance, especially when the contents of these constituent are medium to high. The 520-600 nm wave band gave a better estimate of N and chlorophyll concentrations than the other 2 bands.

Patil and Malewar (1994) studied the effect of zinc levels on different genotypes of wheat and reported that chlorophyll content was maximum between 55 to 60 DAS in wheat crop.

Filella *et al.* (1995) indicated that leaf chlorophyll 'a' content in mainly determined by N availability. They found improved correlation between measured chlorophyll 'a' content and leaf reflectance at 550 and 680 nm, whereas, Gamon *et al.* (1992) used a physiological Reflectance Index (PRI) to track diurnal changes in photon efficiency (CO2 uptake / absorbed photo-synthetically active radiation = Rref = R531 / Rref + R531, where Rref was a reference wavelength and R531 represented spectral radiance at 531 nm.

Schepers *et al.,* (1996) concluded that in greenhouse experiments analytical and optical methods to monitor crop N status were evaluated with maize Pioneer hybrids 3398 and 3379 at silking growth stage. A portable chlorophyll meter was used to measure chlorophyll content of leaves by means of transmittance measurements. Leaf N concentration and chlorophyll meter readings were positively correlated, but were also affected by water stress and hybrid differences. Water stress decreased chlorophyll meter readings but increased leaf N content and diffusive resistance. Nitrogen stress decreased leaf N concentration, chlorophyll meter readings, and diffusive resistance. Both water and N stresses affected crop reflectance measurements. Reflectance values in the green and near IR portions of the spectrum were inversely related to crop N status. Water stress increased reflectance in red, green, and near IR wavelengths. Water stress by N status interaction was significant for chlorophyll meter readings as well as reflectance measurements. Both leaf reflectance and chlorophyll meter measurements provided a good indication of N status for adequately watered plants, but the relationships were poor for plants grown under prolonged water stress.

Rundguist *et al.*, (1996) measured algal chlorophyll concentrations in surface waters, this provides hyper spectral signatures, in the visible and near infrared, associated with two experiments conducted out doors in large water tanks. One involving relatively high amounts over a wide range. The principle finding was that the commonly used near infrared / red ratio is best for estimating pigment amount when the concentration of chlorophyll is relatively low, and the first derivative of reflectance around 690 um in best when the concentration is relatively high.

Gitelson and Merzlyak (1997) formulated indices for the nondestructive estimation of chlorophyll content using various instruments to measure reflectance and absorption spectra in visible and near infrared ranges, as well as chlorophyll contents from several non related species from different climatic regions. The proposed new algorithms are simple ratios between percentage reflectance at spectral regions that are highly sensitive (540 to 630 um and around 700 um) and insensitive (near infrared) to variations in chlorophyll content (R NIR / R 700 and R NIR / R 556). The algorithms developed for predicting leaf chemistry from the leaf optics were validated for 9 plant species with a range of chlorophyll contents from 90.27 to 62 um $cm^{-2.}$ The use of green and red (near 700 um) channels increases the sensitivity of NDVI to chlorophyll content by about 5 fold.

Blackburn (1998) investigated the effectiveness of a range of hyper spectral approaches for estimating the concentrations of chlorophyll 'a', chlorophyll 'b' and carotenoids at the plant canopy and leaf scales. The resulted indicated that narrow band reflectance indices can be developed, such as the pigment specific simple ratio, which have extremely strong relationships with the concentration per unit area of individual pigments at the canopy scale. Spectral derivative approaches, particularly these base don pseudo absorbance (log 1/R), are also closely related to canopy pigment concentration per unit area but are more useful for deriving estimates of the concentration per unit mass of photosynthetic pigments at both canopy and leaf scales.

Shadchina *et al.,* (1998) found the correlation between chlorophyll and leaf nitrogen contents. Leaf chlorophyll content, leaf nitrogen contents and grain yields were closely correlated and during grain ripening, these correlations were cultivar specific and were maintained although leaf chlorophyll and nitrogen contents were declining.

Datt (1999) studied the visible / near infrared reflectance properties of leaves from 21 *Eucalyptus spp.* To determine appropriate indices for remote sensing of chlorophyll content. A scatter correction technique was applied to reflectance spectra, which gave improved calibrations for the estimation of chlorophyll content. Reflectance near 710 un wavelength showed maximum sensitivity to chlorophyll content with reflectance near 550 um a less sensitive indicator. Among several reflectance indices tested, the ratio (R 850-680) or (R 850 – 680) performed best and is proposed as new index for the remote estimation of chlorophyll content in higher plant. From the first derivatives of reflectance, the ratio D1 (754) / D1 (704) and the wavelength position of the red edge were best correlated with chlorophyll content. The ratio D2 (712) / D2 (688) from the second derivatives of reflectance was also and equally good indicator chlorophyll content.

Gitelson *et al.*, (1999) presented a data on remote sensing technique to estimate the chlorophyll content in higher plants. The ratio between chlorophyll fluorescence at 735 um and in the range 700 - 714 um. F 735 / F 700 (measured in detached intact leaves using a spectrofluorometer), was found to be linearly proportional to the chlorophyll content and thus this ratio can be used as a precise indicator of chlorophyll content in plant leaves. This new chlorophyll fluorescence ratio indicates chlorophyll levels with high precision.

Samson *et al.*, (2000) found that the capability of laser induced fluorescence (LIF) to discriminate between nitrogen and sulfur deficiencies in corn that result in similar effects on leaf colour, and to determine the relationship between changes in LIF emission spectra and plant growth inhibition. Corn plants were grown in the greenhouse and fertilized for 35 days with nutrient solutions of different nitrogen (N) and sulfur (S) concentrations. Every 6 to 8 days during the experiment, fluorescence data were remotely obtained using an FLS-PL compact multi-wavelength fluorescence LIDAR developed by our group. Excitation wavelengths varied from the UV to the VIS spectral range over the measurements cycle. The corresponding fluorescence spectra were recorded at each laser pulse with a CCD-based multi-channel detector. On the same day as the fluorescence measurements were made, the leaf reflectance was measured and leaf samples were collected for the N, S and chlorophyll {a+ b} Chl{a+b} content analysis.

N deficiency caused a significant decrease in the accumulation of plant biomass and the Chl{a+b} leaf contents after 6 days of treatments, whereas S deficiency led to significant decrease in Chl{a+b} leaf contents and the accumulation of plant biomass after 6 and 18 days of treatments, respectively. The losses of Chl{a+b} in N- and S- deficient plants were similarly correlated to the changes in the R 740 / R 540 reflectance ratio and in the F 690 / F 740 fluorescence ratio (the numbers shown are the corresponding wavelengths at which fluorescence intensities were measured). The R 740 / R 540 and F 690 / F 740 ratios proved useful for the detection of decreases in the leaf Chl{a+b} contents but they could not discriminate between N and S deficiencies. On the other hand, throughout the experiment we observed large differences between the effects of N and S deficiencies on LIF emission spectra. Only the N deficiency induced changes, which could be related to decreased transmittance of the UV radiation through the leaf epidermis. The value of this transmittance was estimated using ratios of the far-red fluorescence intensities induced by 360 nm- and 440 nm-laser pulses (FRF ex360/FRF ex440).The FRF ex 360 / FRF ex 440 ratio correlated linearly ($R^2 = 0.93$) with the leaf nitrogen content. These results demonstrate that LIF offers potential for early nutrient stress discrimination.

Heege (2001) studied the signals of crop N obtained by the use of a ground based sensor, measuring changes in the green color of crops, preferably identified by means of the reflectance of red and near infrared. This is most likely due to changes in the chlorophyll content of the plant, which is most often attributed to the N supply of the crop, and thus claimed to be suitable information for the design of N dressing by commercial approaches (Hydro-N-sensor, Miles Opti-Crop Division, Owensboro, KY).

Sadhu and Chattopadhyay (2001) stated that specific absorption rate of N, K and Fe were consistently higher under nitrate nitrogen on the other hand, the specific absorption rate of P was less affected by ammonium form of N. The root-shoot data revealed that under both forms of N, the proportion of N in the shoot steadily declined with time. The total chlorophyll concentration was initially higher with ammonium than obtained under nitrate nutrition due to both high chlorophyll 'a' and 'b' concentration. Nitrogen use efficiency was highest under ammonium nutrition.

A field experiment on detection of nutrient deficiencies in soybean (Glycine max L.) crop by remote sensing was conducted at Departmental farm of Marathwada Agricultural University, Parbhani, during monsoon 2001-02 by Kolte, 2002 to find out the feasibility of nutrient deficiency diagnosis by studying the spectral reflectance. There were sixteen treatment combinations of four main plot treatments (two moisture levels – no irrigation and irrigation and two plant protection levels – no plant protection and plant protection) and four sub plot treatments of fertility levels viz; No, N and P, N @ 30 Kg N ha^{-1}, P @ 60 Kg P_2O_5 ha^{-1} and N + P. The experiment was laid out in split plot design with three replication. Spectral reflectance in four bands i.e. visible blue green (0.45 – 0.52 μm), visible green (0.52-0.59 μm), visible red (0.62-9.68 μm) and near infra red (0.77-0.68 μm) were recorded between 10:30 to 13:30 hrs IST in direct sunlight by multi band radiometer. The physiological changes in canopies (Leaf area, biomass and chlorophyll content of leaves) caused by irrigation, plant protection and N+P deficiency resulted in detectable reflectance variation.

Reduced LAI, chlorophyll content and biomass were among the effects seen in N, P and N + P deficient canopies. RVI and NDVI ratios found better in discriminating stressed soybean and normal soybean crop. RVI established positive relationship with total biomass and LAI.

Han *et al.,* (2002) showed that in season site-specific nitrogen application has the potential to improve the efficiency of nitrogen use and reduce environmental contamination. Corn nitrogen stress is frequently associated with leaf chlorophyll content, which can be characterized by spectral reflectance measurements. To evaluate the use of satellite imagery to detect nitrogen deficiency in corn during the growing season, the study compared spectral variables extracted from SPOT satellite imagery and digital aerial imagery, and investigated the relationship between spectral variables of the SPOT imagery and the measurement data of a hand-held chlorophyll meter (or SPAD meter). The correlation coefficient (r) between the NDVIs derived from the SPOT image and the aerial image was 0.73 in 1999 and 0.54 in 2000, respectively. When the field had large spatial variability in crop development in 1999, the spectral variables from the SPOT image were strongly correlated with those from the aerial image. However, when the crop development was more uniform in 2000, the correlation between the SPOT image and the aerial image was not as good. The limited range of available digital counts that can be used to represent the reflectance from an individual farm field for the SPOT system limited its sensitivity in detecting crop stress. Nevertheless, the NDVIs from SPOT images were significantly correlated with SPAD data in both fields, with correlation coefficients of $r = 0.90$ in 1999 and $r = 0.68$ in 2000. The correlation between SPOT images and SPAD data was similar to that between aerial images and SPAD data, indicating that SPOT imagery may have potential for detecting chlorophyll levels and nitrogen stress in corn during the growing season.

Zhao *et al.,* (2003) in an experiment in sunlit, controlled environment chambers in the 2001 growing season to determine responses of corn (*Zea mays* L.cv.22A14) growth and leaf hyper spectral reflectance properties to varying N supply. Four N treatments were: (1) half-strength Hoagland's nutrient solution applied throughout the experiment (control); (2) 20% of control N starting 15 days after emergence (DAE); (3) 0% N starting 15 DAE; and (4) 0% N starting 23 DAE (0% NL). Plant height, the number of leaves, and leaf lengths were examined for nine plants per treatment every 3-4 days. Leaf hyper spectral reflectance, concentrations of chlorophyll a, chlorophyll b, and carotenoids, leaf and canopy photosynthesis, leaf area, and leaf N concentration were also determined during the experiment. The various N treatments led to a wide range of N concentrations (11-48 g kg^{-1} DW) in uppermost fully expanded leaves. Nitrogen deficiency suppressed plant growth rate and leaf photosynthesis. At final harvest (42 DAE), plant height, leaf area and shoot biomass were 64-66% of control values for the 20% N treatment, and 46-56% of control values for the 0% N treatment. Nitrogen deficit treatments of 20% N and 0% N (Treatment 3) could be distinguished by changes in leaf spectral reflectance in wavelengths of 552 and 710 nm 7 days after treatment. Leaf reflectance at these two wavebands was negatively correlated with either leaf N (r = - 0.72 and – 0.75**) or chlorophyll (r = 0.60 and -0.72 **) concentrations. In addition, higher correlations were found between leaf N concentration and reflectance ratios. The identified N-specific spectral algorithms may be used for image interpretation and diagnosis of corn N status for site-specific N management.

Patil (2003) investigated that physiological changes cotton crop canopies caused by N and P deficiency resulted in detectable spectral reflectance variation. Reduced LAI, chlorophyll content and biomass were among the effects seen in N and P deficient crop.

Baret *et al.*, (2006) showed that the remote sensing techniques offer a unique solution for mapping stress and monitoring its time-course. The combination of remote sensing observations with crop models provides an elegant solution for stress quantification through assimilation approaches. It fuses several sources of information within our knowledge of the processes involved and accounts for the environmental budget which can be integrated when making decisions about cultural practices. Conclusions are drawn on the issues related to the retrieval of canopy state variables from remote sensing data, to the link between these observables and crop models, and to the assimilation approaches. Avenues for further research are finally discussed along with the required observation system.

Bhavanarayana (2006) in a review reported that the carotenoids pigments are masked by chlorophyll and once chlorophyll breaks down later in the growth period, they become prominent and affect the light reflectance. Young leaves contain less water than mature leaves because immature cells in young leaves are primarily protoplasmic with little vacuolated water storage. The correlation of leaf water content with reflectance is strongest in the near infrared region of the spectrum. Effects of pigments changes and leaf moisture are closely related and sometimes can not be separated. Changes in leaf moisture affect the pigments in the leaf within a very short period of time. As leaves senescence, their light reflection usually increases markedly in the green visible light wavelength region peaking at 0.5 um, because of chlorophyll degradation leaf senescence decreases near infrared light reflectance over the 0.75 to 1.35 um wavelength interval. He further reported that the effects of structural compounds of plant leaves on reflectance are varied. To facilitate interpretation the 0.5 to 2.5 um wavelength interval can be divided into three categories.

1. The 0.5 to 0.75 um visible light absorbance region, dominated by pigments primarily chlorophyll 'a' and 'b' (chlorophyll 'c' occurs in brown algae), carotenes and xanthophylls.
2. The 0.75 to 1.35 um near infrared region of high reflectance and low absorbance affected considerably by internal leaf structure, and
3. 1.35 to 2.5 um region influenced some by leaf structure, but affected greatly by water concentration in the tissue with strong water absorption bands occurring at both 1.45 and 1.95 μ m.

Pandya *et al.,* (2007) suggested that chlorophyll content can be estimated using various methods varying from regression models between red edge position and chlorophyll concentration; chlorophyll fluorescence ratio at 735 nm to 710 nm; or using the combination of spectral vis. Predictive regression equations to map chlorophyll concentration (Chl) from the red edge positions have also been shown. The ratio between chlorophyll fluorescence at 735 nm and 700-710 nm, F 735 / F700 was found to be linearly proportional to the Chl and this ratio can be used as an indicator of Chl in plant leaves. Some spectral vegetation indices (e.g. NIR/Red, OSAVI) minimize background reflectance contribution while other indices (e.g. NIR/G) respond more to leaf chlorophyll concentrations. The ratio of pairs of these spectral vegetations indices was linearly related to leaf chlorophyll concentration over a wide range of foliage cover and background reflectance.

2.3 Spectral reflectance studies in relation to plant pigments

Vesk *et al.* (1966) observed that variation in spectral reflectance among N treatments also resulted from charges in leaf structure and composition, including pigment concentration, cell size and cell wall composition and structure, all of which are altered by N treatment.

Guasman (1982) reported that reflectance in the visible region decreases towards full leaf development, increased reflectance in the visible portion can be attributed to chlorophyll degradation, unmasking of carotenoids pigments in the blue and red bands.

Daughtry *et al.,* (1983) observed that combination of spectral and meteorological data are capable of providing estimates of crop yields. Further, Goetz *et al.,* (1983) reported that the band 1, band 2 and band 3 reflectance decreased by 16.40 , 8.08 and 23.05 per cent, respectively between 30 and 45 DAS in the soybean crop due to chlorophyll absorption at the protective site.

Jackson and Pinter (1986) confirmed under stress conditions plants were found to have a lower NIR reflectance, low NIR reflectance spectral were measured in May at the highly fertilized plot, because excessive N fertilization of 175-300 kg ha^{-1} N resulted in plant lodging.

Jackson *et al.,* (1987) founded that the content of carotenoids has also shown a positive relationship with growth stages of maize, groundnut and soybean crops. Chlorophyll masks the contribution of carotenoids to spectral reflectance. During the process of senescence, the chlorophyll concentration decreases and allows dominance of carotenoids soybean crop has shown an accumulation in carotenoid concentration towards physiological maturity.

Lichtenthaler (1996) investigated that the changes in chlorophyll (a and b), which is the primary pigment of leaves and stimulates photosynthesis are determined by spectral indices like NDVI. Remote detection of chlorophyll content from visible and NIR reflectance by non-destructive means is a good measure to detect stress in plants. Thus remote sensing has potential to revolutionalise the detection and characterization of agricultural phenomena towards condition assessment of crops.

The spectral reflectance of a well developed leaf is dependent on structural fibrous organic matter, water molecules and air spaces. Each of the three features- pigments, structure and water content has an effect on reflectance absorbance and transmittance properties the green leaf. The primary pigments of chlorophyll 'a' chlorophyll 'b' carotene and xanthophylls absorb incident light for photosynthesis, chlorophyll 'a' absorbs at wavelengths of 0.45 and 0.66 um and chlorophyll 'b' absorbs at wavelength of 0.45 and 0.65um. The carotenoids blue and green light at a number wavelengths (Gupta and Badarinath, 1992).

Penuelas *et al.,* (1993) examined a water Bank Index (WBI) defined as R 950 /R900, because the reflectance spectrum was associated with a water absorption band they reported that normalized total pigment to chlorophyll a ratio index was highly correlated with chlorophyll content and was a rough estimate of the ratio of total pigments to chlorophyll a decreasing in healthy plants and rising in stressed or senescing plants.

Curran *et al.,* (1995) showed that crop management purposes the two most important wavelength regions are located in the red (640 to 740 nm), and shoulder of the NIR (760 to 800 nm) where light reflectance is found to be negatively correlated to leaf chlorophyll content at wavelengths near 50 to 860 nm and positively related to the amount of leaf per unit area at wavelengths near 800 nm.

Bausch *et al.* (1996) estimated plant N in irrigated maize using a previously developed N Reflectance Index (NRI) calculated from measured green (G) and near infrared (NIR) canopy reflectance. The field study site considered of continuous plots with four imposed by N fertilizer treatments. Chlorophyll meter measurements, whole plant sampling for N analysis and canopy reflectance measurements were made throughout the 1995 growing season of maize Cv. Pioneer 3790. Meausred plant total N (all leaves) and estimated plant total N based on the NRI compared favourably as long as the soil background was observed. Consequently, the NRI is assumed to be a better indicator of N sufficiency than the N sufficiency index calculated from chlorophyll meter measurements, because a plant community consisting of many plants is monitored instead of a single point on a single maize leaf. Further more, assessment of plant N status by the NRI is rapid and can be easily mapped with GIS tools to show areas that are N deficient.

Naresh Kumar and Singh (1996) reported that the chlorophyll content of leaf tissue is different for different species and growth stages of plant. The content of total chlorophyll increased by 40.61 per cent and 24.07 per cent for maize and groundnut respectively up to flowering (60 DAS) and declined gradually towards physiological maturity. The duration of the three crops are different and maize and groundnut are grown upto 105 DAS while soybean is grown upto 75 DAS. The content of total chlorophyll was maximum at tassel formation stage (60 DAS) in maize.

Choubey and Choubey (1999) reported that relations among spectral reflectance, chlorophyll 'a' and growth of rice plants grown on irrigated course-textured soil in a semiarid region of India. There was a linear relation between spectral reflectance and rice plant height (r-0.97), for band 1 (0.45 – 0.52 μm) reflectance values. On the other hand, in band 2 (0.52 -0.60 μm) and band 3 (0.63 – 0.69 μm), reflectance values decreased until 70 days after planting (DAP) and then increased during the reproductive phase of the crop. The near IR band 4 (0.76 -0.90 μm) showed a maximum reflectance at 59 DAP (Panicle initiation stage) and a decline in reflectance thereafter through maturity. The peak value of IR:R ratio was 16.39 at 62 DAP during the early reproductive phase, thereafter, it declined gradually with the maturity of the crop. Chlorophyll 'a' concentration was high during early growth (Vegetative and early reproductive stages) and decreased during the flowering and maturity stages. The rice plant canopy showed a high chlorophyll 'a' concentration at 64 and 59 DAP for sites A and B respectively. Chlorophyll 'a' concentration was higher in site A plant canopy than it was in site B during the entire crop cycle. A good inverse correlation (r = 0.91) was found between chlorophyll 'a' and band 1, while the IR:R ratio and the NDVI showed a relationship (r = 0.78) with the chlorophyll 'a' concentration during the crop cycle. Band 2, 3 and 4 radiance values show a biphasic linear relationship with chlorophyll 'a' concentrations, negative for early growth and positive for flowering and maturity stages. Results showed that the period between 66 to 70 DAP were most suitable for the assessment of rice crop yield, based on chlorophyll 'a' concentration.

Lilienthal *et al.*, (2000) reported that Precision-Agriculture will be an integral part of agricultural farm management practices in future and the exploitation of this tool for fertilizer use is essential for the harmonization of agricultural and ecological demands. The utilization of remote sensed images for the prognosis of the nitrogen demand could be an efficient method to assist the determination of variable fertilizer rates. In the presented studies hyper spectral earth observation (E/O) data were evaluated together with plant samples collected in a ground truth with view to their suitability to determine the spatial variability of the N status of winter wheat and to their implementation in precision agriculture for the calculation of variable N- rates.

Reddy *et al.,* (2001) observed that spectral reflectance of leaves is influenced primarily by plant pigments, chlorophyll and carotenoids. Such reflectance can be used to study the changes in chlorophyll content and nitrogen status and in turn measures the amount of biomass accumulation. A field experiment was laid out at the Research Farm of ANGR Agricultural University, Hyderabad, Andhra Pradesh, India. The reflectance observations were taken using a hand-held ground radiometer at an interval of 15 days beginning from 30 days after sowing (DAS) unit harvest of the crops. The plant pigments were determined simultaneously using DMSO (Dimethyl Sulfoxide) method in the laboratory. The experimental results revealed the influence of plant pigments on spectral reflectance of maize, groundnut and soybean. It was observed that there was in increase in chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids content up to flowering and thereafter chlorophyll-a content declined at a faster rate than chlorophyll-b towards physiological maturity. With the increase in concentrations of chlorophyll and carotenoids, there was a decline in spectral reflectance of the blue band (450 – 520 nm) and the red band (620-680 nm). Whereas, NIR (near infrared) reflectance in the case of soybean and groundnut was found to be higher than that of maize by 11 and 2%, respectively. This was attributed to canopy cover of soybean and groundnut crops where the soil was fully covered with vegetation. In case of maize, due to wider spacing, the soil exposure is greater, which results in low reflectance values of the NIR band. Normalized Difference Vegetations Index (NDVI) is linearly related to total chlorophyll content and the growth stages of a crop up to flowering. The NDVI differs significantly during the peak vegetative growth period, among the three crops type. The study revealed that the significant differences in reflectance of maize, groundnut and soybean in the red and NIR bands were influenced by concentrations of chlorophyll-a, chlorophyll-b and carotenoids, which indicates the photosynthetic behaviour of the crops.

Gitelson (2001) suggested that chlorophyll is strongly related to N supply, it is appropriate to say that higher reflectance at the visible region displayed from P_1 and P_2 compared to P_3, P_4, P_5 and P_6 was due to N deficiency. This is because in the visible (blue, green and red) spectral region, high absorption or reflectance of radiation energy is dominantly influenced by leaf pigments, primarily the chlorophylls, although carotenoids, xanthophylls and anthocyanins also have an effect.

Tumbo *et al.,* (2002) investigated that the remote sensing of chlorophyll levels in maize at V6 growth stage based on spectral measurements has been limited by soil background, changes in cloud cover, and solar angles. Chlorophyll levels at V6 growth stage are a strong indicator of nitrogen status. This articles suggests a solution based on an understanding of the hyper spectral characteristics of maize plants at different chlorophyll levels, under variable cloud cover, and with variable solar angles are presented. The chlorophyll levels were measured using the Minolta 502 chlorophyll meter. Investigation has found that spectral reflectance response patterns (SRRPs) of maize plants at the same chlorophyll levels, acquired during cloud cover changes, still show consistent spectral pattern characteristics. The same. characteristics were observed for SRRPs acquired as solar angles changed between 9.50 and 14.50 h. The results suggest that the SRRPs can be used to develop a robust model for predicting chlorophyll in maize at V6 growth stage. Furthermore a strong correlation was obtained between chlorophyll reading (in SPAD units) and the near infrared / green (NIR/G) ratio (r^2 = 0.94, root mean square error = 1.44 SPAD units) at constant solar irradiance.

Sims and Gamon (2002) concluded that leaf pigment content can provide valuable insight into the physiological performance of leaves. Measurement of spectral reflectance provides a fast, nondestructive method for pigment estimation. A large number of spectral indices have been developed for estimation of leaf pigment content. However, in most cases these indices have been tested for only one or at most a few related species and thus it is not clear whether they can be applied across species with varying leaf structural characteristics. One objective in this study was to develop spectral indices for prediction of leaf pigment content that are relatively insensitive to species and leaf structure variation and thus could be applied in larger scale remote sensing studies without extensive calibration. They also quantified the degree of spectral interference between pigments when multiple pigments occur within the same leaf tissue. Leaf surface reflectance appeared to be the most important factor in this variation. By developing a new spectral index that reduces the effect of differences in leaf surface reflectance. The presence of other pigments did not significantly affect estimation of chlorophyll from spectral reflectance. Previously published carotenoids and anthocyanin indices performed poorly across the whole data set. However, it was noticed that the photochemical reflectance index (PRI, originally developed for estimation of xanthophylls cycle pigment changes) was related to carotenoid / chlorophyll ratios in green leaves. This result has important implications for the interpretation of PRI measured at both large and small scales. Our results demonstrate that spectral indices can be applied across species with widely varying leaf structure without the necessity for extensive calibration for each species. This opens up new possibilities for assessment of vegetation health in heterogeneous natural environments.

Patil (2003) reported that RVI and NDVI were found to be reliable indices for discriminating stressed vegetation and normal vegetation. However NDVI showed superiority in smoothening of reflectance curves.

Gitelson *et al.,* (2003) observed that leaf chlorophyll content provide valuable information about physiological status of plants. Reflectance measurements makes it possible to quickly and non-destructively asses, in situ, the chlorophyll content in leaves. The objective was to investigate the spectral behaviour or the relationship between reflectance and chlorophyll content and to develop a technique for non-destructive chlorophyll estimation in leaves with a wide range of pigment content and composition using reflectance in a few broad spectral bands. Spectral reflectance of maple, chestnut, wild vine and beech leaves in wide range of pigment content and composition was investigated. It was shown that reciprocal reflectance (R lambda)- in the spectral range lambda from 520 to 550 nm and 695 to 705 nm related closely to the total chlorophyll content in leaves of all species. Subtraction of near infra-red reciprocal reflectance, (RNIR) -1, from (R lambda) -1 made from 695 to 725 nm with coefficient of determination $r^2 > 0.94$. To adjust for differences in leaf structure, the product of the latter index and NIR reflectance {(R lambda)-1-(RNIR)-1}*

(RNIR) was used; this further increased the accuracy of the chlorophyll estimation in the range lambda from 520 to 585 nm and from 695 to 740 nm. Two independent data sets were used to validate the developed algorithms. The root mean square error of the chlorophyll prediction did not excess 50 micro mol/m^2 in leaves with total chlorophyll ranged from 1 to 830 micro mol/m^2

Dhoble *et al.,* (2004) identified that nutrient stress is one of the important growth and yield limiting factors. Nitrogen is the primary nutrient required by crops and its deficiency is wide spread in India. To find out the feasibility and applicability of spectral data in detection of nutrient stress, field experiment was carried out in split plot design in 4 replications on wheat, consecutively for 3 years from (2001 and 2003). Growth of wheat was monitored by collecting data on LAI, chlorophyll content and total biomass. Reduced LAI, chlorophyll content and biomass were among the effect seen in nitrogen deficient crop.

Yallin *et al.,* (2004) showed that the hyper spectral reflectance of the canopies and the leaves on the main stem of six cultivars of rice, maize and cotton were measured at different stages, and the chlorophyll and carotenoid contents of the canopies and leaves corresponding to the spectra were determined. The characteristics of their hyper spectra and their red edge, the correlation between their red edge parameters and the pigment contents, leaf are indices, above ground biomass and fresh leaf mass were analyzed. The canopy hyper spectral reflectance of the three crops was correlated with their growth stages. The maximum value of canopy spectral reflectance was highest for cotton and lowest for rice, while that of leaf spectral reflectance was highest for rice and lowest for maize. There were "double peak" phenomena for the red edge of canopy spectra in all crops. Increasing and decreasing trends for the red edge slope (D lambda r) and red edge area (Sr) of the canopy spectra were observed for the three crops, but the extent of variation and trends for their red edge position lambda r were different. The leaf area indices and fresh leaf mass were significantly correlated with the lambda r, D lambda r and Sr of their Canopy spectra, but the correlation between the above ground fresh biomass and above ground dry biomass was different. The contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid of the leaves were all significantly correlated with their red edge position lambda r, but their correlations with other red edge parameters (D lambda r and Sr) were different.

Patil *et al.,* (2004) reported that chlorophyll and carotene pigment absorption radiation lies in B_1 (0.45-0.52 μm) and B_3 band (0.62 – 0.68 μm). Hence these chlorophyll pigment concentration established significant relationship with spectral reflectance in blue and red band of EMR. Further it was observed from R^2 values that both indices (RVI and NDVI) were highly correlated with chlorophyll and nitrogen concentration of leaves. Further it was reported that NDVI found a better index far diagnosing chlorophyll and nitrogen leaf connection.

Stiegler *et al.,* (2005) found that the use of optical sensors to assess plant status may play an integral part in the future of turf grass management. Varying pigment concentrations affect the amount of reflectance obtained from the plant canopy and may affect the reliability of sensor-based indirect measurements. The objectives of this study were to determine the influence of the chlorophylls and carotenoids, including xanthophylls, on the spectral reflectance from a dense turf canopy. Reflectance measurements were converted to normalized difference vegetation indices (NDVI) and compared with pigment concentrations in leaf tissue collected from a creeping bent grass (*Agrostis stolonifera* L.) putting green. Variable pigment concentrations in the creeping bent grass tissue were established using applications of nitrogen (N), magnesium (Mg), and iron (Fe). Pigment concentrations were measured following extraction from leaf clippings using high performance liquid chromatography (HPLC). Chlorophyll b, chlorophyll a, lutein, β□ -carotene, and the Xpool (xpool = xanthophylls pool = violaxanthin + antheraxanthin + zeaxanthin) were highly correlated with one another. Significant relationships were found between NDVI and pigments, but pigment concentrations did not account for more than 36% of the variation associated with NDVI. The results indicated that plant pigment concentrations affected NDVI. However, the results also suggested that another factor or combination of factors provided a stronger influence on NDVI than plant pigments.

Schlemmer *et al.,* (2005) observed that the normalized difference between the first derivatives at 525 and 570 nm, and the wavelength location of the red edge, showed a strong association with chlorophyll content ($r^2 = 0.81$ and 0.80, respectively). Even stronger relationships to chlorophyll content were observed with the ratio of 600 / 680 ($r^2 = 0.83$) and 630 / 680 nm ($r^2 = 0.83$). Thus, the results suggest that spectral reflectance measurements hold promise for the assessment of some physiological parameters at the leaf level. Further investigations are needed to evaluate the effectiveness of such techniques at the canopy level.

Singh *et al.,* (2006) concluded from field experiments conducted during 1998-99 and 1999-2000 at research farm of the Department of Agricultural Meteorology. CCS Haryana Agricultural University, Hisar. Five wheat cultivars: WH 542, PBW 343, UP 2338, Raj 3765 and Sonak were sown on 25th November. 10th and 25th December with four nitrogen levels viz. no nitrogen, 50, 100 and 159% of recommended dose. Leaf area index, dry matter at anthesis, final dry biomass and grain yield were recorded in all the treatments. Chlorophyll and wax contents of wheat leaves were estimated at different growth stages. Multiband spectral reflectance was measured using hand-held radiometer. Spectral indices such as simple ratio, normalized difference transformed vegetation index, perpendicular vegetation index and greenness index were computed using the multiband spectral data. Values of all the spectral indices were maximum in 25 November sown crop with maximum dose of nitrogen (180 Kg Nha^{-1}) O/bw 343 showed higher values of all the spectral indices in comparison with other cultivars. The spectral indices recorded during maximum leaf area index stage were correlated with crop parameters. Using stepwise regression, empirical models for chlorophyll, leaf area index, dry biomass and yield prediction were developed. The 'R^2' values of these models ranged between 0.87 and 0.95.

Patil *et al.* (2008) established a relationship between spectral indices and crop growth parameters and chlorophyll concentration of soybean under different fertilizer treatments. They observed that the growth parameters of soybean like LAI, dry matter and total chlorophyll concentration increased with the increase in N, P and S levels. All these parameters were maximum at grand growth stage of soybean. RVI, NDVI and SAVI established significant correlation with LAI, biomass and yield. Hence, they can be used as a yield stick to identify the crop condition i.e. fertilizer fed crops vs non-fertilized crops.

Material and Methods

CHAPTER 3

MATERIALS AND METHODS

A research project "Relationship between chlorophyll and other pigments and spectral reflectance in various plant species" was conducted during 2007-08 at Marathwada Agricultural University, Parbhani. It was aimed to find out the influence of plant pigments viz., chlorophyll, lycopene, anthocyanin and B-carotene on spectral reflectance by the canopies of plant species. As chlorophyll is a major pigment that contributes towards spectral behaviour of plant species, a field experiment was conducted on maize under various fertility levels so as to obtain different leaf chlorophyll concentrations. Similarly, other plant species like Acalypha and Golden duranta which are dominant in other plant pigments were selected to study the spectral behaviour of other pigments. The details of material used and methods adopted are presented in this chapter.

3.1 Experimental site and climate
3.2 Experimental soil
3.3 Crop weather parameters prevailed during field experimentation
3.4 Field experimentation and observations
3.5 Other plant species
3.6 Spectral observations
3.7 Instruments used for collection of spectral data
3.8 Collection of soil and plant samples and its analysis
3.9 Spectral indices and statistical analysis

3.1 Experimental site and climate

The experiment was conducted at Research Farm, Department of Soil Science and Agricultural Chemistry, Marathwada Agricultural University, Parbhani (Plate 1) during the post monsoon season of 2007-08. The experimental site is situated at 19° 16’ North latitude and 76° 74’ East longitude with 409 meters above mean sea level. It has hot and dry summer and area comes under semi-arid tropics. The rainy season (south-west monsoon) starts from June and recedes in the month of October.

Plate 1. General view of field experiment

3.2 Experimental soil

The Typic Haplustert soils of Parbhani formed from weathering of trap basalt rock rich in iron and manganese. The soil at experimental site was dark brown in colour (10YR 4/3), more than 90 cm deep and clay in texture. The taxonomically it is classified as fine, smectitic calcareous isohyperthermic Typic Haplusterts. The soil sample from root rhizosphere (0-20 cm) was collected as per the prescribed method to find out the initial soil properties. The experimental soil was clay in texture (55% clay), alkaline in reaction (pH_2 7.9), safe in total soluble salt concentration (EC_2 – 0.176) dSm^{-1}) and calcareous in nature ($CaCO_3$ 105.0 g kg^{-1}). Among the fertility constituents, organic carbon was 7.8 g kg^{-1}, available nitrogen, phosphorus, potassium, sulfur, DTPA zinc and DTPA iron were 452.8 kg ha^{-1}, 7.39 kg ha^{-1}, 737.68 kg ha^{-1}, 11.87 mg kg^{-1}, 0.65 mg g^{-1} and 3.72 mg kg^{-1}, respectively.

3.3 Crop weather parameters prevailed during field experimentation

3.3.1 Rainfall and temperature prevailed during crop growth period

Data on weekly weather parameters from October 2007 to February 2008 are presented in Appendix-I. It revealed that during growth period of maize, total rainfall of 18.2 mm was received in 44th metrological weeks. The data on maximum and minimum temperature showed that maximum temperature was prevailed in the 41 meteorological week and minimum in 47 meteorological week (during crop growth period) The other climatic parameters were conducive for crop growth and were fluctuating as per the normal values for the season.

3.3.2 Weather parameters prevailed on observation days

The spectral observations were recorded during growth period of maize on November 12, 19, 25, December 2, 16, 2007 and January 2 and 29, 2008. The weather parameters viz., temperature, relative humidity, rainfall, soil temperature and wind speed prevailed on observation day

(10.30 hrs to 13.30 hrs) were recorded. They are presented in Appendix II as a ready reference for the interpretation of experimental results.

3.4 Field experimentation and observations

In order to meet the objectives of the project, a field experiment was laid out (Fig. 3.1) in Randomized Block Design using maize as a test crop during late monsoon season of 2007-08. There were 6 fertility treatments as detailed below.

Treatment details

Fertility levels

F_0 No fertilizer application

F_1 Only N 150 kg N ha^{-1}

F_2 N + P (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1})

F_3 N + P + S (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1} + 30 kg S ha^{-1})

F_4 N + P + S + Zn (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1} + 30 kg S ha^{-1} + 20 kg $ZnSO_4$ ha^{-1})

F_5 N + P + S + Zn + Fe (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1} + 30 kg S ha^{-1} + 20 kg $ZnSO_4$ ha^{-1} + 2 % Fe foliar spray at 2 stages)

The recommended dose of N, P and K to irrigated maize under Marathwada condition is 150:50:50 kg N, P_2O_5 and K_2O ha^{-1}. Potassium @ 50 kg K_2O ha^{-1} was applied for all six treatments at the time of sowing of maize. The two iron sprays were given one at silking stage and another at cob development stage.

Soil type

Fine, Smectitic calcareous isohyperthermic, Typic Haplusterts.

Other details

Replications : Four
Plot size (m) : 4.2 m x 4.8 m
For spectral observations : 4.8 m x 3.0 m
For biometric observations : 4.8 m x 1.2 m
Sowing date : 9th October, 2007.
Harvesting date : 13th February, 2008
Crop spacing : 60 cm x 30 cm

The details of the intercultural operations and other practices followed during the experimentation are presented in Appendix-III.

Observations were recorded during the growth period of maize. The biometric parameters like height, number leaves, leaf area and biomass were recorded during crop growth. Visual nutrient diagnosis was also made during the crop growth. The initial germination count, final plant population and economic yield under various treatments were recorded.

Growth and other observations

1. Germination percentage
2. Final plant population
3. Number of leaves
4. Leaf area
5. Dry matter yield
6. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll
7. Other pigments viz., anthocyanin, lycopene, B-carotene.

8. Plant N, P, K, S, Zn and Fe concentrations at critical growth stages viz., early vegetative, silking stage and grain ripening stage.

3.5 Other plant species

In the present investigation following special plant species dominating in particular leaf pigments were selected.

Acalypha (*Acalypha batik*)

Acalypha are a group falls under euphorbiaecae family bearing colorful leaves and are used as hedge and also for providing colour in the shrubbery. These are quick growing and can be grown in any soil.

Plate 2. Acalypha (*Acalypha batik*)

Golden duranta (*Duranta plumieri*)

Golden duranta comes under verbenaceae family. It is a large (2.5 to 3 m tall), woody shrub with auxiliary spines. Small, bright, yellowish, green leaves are attractive. A most suitable shrub for hedge and also for topiary.

Plate 3.Golden duranta (*Duranta Plumieri*)

3.6 Spectral observations

OptoMech Multiband Ground Truth Radiometer having four spectral bands similar to Indian remote sensing satellite (IRS-1C) was used for collecting the data on canopy reflectance in Visible and Near Infra Red (NIR) region of Electromagnetic Radiation (EMR). The spectral bands selected were:

1. Visible (blue-green) : 0.45 – 0.52 um
2. Visible (green) : 0.52 – 0.59 um
3. Visible (red) : 0.62 – 0.68 um
4. Near infra Red : 0.77 – 0.86 um.

The radiometric measurements were carried out throughout the growing season keeping approximately 10 to 15 days interval in two observations. The radiometer was kept one feet above the canopy. All spectral data were collected in between 10.30 to 13.30 hrs. Indian standard time in direct sunlight on cloud free days or nearly cloud free day. Irradiance from Barium sulfate standard was measured after each radiance measurements and readings were used to calculate the corrected canopy reflectance. One spectral observation on plant canopy of Acalypha and Golden duranta grown in college garden was also taken to study the contribution of other pigments in leaf reflectance.

3.7 Instruments used for collection of spectral data

3.7.1 Ground Truth Multiband Radiaometer (GTMR)

Ground Truth Multiband Radiometer having four spectral bands similar to Indian Remote Sensing satellite (IRS-1C) i.e. used to collect spectral data.

1. Visible (Blue – Green) : 0.45-0.52 um
2. Visible (Green) : 0.52-0.59 um
3. Visible (Red) : 0.62-0.68 um
4. Near Infra Red (NIR) : 0.77-0.86 um

Plate 4. GTMR

The GTMR collects solar reflectance, radiance and irradiance from this four bands.

3.7.2 Infra Red Thermometer

Infrared thermometers measure temperature using blackbody radiation(generally infrared) emitted from objects. By knowing the amount of infrared energy emitted by the object and its emissivity, the objects temperature can be determined. The most basic design consists of a lens to focus the infrared energy on to a detector, which converts the energy to an electrical signal that can be displayed in units of temperature after being compensated for ambient temperature variation. This configuration facilitates temperature measurement from a distance without contact with the object to be measured.

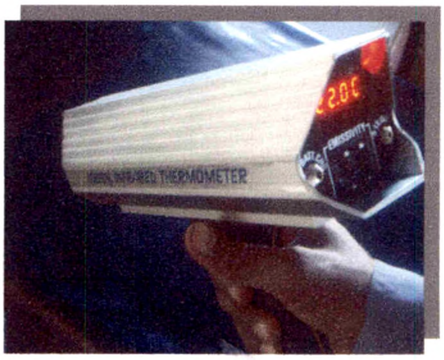


Plate 5. IR Thermometer

3.7.3 PAR/LAI Ceptometer

Plate 6. PAR Ceptometer

It is used to measure light interception in plant canopy and to calculate LAI. It consists of an integrated microprocessor-driven data logger and probe. The probe contains 80 independent sensors, spaced 1 cm apart. The photo-sensors measure PAR (Photo-synthetically Active Radiation) in the 400-700 nm waveband. The AccuPAR displays PAR in units of micromoles per meter squared per second (umol $m^{-2}s^{-1}$). The instrument is capable of hand held or unattended measurement.

3.8 Collection of soil and plant samples and its analysis

The soil samples were collected from 0-20 cm depth and dried in shade. The samples were processed, labeled and kept in clean cloth bag for further analysis. For each observations, two maize plants were uprooted to record biomass, leaf area and chlorophyll content. Some part of plant samples were dried and processed for chemical constituent analysis. Similarly, to study the concentration of other leaf pigments 500 g fresh leaves were collected from Acalypha and Golden Duranta and were analyzed for chlorophyll, Anthocyanin, Lycopene and B-carotene. Methods adopted for soil and plant samples analysis are listed in Table 3.1.

Table 3.1 Methods adopted for soil and plant analysis

Property / nutrient	Method	Reference
Soil analysis		
pH$_2$	Potentiometric	Jackson (1973)
EC $_2$	Conductometric	Jackson (1973)
Free CaCO$_3$	Rapid titration	Piper (1966)
Organic carbon	Wet digestion	Walkely and Black (1934)
Available nitrogen	Alkaline potassium permagnate	Subbiah and Asija (1956)
Available phosphorus	0.5 M sod. biocarbonate	Olsen *et al.* (1954)
Available sulfur	Trubidimetric method	Chopra and Kanwar (1976)
DTPA-Zn and Fe	By DTPA extraction	Lindsay and Norvell (1978) using AAS
Plant analysis		
Nitrogen	Microjeldahl's	AOAC (1965)
Phosphorus	Vandomolybdate phosphoric acid yellow colour method	Jackson (1973)
Sulfur	Trubidimetric method	Tabatabai and Bremner (1970)
Zn, Fe	Acid digestion	AAS
Plant pigments		
Total chlorophyll	DMSO method	Hiscox and Israelstan (1979)
Chlorophyll 'a'	DMSO method	Hiscox and Israelstan (1979)
Chlorophyll 'b'	DMSO method	Hiscox and Israelstan (1979)
Other pigments		
Anthocyanin	Spectorphotometric	Syed (2007)
Lycopene	Spectorphotometric	Syed (2007)
B-carotene	Spectorphotometric	Syed (2007)
Leaf reflectance	GTMR	--

3.8.1 Estimation of total chlorophyll

Quantitative analysis of chlorophyll by using DMSO as an extractant

Material required

DMSO, mortar and pestle, measuring cylinder, distilled water, pipette, Spectrophotometer leaf punch, electronic balance.

Procedure

- 0.25 g of leaf sample was taken by using leaf punch, then the sample was kept in 10 ml DMSO in the test tube.
- The sample was kept for 2 hrs at 60°C for complete extraction of the chlorophyll from leaf
- A blank solution without leaf sample was prepared for comparison. The optical density of coloured extracted solution was measured at 645 nm, 663 nm and 652 nm for chlorophyll 'a', chlorophyll 'b' and total chlorophyll.

$$\text{Chlorophyll – a (mg g}^{-1}) = \left[12.7 \times \overset{\text{OD at}}{\text{663nm}}\right] - \left[2.69 \times \overset{\text{OD at}}{\text{645 nm}}\right] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll – b (mg g}^{-1}) = \left[22.9 \times \overset{\text{OD at}}{\text{645nm}}\right] - \left[4.68 \times \overset{\text{OD at}}{\text{663 nm}}\right] \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg g}^{-1}) = \left[20.2 \times \overset{\text{OD at}}{\text{645nm}}\right] + \left[8.02 \times \overset{\text{OD at}}{\text{663 nm}}\right] \times \frac{V}{1000 \times W}$$

Where,

OD = Optical density

V = Volume of extract in ml

W = Weight of sample in g

3.8.2 Anthocyanins contents

The quantification of anthocyanin pigment was done spectro-photo - metrically by using absorbance at 535 nm wavelength. Weighed 25 g of sample was well mixed with 25 ml of suitable solvent i.e. Methanolic HCl (Meol (95%) : 1.5 N HCl (85:15) and it was stored overnight in glass stopper bottle. Then the sample was filtered through Whatman No. 1 paper . The residue was washed then till 100 ml extract was collected. The volume was made 125 ml with methanolic Hcl. The small amount from it was diluted and stored in dark for 2 h and optical density was measured at 535 nm. The readings were taken in triplicate.

25 g of sample made to 125 ml, 2 ml of which was made to 100 ml with methonolic Hcl, the absorbance (x) was measured at 535 nm.

$$\text{Total OD per g sample} = \frac{\text{Absorbance at 535 nm} \times \text{Volume made for colour measurement} \times \text{Total volume}}{\text{ml of extract used} \times \text{wt of sample taken}} \times 100$$

$$= \frac{X \times 100 \times 125 \times 100}{2 \times 25} = 25000\,x$$

$$\text{Total anthocyanins} = \frac{25000\,x}{98.2} = \left(\frac{\text{Total OD}}{98.2}\right) \text{mg/g}$$

The E value (extinction coefficient) for 1 % solution i.e. 10 mg per 1 ml at 535 nm is equal to 982.

Therefore, the absorbance of a solution containing 1 mg per ml is equal to 98.2

$$\text{There fore , total anthocyanin content in mg/g of sample} = \frac{25000\,x}{98.2}$$

3.8.3 Estimation of lycopene pigment in plant samples

The sample was blended in a wearing blender to a uniform pulp (5'). Then the samples was removed from the blender and weighed a 5 g sample. It was transferred to the cup of a clean blender, to which 10 ml of Benzol was added and blend exactly 5'. 4 ml of the benzol extract was taken to a 15 ml centrifuge tube, as volume made make up to a 15 ml with Benzol and Centrifuged at 100 rpm for 10'

The supernatant coloured liquid was poured off the centrifuge into the glass cell for the spectrophotometer and per cent transmittance at 484 nm was measured.

3.8.4 Quantitative determination of plant pigments (Lycopene and Beta carotene)

The sample was blended in weaning blender to a uniform pulp. The blended sample was then removed from the blender and accurately weighed a 5 g sample was transferred to a clean blender cup to which 75 ml of acetone and 60 ml petroleum ether (65-100 °C) was added and blended for exactly 5'. The content then transferred to a 500 ml reparatory funnel. A 9 cm funnel loosely plugged with glass wool and a wash bottle containing acetone facilitated this transfer and prevented blended sample from entering the reparatory funnel.

The extract was washed for three times with distilled water and then shake in separatory funnel gently in an inverted position for 0-5'/wash. This step removes the acetone whose function is to remove the water of the sample, thus helping to prevent the formation of stable emulsions. Sample was drained and water was discarded.

20 ml of 90 % methanol added and mixed for 0-5' then 20 ml of 20 % KOH was added in methanol and mixed for 0.5 minutes. This specifications removes all but the nonsaponifiable fraction, which includes the carotenoids. Again 20 ml of 95 % methanol added and mixed for 0-5'. Then extract was washed for three times with distilled water. The separating funnel stem is dried with absorbent cotton. Petroleum ether was taken (hyper phase) into a 100 ml volumetric flask through 9 cm funnel, which has been loosely plugged with glass wool on which approximately 4 g of sodium sulfate had placed. This step removes and moisture which tends to cloud the extract. Final 100 ml volume was made with petroleum ether.

1. 20 ml of the cleared extract into a glass absorption cell was transferred to measure the transmittance on the spectrophotometer.
2. Per cent transmittance and optical density of the extract was measured over the visible spectrum at 500 nm.

3.9 Spectral indices and statistical analysis

3.9.1 Calculations for spectral indices

Four spectral indices viz., Relative Vegetative Index (RVI), Normalized Difference Vegetative Index (NDVI), Soil Adjusted Vegetation Index (SAVI) and Infrared Percentage Vegetation Index (IPVI) were computed from the spectral reflectance data. The Red (R) and Near Infrared (NIR) spectral reflectance for each treatment were transformed into RVI, NDVI, SAVI and IPVI ratios.

Calculation of corrected NIR and R reflectance.

NIR = NIRc/NIRb

Where,

NIRc = Canopy radiance in NIR band

NIRb = Irradiance on barium plate in NIR band

Rc = Canopy radiance in R band

Rb = Irradiance on barium plate in R band

Relative vegetation index (RVI)

Relative vegetation index is the ratio vegetation index which was first described by Jordan (1969). Relative vegetation index is a corrected ratio of canopy reflectance in NIR (Band 4) over canopy reflectance in Red (Band 3) radiation.

RVI = NIR/R

Normalized difference vegetation index (NDVI)

NDVI is described to Rouse *et al.* (1973) but the concept of NDVI was first presented by Kriegler *et al.* (1969).

$$\text{NDVI} = \frac{\text{NIR}-\text{R}}{\text{NIR}+\text{R}}$$

Soil adjusted vegetation index (SAVI)

SAVI is the soil adjusted vegetation index which was introduced by Huete (1988).

$$\text{SAVI} = \frac{\text{NIR}-\text{Red}}{\text{NIR}+\text{Red}+\text{L}}(1+\text{L})$$

Where, L is a correction factor which ranged from 0 for very high vegetation cover to 1 for every low vegetation cover. The most typically used value is 0.5 which is for intermediate vegetation cover.

Infrared percentage vegetation index (IPVI)

IPVI was first described by Crippen (1990).

IPVI = NIR / NIR + Red

Leaf area index

$$LAI = \frac{\text{Leaf area per plant in cm}^2}{\text{Spacing per plant in cm}^2}$$

3.9.2 Statistical analysis

The data emerged out from the field experiment on spectral reflectance, biometric parameters, chlorophyll content, other plant pigment, available soil nutrients, plant nutrient concentrations and various spectral ratios were computed and correlation regression techniques were carried out (Panse and Sukhatme, 1985). The biomass and economic yield data was also exposed for design analysis and critical difference was calculated for treatment comparison. The relationship between plant pigments and spectral indices were established by fitting trend line curves. These curves were then smoothened using MS-Excel.

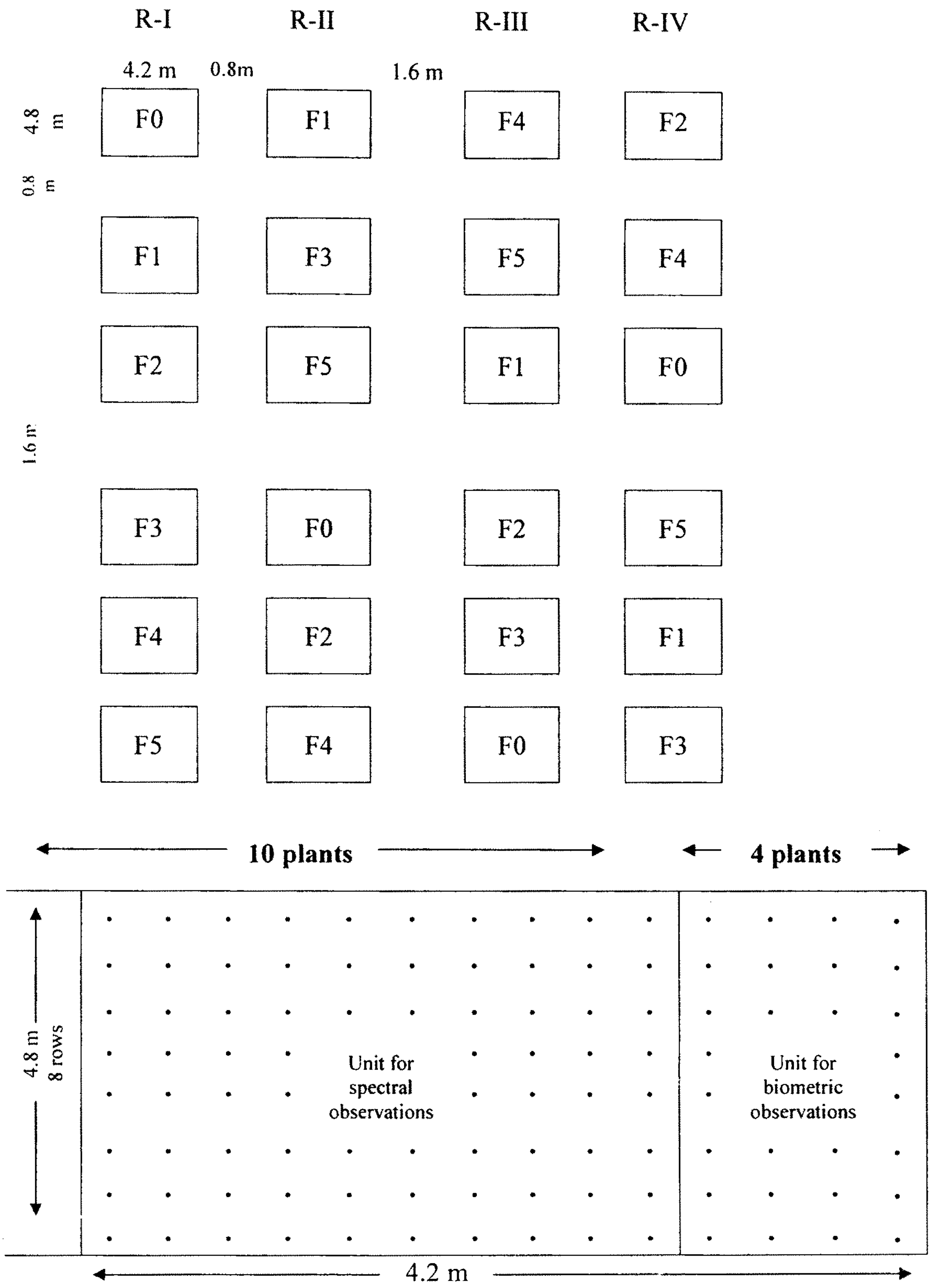


Fig. 3.1 Plan of layout and unit of observations

Results and Discussion

Chapter 4

RESULTS AND DISCUSSION

Plant pigments play important role in photosynthesis. The relationship between plant pigments with spectral reflectance is established by various research workers. The spectral reflectance of leaves is primarily influenced by chlorophyll content As chlorophyll stimulate the photosynthesis, its contribution towards the manufacture of photosynthates leads to development of crop species. However, chlorophyll content is not only a pigment that contribute towards canopy reflectance, but other pigments like carotenoids, anthocyanin and so found to contribute in spectral reflectance by crop plants. Therefore, research of the present study is centralized to find out relationship between chlorophyll and other pigments and spectral reflectance under varied fertility levels and in selected plant species. As described in material and methods there were six fertility levels maintained through the application of recommended dose of N, P, S, Zn and Fe, using maize as a test crop. The data on various growth, physiological and spectral parameters were compiled for critical difference analysis, spectral ratios, correlation and regression models using trend line curves. The results emerged out are presented under the following subheads.

4.1 Soil nutrient status of the experimental site

4.2 Weather parameters prevailed during the course of field experimentation

4.3 Effect of fertility levels on germination and final plant stands of maize

4.4 Effect of fertility levels on growth, yield and physiological parameters of maize

4.4.1 Effect of fertility levels on plant height

4.4.2 Effect of fertility levels on number of leaves

4.4.3 Effect of fertility levels on leaf area index

4.4.4 Effect of fertility levels on total biomass and economic yield

4.5 Effect of fertility levels on chlorophyll content of maize

4.5.1 Effect of fertility levels on chlorophyll 'a'

4.5.2 Effect of fertility levels on chlorophyll 'b'

4.5.3 Effect of fertility levels on total chlorophyll

4.6 Concentration of chlorophyll and other plant pigments in Acalypha and Golden duranta

4.7 Effect of fertility levels on spectral reflectance by maize canopy

4.7.1 Effect of fertility levels on canopy reflectance of maize in Red and NIR bands

4.7.2 Effect of fertility levels on RVI, NDVI, SAVI and IPVI

4.8 Influence of various plant species on spectral reflectance in Red and NIR wave length bands and spectral indices

4.8.1 Influence of various plant species on spectral reflectance in Red and NIR bands

4.8.2 Influence of various plant species on spectral indices

4.9 Influence of fertility levels on thermal behaviour of maize canopy

4.10 Relationship between physiological and spectral parameters in maize

4.10.1 Relationship between LAI and spectral indices in maize

4.10.2 Relationship between total chlorophyll and spectral indices in maize

4.10.3 Relationship between plant pigments and spectral indices in Acalypha

4.10.4 Relationship between plant pigments and spectral indices in Golden duranta

4.11 Visual nutrient deficiency symptoms, plant nutrient concentration and nutrient uptake by maize

4.1 Soil nutrient status of the experimental site

The soil analysis was carried out before the establishment of field experiment and after the harvest of crop. The data thereof presented in Table 4.1.The experiment was conducted on research farm of Department of Soil Science and Agricultural Chemistry, College of Agriculture, Marathwada Agricultural University, Parbhani. The experimental soil was fine, smectitic (calcareous), iso hyperthermic Typic Haplusterts. It was alkaline in reaction (pH 7.9), safe in soluble salt concentration (EC 0.176 dSm^{-1}) and high in organic carbon content (7.8 g kg^{-1}). The free calcium carbonate content was 105.0 g kg^{-1}. After harvest of the crop pH and electrical conductivity of soil was not influenced significantly due to administration of various fertility level treatments. However, there was numerical increase in organic carbon content in all the treatments except absolute control where the organic carbon was reduced by 0.1 g kg^{-1}. In rest of the treatments the increase was inconsistent. The free calcium carbonate equivalent was varied in consistently due to application of various treatments. However, the mean calcium carbonate content after harvest of crop was 105.4 g kg^{-1} which was almost same as that of initial $CaCO_3$ content.

Table 4.1. Soil properties of the experimental site before sowing and after harvest of crop

Treatments	pH	EC (dSm^{-1})	Organic carbon (g kg^{-1})	$CaCO_3$ (g kg^{-1})
Before sowing				
Initial	7.9	0.176	7.8	105.0
After harvest				
F_0	7.5	0.167	7.70	105.7
F_1 (N)	7.5	0.171	8.06	105.0
F_2 (NP)	7.3	0.169	7.84	104.5
F_3 (NPS)	7.6	0.175	7.89	103.0
F_4 (NPSZn)	7.7	0.176	8.40	107.0
F_5 (NPSZnFe)	7.4	0.179	8.43	108.5
Mean	7.5	0.172	8.05	105.6
SEm±	0.08	0.08	1.21	4.39
CD at 5%	NS	NS	NS	NS

Nutrient status of experimental soil before sowing and after harvest of crop is presented in Table 4.2. The available nitrogen, phosphorus, potassium and sulfur status of experimental soil was 452.8, 7.39, 737.68 and 5.30 kg ha^{-1}, respectively. While the zinc and iron content before administration of treatments was 0.65 and 3.72 mg kg^{-1}, respectively. The mean data on final nutrient status shows that their was increase in nitrogen, phosphorus, sulfur and zinc status of soil after harvest of crop.

Table 4.2. Nutrient status of the experimental site before sowing and after harvest of crop

Treatments	**Available Macro-nutrients (kg ha^{-1})**				**DTPA Micronutrients (m g kg^{-1})**	
	Nitrogen	**Phosphorus**	**Potassium**	**Sulfur**	**Zinc**	**Iron**
Before sowing						
Initial	452.8	7.39	737.68	11.87	0.65	3.72
After harvest						
F_0	433.1	9.2	739.4	14.00	0.66	3.44
F_1 (N)	503.8	9.2	729.4	14.24	0.61	3.17
F_2 (NP)	503.7	9.0	724.0	15.38	0.67	3.04
F_3 (NPS)	656.6	9.0	733.6	19.08	0.81	3.99
F_4 (NPSZn)	660.7	9.0	735.0	17.53	0.77	3.66
F_5 (NPSZnFe)	662.0	9.3	740.8	16.66	1.11	3.16
Mean	569.9	9.1	731.9	16.15	0.77	3.41
SEm±	50.07	1.152	7.593	1.79	0.105	0.873
CD at 5%	150.7	NS	22.84	NS	0.318	NS

The results presented above revealed that the experimental soils was safe in pH and EC. However, the calcium content was high. The high calcium carbonate content of sub-tropical black soils pose problem in nutrient management. In the present study this might be the reason of inconsistent soil nutrient status recorded after harvest of crop under varied fertility treatments. Further, it was also inferred that one season cropping did not influence the soil properties significantly. However, numerical increase in nutrient status was recorded. Application of recommended dose of fertilizer further not only protected the soil nutrient status but it has improved it. Confirming the application of recommended dose of fertilizer is a key factor in sustainable agriculture development and protection of soil health. Summarizing the soil of the experimental site was suitable for maize cultivation and for conducting the experiment.

4.2 Weather parameters prevailed during the course of field experimentation

Daily sunshine hours and sun elevation are of particular importance for sensor measurements. Because the accuracy of the measurement is influenced by high variation of these parameters. Therefore, observations are recorded on cloud free days and between 10.30 to 13.30 hrs. Sensor measurements get impaired by cloud and rainy weather conditions.

At Parbhani, the bright sunshine hours decrease as monsoon proceeds and further the sunshine hours increase as winter ceases. The average bright sunshine hours where highest in 45 to 47th meteorological weeks. The experiment was sown in the month of October so as to avoid cloudiness and rainy weather conditions. In general, weather parameters were favorable for maize cultivation

4.3 Effect of fertility levels on germination and final plant stand of maize

The data on germinated seedlings and final plant (population) stand at harvest are presented in Table 4.3.

The effect of different fertility levels recorded non significant effect on germination and final plant population of maize.

Table 4.3. Effect of fertility levels on germination and final plant stand of maize (per cent)

Treatments	**Germinated seedlings (%)**	**Final plant stand (%)**
F_0	93.75	89.73
F_1 (N)	95.53	92.41
F_2 (NP)	96.42	93.75
F_3 (NPS)	97.32	95.08
F_4 (NPSZn)	98.21	96.42
F_5 (NPSZnFe)	99.10	97.32
Mean	96.72	94.11
SEm±	2.83	3.00
CD at 5%	NS	NS

Germination of the maize crop was more than 96 per cent and final plant stand recorded was more than 94 per cent in each treatment. This explains good conduct of experimentation. Due to non significant plant population, treatments expressed their full potential with fertility levels. Panse and Sukahtme (1985) reported that plant population of the field experiment must be non significant to get clear effect of treatments administered.

4.4 Effect of fertility levels on growth, yield and physiological parameters of maize

In the present project, observations were recorded on height, number of leaves, leaf area index, total biomass production and economic yield. The statistically analyzed data are presented in Table 4.4 to Table 4.7.

4.4.1 Effect of fertility levels on plant height

The height of maize was monitored at critical growth stages of the crop (early vegetative 20 DAS, silking 73 DAS and grain ripening 103 DAS). Periodical observations recorded on various dates under various fertility level treatments are presented in Table 4.4. It was observed that in first phase of crop i.e. upto 20 DAS growth rate was rather slow but from 20 DAS to 73 DAS it was fast. Various fertility level treatments significantly increased the height of maize as compared to control plots. Application of N150, P50, K50, S30, Zn20 and foliar spray of 2 % Fe at 2 (F_5) stages significantly increased plant height upto 162.1 cm at 103 DAS. Each fertility levels significantly increased the height of plant over control at 103 DAS.

Table 4.4. Effect of fertility levels on plant height (cm plant^{-1})

Treatments	**Height (cm plant^{-1})**			
	20 DAS	**73 DAS**	**103 DAS**	**Mean**
F_0	5.68	84.12	89.00	59.6
F_1 (N)	6.68	102.12	113.20	74.0
F_2 (NP)	6.68	107.50	132.80	82.3
F_3 (NPS)	6.81	118.75	142.50	89.35
F_4 (NPSZn)	6.93	123.75	147.30	92.66
F_5 (NPSZnFe)	7.31	132.62	162.10	100.67
Mean	6.68	111.48	131.19	83.11
SEm±	0.326	3.885	1.868	--
CD at 5%	0.982	11.690	5.623	--

4.4.2 Effect of fertility levels on number of leaves

The data on number of leaves per plant are presented in Table 4.5. The observations were taken at three growth stages from 20 days after sowing to 103 days after sowing i.e. at early vegetative (20 DAS). Silk formation (73 DAS) and grain ripening (103 DAS). Maximum number of leaves was found at 103 DAS. The application of each additional nutrient increased number of leaves per plant significantly at 103 DAS. Further treatments F_1 to F_5 showed significantly more number of leaves per plant at all growth stages over control (F_0).

Table 4.5. Effect of fertility levels on number of leaves

Treatments	Number of leaves per plant			
	20 DAS	73 DAS	103 DAS	Mean
F_0	5.37	8.25	11.87	8.49
F_1 (N)	5.87	10.37	14.37	10.41
F_2 (NP)	6.12	11.00	15.75	10.74
F_3 (NPS)	6.37	11.62	18.12	12.03
F_4 (NPSZn)	6.75	11.12	18.75	12.20
F_5 (NPSZnFe)	7.25	12.37	20.50	13.37
Mean	6.29	10.78	16.56	11.21
SEm±	0.176	0.535	0.516	--
CD at 5%	0.531	1.611	1.555	--

The increase was from 5.37 to 27.25, 8.25 to 212.37 and 11.87 to 20.50 at 20, 73 and 103 DAS with mean number of leaves 6.29, 10.78 and 16.56, respectively.

4.4.3 Effect of fertility levels on leaf area index

The crop leaf area is one of the important attributes that influences the growth, development and spectral parameters. Hence, the measurement of leaf area was carried out at all growth stages. The data pertaining to these observations are presented in Table 4.6 and Figure 4.1. There was average increase of leaf area index from just 0.07 to 3.3 from 20

DAS to 103 DAS. Similarly, the application of each additional nutrient had significant influence on leaf area index of maize. The application of nitrogen alone had profound influence on leaf area and contributed nearly 50 % more increase in leaf area over no nitrogen application. Further addition of phosphorus, sulfur and zinc to soil and foliar sprays of iron increased the leaf area index. There was boost in leaf area index from 20 DAS to 73 DAS. This period coincides with top dressing of maize with remaining dose of nitrogen and increase in bright sunshine hours (Appendix-I).

Table 4.6. Effect of fertility levels on leaf area index (LAI)

Treatments	**Leaf Area Index**			
	20 DAS	73 DAS	103 DAS	Mean
F_0	0.06	2.2	2.7	1.65
F_1 (N)	0.07	3.0	3.3	2.12
F_2 (NP)	0.07	3.1	3.4	2.19
F_3 (NPS)	0.08	3.1	3.5	2.22
F_4 (NPSZn)	0.08	3.1	3.6	2.26
F_5 (NPSZnFe)	0.09	3.5	3.9	2.49
Mean	0.07	3.0	3.4	2.12
SEm±	0.004	0.173	0.08	--
CD at 5%	0.013	0.353	0.24	--

4.4.4 Effect of fertility levels on total biomass and economic yield

The total biomass (oven dried) and economic yield of maize crop under various fertility levels are presented in Table 4.7 and Figure 4.2a and Figure 4.2b. The data indicated the periodical increase in total biomass of maize, the biomass found to be increased with the advancement of crop

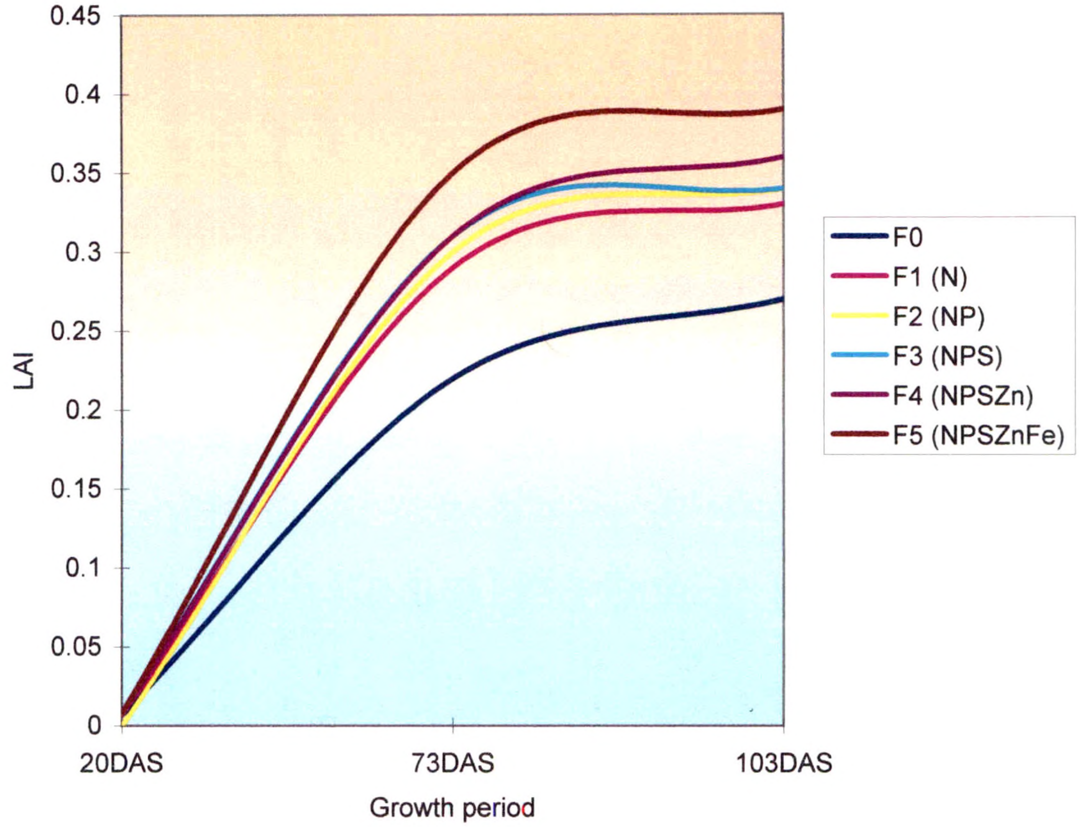


Fig. 4.1. LAI as influenced by fertility levels

growth. The average increase in biomass recovery was from 0.73 g plant^{-1} to 365.6 g plant^{-1} within 83 days. Influence of fertility levels was recognizable and seen in the data presented in the Table 4.7 on an average lowest biomass of 0.73 g plant^{-1} was recorded in control treatment at 20 DAS which was increased upto 365.6 g plant^{-1} at 103 DAS in the treatment received additional nutrient application. At all growth observations application of additional nutrient produced higher biomass. The biomass production under treatment T5 (100:50:50:30:20 N, P_2O_5, K_2O, S, $ZnSO_4$ kg ha^{-1}, respectively), with two iron sprays (at 51 and 73 DAS) produced maximum biomass. It was also evidenced that when sulfur was added to the soil there was boost in growth of maize crop. This indicates that sulfur is becoming deficient in growing media and due care must be taken in future fertilizer programmes. Between the two micronutrients foliar spray of iron contributed more to the growth of maize as compared to zinc application. This might be because of highly calcareous nature of soil, which reduced the soil iron availability and crop responded to foliar application of iron.

Further scrutiny of data of Table 4.7 revealed that the total biomass and cob yield was lowest (143.2 and 41.26 q ha^{-1}, respectively) in unfertilized plot and yield was improved in nutrient added plots. The total biomass and cob yield increase in N applied treatment was from 37.75 q ha^{-1} and 20.24 q ha^{-1}. In addition to N, when P was applied the biomass and cob yield was increased to the level of 191.0 and 61.01 q ha^{-1}, respectively. It was noted in the present investigation that in addition to N and P when sulfur was added there was boost in total biomass and cob yield. The additional application of zinc and iron further increased the total biomass and cob yield. Among the two micronutrients applied, spraying of iron contributed more in respect of total biomass and cob yield, which was to the tune of 197.1 and 81.59 q ha^{-1}. These results showed that foliar sprays of iron satisfied the iron hunger of plants which was because of high calcium carbonate content in the soil (lime induced iron deficiency).

Table 4.7. Effect of fertility levels on biomass and economic yield (Cob yield)

Treatments	**Biomass (g plant^{-1})**				**Total biomass at harvest (q ha^{-1})**	**Cob yield (q ha^{-1})**
	20 DAS	**73 DAS**	**103 DAS**	**Mean**		
F_0	0.63	25.81	285.60	104.0	143.2	41.26
F_1 (N)	0.65	28.92	369.70	133.09	189.5	60.86
F_2 (NP)	0.71	33.18	374.70	136.19	187.0	61.01
F_3 (NPS)	0.80	47.73	385.30	144.61	194.9	73.06
F_4 (NPSZn)	0.80	48.68	386.70	145.39	195.6	74.15
F_5 (NPSZnFe)	0.84	52.30	391.70	148.28	197.1	81.59
Mean	0.73	39.43	365.60	135.25	84.55	65.32
SEm±	0.014	0.544	6.795	--	33.97	7.68
CD at 5%	0.042	1.638	20.44	--	102.38	23.21

The results discussed in above paragraphs (Table 4.4 to 4.7) on various growth parameters viz., height of plant, number of leaves, leaf area index (LAI), total biomass per plant, total biomass per hectare at harvest and cob yield of maize revealed that all the listed growth parameters and yield were found to be improved due to each additional nutrient viz., N, P, S, Zn and Fe. The height of plant boosted after 20 DAS

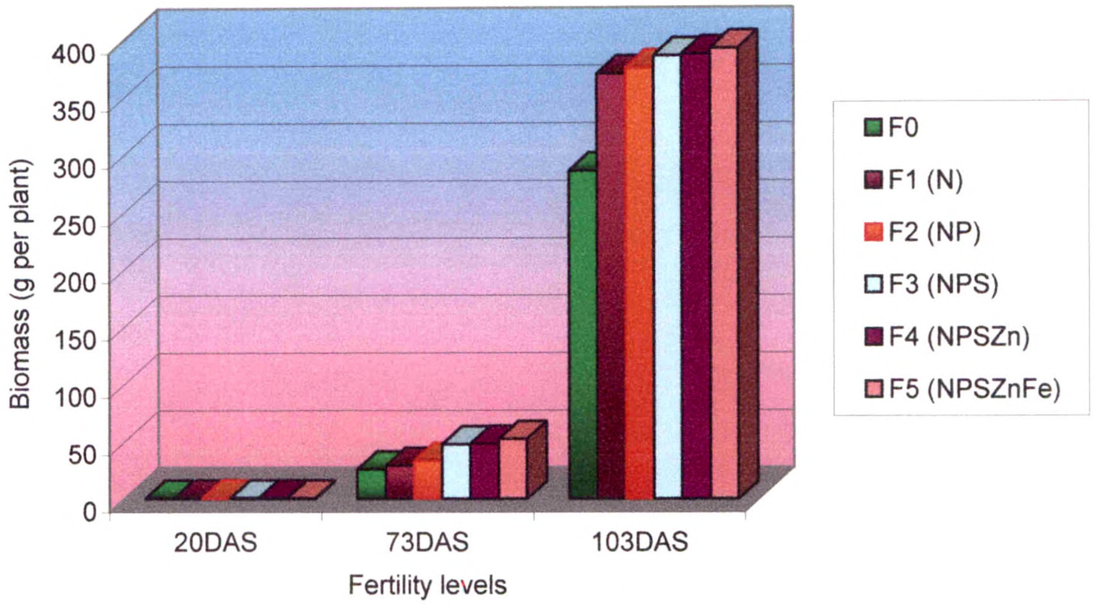


Fig. 4.2a. Biomass per plant as influenced by fertility levels

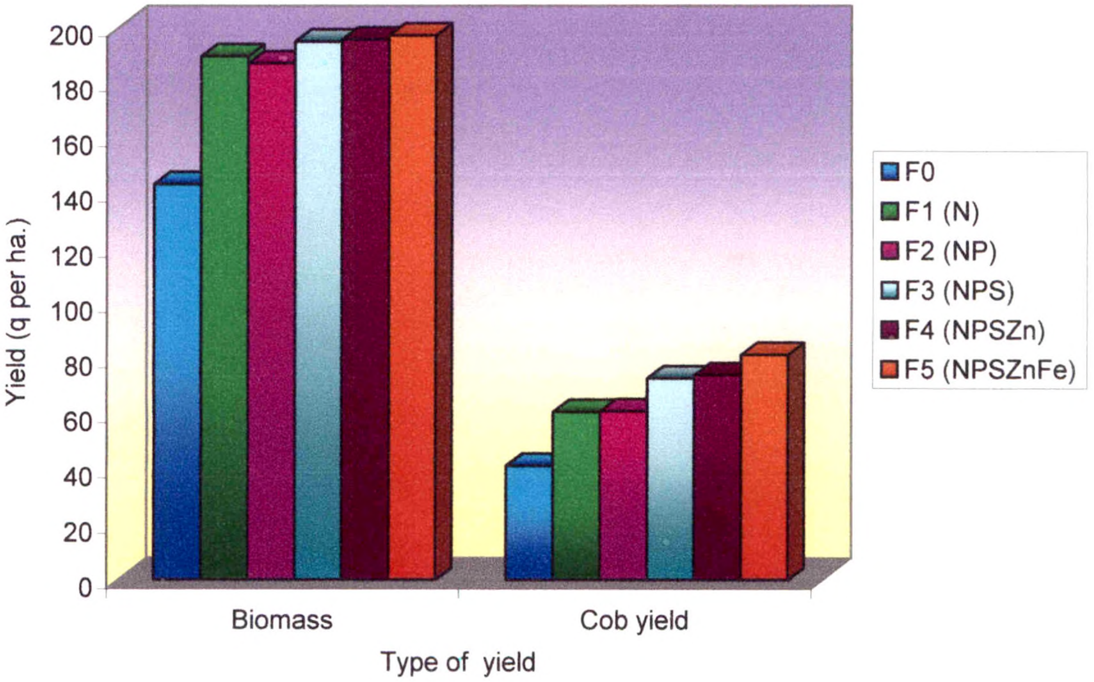


Fig. 4.2b. Total biomass and cob yield as influenced by fertility levels

in all the treatments. This may be because of top dressing of remaining dose of nitrogen. Further, it was also noticed that application of N, N + P, N + P + S, N + P + S + Zn, N + P + S + Zn + Fe sprays significantly increased height of maize over control. Shivay and Singh (2000) and Petkar (2004) recorded increase in height and growth parameters of maize due to application of nitrogen. Even though the rate of increase of height of fertilized crop was always higher than the control plot at all observations, the increase of height was at its lower magnitude till 20 DAS. The treatment differences were broadened at 73 DAS and at 103 DAS. This low initial growth rate might be because of initial time taken by crop for its acclimatization with soil and climate. Among P, S, Zn and Fe application, application of sulfur and iron showed higher increase in height, number of leaves, leaf area and total biomass at all growth stages and even cob yield. These results confirmed the response of sulfur and iron in the experimental soil. Day by day soils are becoming deficient in sulfur. Patil and Mali (2000) reported nearly 30 % soils of Parbhani and Latur districts of Marathwada are deficient in sulfur. They also observed the response of sulfur application to sunflower, soybean, safflower and groundnut. Visual observations confirmed the deficiency of N, S and Fe. The maize leaves in F_0 treatment were very small, yellowish internodes were shortened. The poor growth of maize in control plot is attributed to the low supply of nitrogen, sulfur and iron. Such effects due to low nutrient supply were noticed by Petkar (2004). Leaf area index is one of the important parameters that affect the growth of plant. Application of nitrogen had profound influence on leaf area index. There was nearly 50% leaf area increase over no nitrogen application. Further addition of phosphorus, sulfur and zinc to soil and foliar sprays of iron increased the leaf area index. Relatively more increase was observed from 20 DAS to 73 DAS. This period coincides with grand growth period of maize and top dressing. The balanced fertilization with increase in treatment number improved the growth parameters and biomass production. The increase was from 0.73 g plant^{-1} to 365.60 g plant^{-1} within 83 days. Similarly, with addition of nitrogen, phosphorus, sulfur, zinc and iron to soil there was increase in total biomass. It is established fact that the application of fertilizer nutrient improves the growth parameters (Heege, 2001).

4.5 Effect of fertility levels on chlorophyll content of maize

Among the various pigments that influence the spectral signature of vegetation is chlorophyll concentration in the leaves. It contributes more than 70 % to spectral reflectance and hence in the present investigation efforts were made to find out the effect of nutrient applications on chlorophyll 'a', chlorophyll 'b' and total chlorophyll and to study the relationship between chlorophyll concentration and spectral reflectance. The relevant data is presented in Table 4.8, 4.9 and 5.0 and Figure 4.3.

4.5.1 Effect of fertility levels on chlorophyll 'a'

On an average chlorophyll 'a' concentration of maize was found to be increased with growth of maize crop upto 73 DAS. Thereafter there was decrease in chlorophyll 'a' concentration. The increase was from

0.182 to 0.613 mg g^{-1}. It was further noted that application of nitrogen over no nitrogen. (Treatment F_1) enhanced the chlorophyll content at all growth stages. The involvement of nitrogen in chlorophyll synthesis is of vital importance. The nitrogen increases the chlorophyll concentrations in plants. Further, treatments i.e. F_2, F_3 and F_4 did not show significant influence on chlorophyll 'a', synthesis. However, F_5 treatment i.e. F_4 spraying of 2 % iron increased the chlorophyll concentration of leaves. The lowest chlorophyll 'a' content was recorded in control treatment and highest chlorophyll content was observed in the treatment receiving complete nutrient package (N+ P + S + Zn + Fe).

Table 4.8. Effect of fertility levels on chlorophyll 'a' content (mg g^{-1})

Treatments	**Chlorophyll 'a' (mg g^{-1})**			
	20 DAS	**73 DAS**	**103 DAS**	**Mean**
F_0	0.14	0.50	0.45	0.36
F_1 (N)	0.18	0.61	0.60	0.46
F_2 (NP)	0.19	0.65	0.62	0.48
F_3 (NPS)	0.18	0.65	0.65	0.49
F_4 (NPSZn)	0.18	0.60	0.57	0.45
F_5 (NPSZnFe)	0.21	0.67	0.62	0.50
Mean	0.18	0.61	0.58	0.42
SEm±	0.022	0.037	0.067	--
CD at 5%	0.068	0.111	0.201	--

4.5.2 Effect of fertility levels on chlorophyll 'b'

The data on chlorophyll 'b' content in maize leaves are presented in Table 4.9. At various growth stages and under various fertility levels chlorophyll 'b' showed a similar pattern as that of chlorophyll 'a'. However, chlorophyll 'b' concentration was relatively more than chlorophyll 'a'.

Table 4.9. Effect of fertility levels on chlorophyll 'b' content (mg g^{-1})

Treatments	Chlorophyll 'b' (mg g^{-1})			
	20 DAS	**73 DAS**	**103 DAS**	**Mean**
F_0	0.12	0.43	0.47	0.34
F_1 (N)	0.16	0.69	0.52	0.45
F_2 (NP)	0.17	0.75	0.75	0.55
F_3 (NPS)	0.18	0.90	0.65	0.57
F_4 (NPSZn)	0.20	0.74	0.65	0.53
F_5 (NPSZnFe)	0.28	0.74	0.58	0.53
Mean	0.18	0.70	0.60	0.49
SEm±	0.022	0.007	0.141	--
CD at 5%	0.067	0.022	0.427	

4.5.3 Effect of fertility levels on total chlorophyll

Total chlorophyll concentration of fresh maize leaves are given in Table 4.10 and Figure 4.3. The data indicated that chlorophyll concentration in maize leaves ranged from 0.37 to 1.36 mg g^{-1} with an average of 1.00 mg g^{-1}. With the advancement of growth of maize, the total chlorophyll concentration was increased upto 73 DAS. At 103 DAS the total chlorophyll concentration was reduced from 1.52 to 1.28 mg g^{-1}. Further, only nitrogen fertilization contributed significantly to total chlorophyll production followed by the additional P + S + Zn + Fe fertilization (Treatment 5). It was to the extend of 23.75 and 17 per cent.

Table 4.10. Effect of fertility levels on total chlorophyll (mg g^{-1})

Treatments	**Total chlorophyll (mg g^{-1})**			
	20 DAS	73 DAS	103 DAS	Mean
F_0	0.31	0.91	1.11	0.77
F_1 (N)	0.38	1.37	1.24	0.99
F_2 (NP)	0.39	1.42	1.27	1.02
F_3 (NPS)	0.39	1.51	1.31	1.07
F_4 (NPSZn)	0.36	1.53	1.31	1.03
F_5 (NPSZnFe)	0.42	1.43	1.56	1.13
Mean	0.37	1.52	1.28	1.00
SEm$\pm$	0.050	0.100	0.219	--
CD at 5%	0.151	0.302	0.659	--

Chlorophyll concentration of the leaves influences the leaf biochemical properties and biochemical interactions are the results of molecular / atomic composition of the leaf. In turn they are responsible for colour changes resulting from differences in pigment concentration. In this whole chain, nitrogen, sulfur and iron play important role. In the present study chlorophyll 'a', chlorophyll 'b' and total chlorophyll was found to be influenced by the application of essential nutrients particularly N, S and Fe. With the advancement of growth upto silk formation of maize the chlorophyll concentration in the leaves has increased. Patil and Malewar (1994) reported similar findings in cotton crop. Similarly, Gausman *et al.* (1973) showed the role of nitrogen in chlorophyll synthesis.

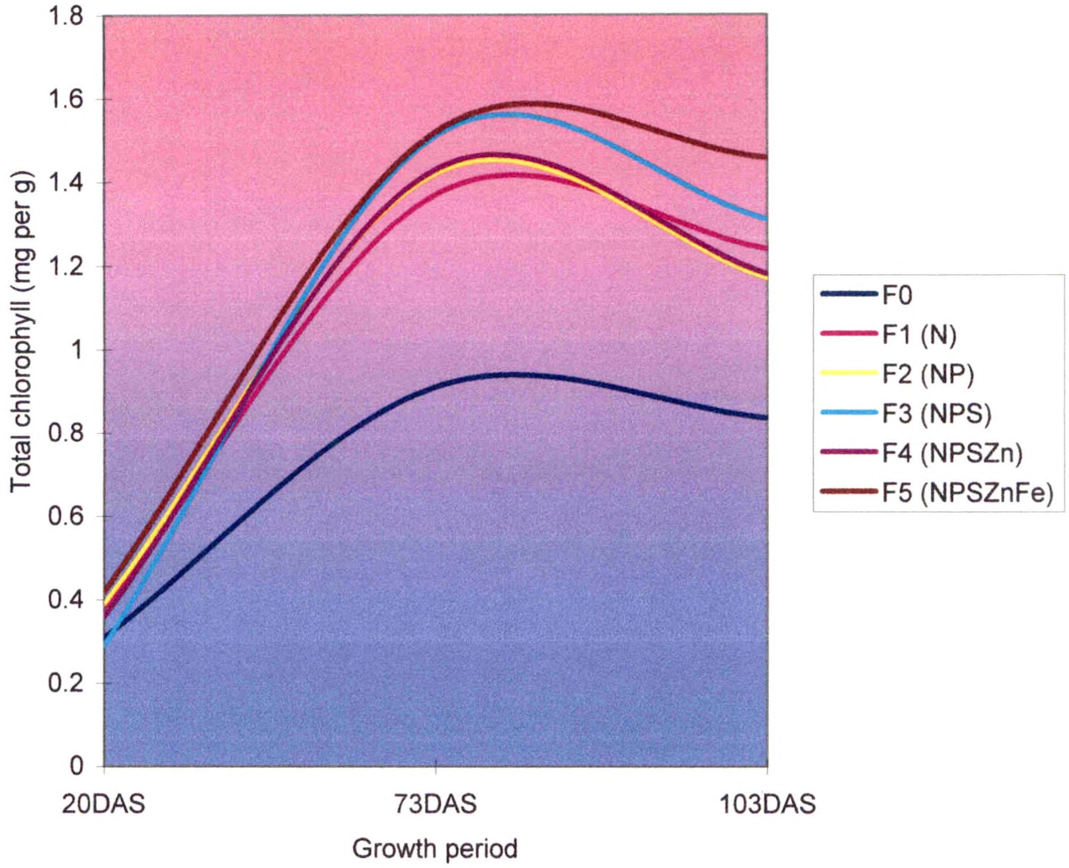


Fig. 4.3. Total chlorophyll concentration as influenced by fertility levels

4.6 Concentration of chlorophyll and other plant pigments in Acalypha and Golden duranta

Leaf pigment provides valuable insight to the physiological performance of leaves. Measurement of pigment concentration focus light on the behaviour of crop canopies towards dry matter production. Therefore, the data related to the various plant pigments like chlorophyll 'a', chlorophyll 'b', total chlorophyll, anthocyanin, lycopene and B-carotene in Acalypha and Golden duranta plant species are presented in Table 5.1. The four species of Acalypha and four locations of Golden duranta species were selected and the pigments were determined. It is interested to note that Acalypha species had very high chlorophyll concentrations as compared to Golden duranta. The chlorophyll 'a', 'b' and total chlorophyll in Acalypha species were ranged from 0.46 to 0.58, 0.46 to 0.60 and 0.92 to 1.263 mg g^{-1}, respectively. While in Golden duranta the chlorophyll a, b and total chlorophyll was varied between 0.05 to 0.09, 0.03 to 0.05 and 0.09 to 0.14 mg g^{-1}. it shows nearly ten times more chlorophyll content in Acalypha. Further, the anthocyanin, a group of plant pigments was more in Acalypha and it is at lower magnitude in Golden duranta. The Acalypha leaves are thick, dark red in colour with varied degrees of red hues (Plate 5). The anthocyanin content in these leaves was varied between 53.09 mg g^{-1} to 43.27 mg g^{-1}. While Golden duranta leaves are thin and visible yellowish in hues (Plate 6). It shows low anthocyanin pigment (2.54 to 7.63 mg g^{-1}) concentrations as compared to Acalypha.

The lycopene content in Acalypha ranged between 0.83 to 0.98 mg g^{-1} with an average of 1.44 mg g^{-1}. Whereas relatively more lycopene content (0.187 to 2.45 mg g^{-1}) was recorded in Golden duranta plants. The average lycopene content in Golden duranta was 1.64 mg g^{-1}.

The data on carotenoids pigments in the Acalypha and Golden duranta are presented in Table 5.1. It is very clear that the carotenoids pigment concentration was at its higher range in Golden duranta. The average carotenoids pigment concentration in Golden duranta and Acalypha was 1.24 and 0.93 mg g^{-1}, respectively.

Table 4.11. Concentration of chlorophyll and other plant pigments in Acalypha and Golden duranta (mg g^{-1})

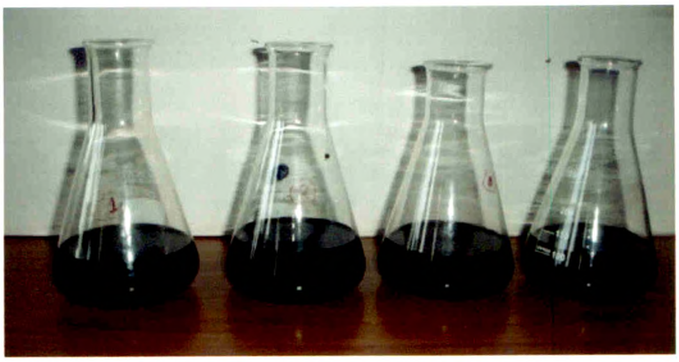
Plant Species	Chloro-phyll 'a'	Chloro-phyll 'b'	Total chlorophyll	Antho-cyanin	Lycopene	Carotenoids pigments
Acalypha						
***Aca1**	0.46	0.46	0.92	35.64	0.83	0.39
Aca2	0.56	0.52	1.16	43.27	1.46	0.96
Aca3	0.58	0.60	1.26	33.09	1.52	0.97
Aca4	0.58	0.55	1.23	35.64	1.98	1.43
Mean	0.54	0.53	1.14	36.91	1.44	0.93
Golden duranta						
****GD 1**	0.05	0.03	0.09	5.09	0.87	0.57
GD 2	0.08	0.05	0.14	2.54	1.68	1.13
GD 3	0.09	0.05	0.14	7.63	1.57	1.27
GD 4	0.07	0.04	0.12	2.54	2.45	1.95
Mean	0.07	0.04	0.12	4.45	1.64	1.23

* Aca – Acalypha ** GD – Golden duranta

The results presented in above paragraphs showed that the Acalypha species posses high concentration of chlorophyll and anthocyanin while Golden duranta had high concentrations of lycopene and carotenoids. The visual observations of Acalypha and Golden duranta species also support the quantitative determinations of these pigments. Acalypha being

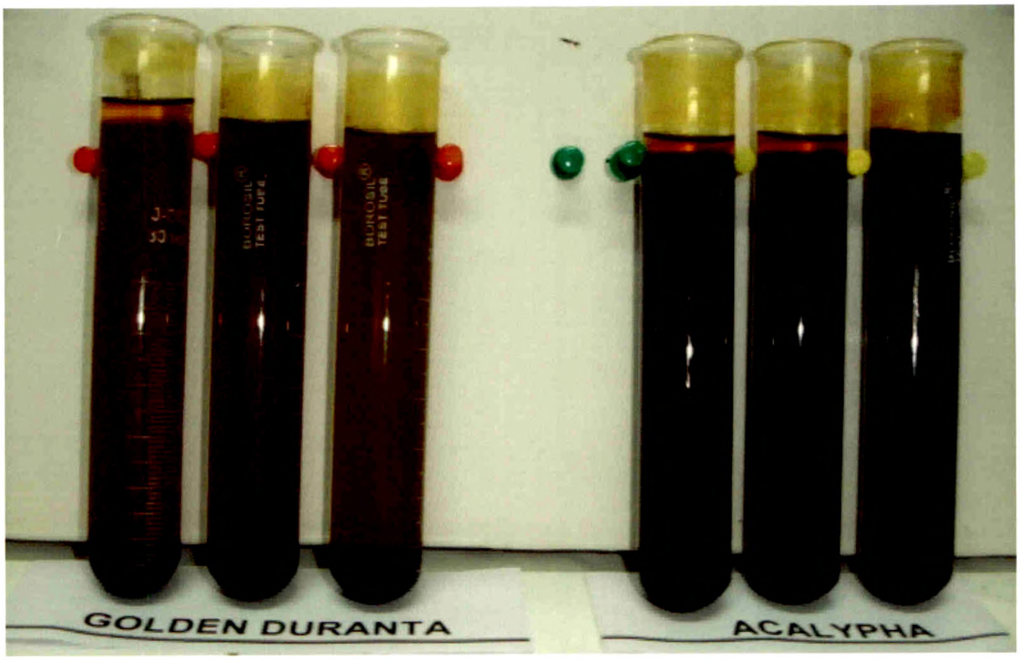
PLATE-7

Chlorophyll in Golden duranta



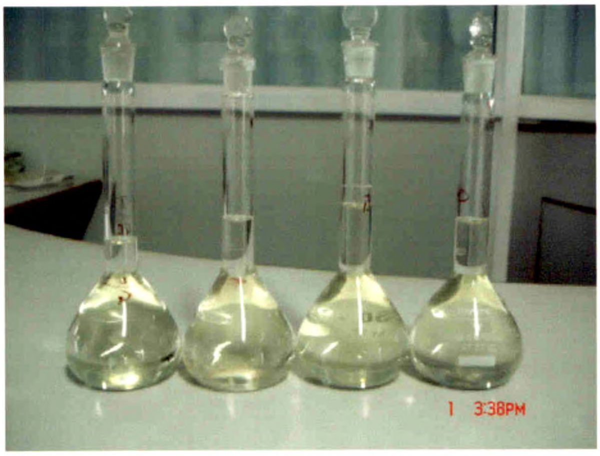
Chlorophyll in Acalypha

PLATE-8

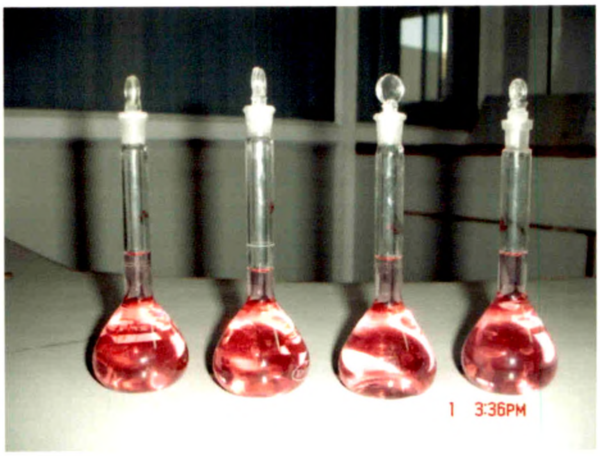


Carotenoids + lycopene in Golden duranta and Acalypha

PLATE-9



Anthocyanin in Golden duranta



Anthocyanin in Acalypha

dominant in red hue showed higher anthocyanin content while the lower portion of Acalypha leaves green in colour which might be responsible for higher concentration of chlorophyll in Acalypha. The yellowish hue of Golden duranta support the higher concentration of carotenoids pigments which was observed in the present research. In general in early stage of crop growth the chlorophyll pigment dominates and masks the effect of anthocyanin pigments while at later stages the chlorophyll concentration reduce down and anthocyanin pigments dominates and impart reddish colour to the leaves by unmasking the anthocyanin and carotenoids pigments (Gausman *et al.*, 1973).

4.7 Effect of fertility levels on spectral reflectance by maize canopy

Spectral reflectance of leaves is influenced primarily by plant pigments and leaf structure. The concentration of plant pigments is influenced by the availability of nutrients in the growth supporting media. In the present study the spectral reflectance by maize canopy was studied. Under varying levels of fertility, so as to create varied chlorophyll concentrations in the leaves of maize crop. Among the various four spectral bands available with Optomech Ground Truth Radiometer band 3 and band 4 i.e. Red wavelengths and NIR wavelength bands were used. As chlorophyll absorbs red wavelength and healthy vigorous leaves reflect NIR and hence Band 3 and Band 4 were selected. The data collected on spectral radiance in band 3 and band 4 are converted into reflectance and the observations are depicted in Figure 4.4 and 4.5

4.7.1 Effect of fertility levels on canopy reflectance of maize in Red and NIR bands

The Figure 4.4 depicts the behaviour of maize canopy during its crop growth in red band. It is observed that the red wavelength (0.62 to 0.68 um) reflectance was more till the maize attain 40 days growth thereafter the red wavelength absorption increased by maize canopy upto 53 days after sowing. Again as growth proceeded beyond 53 days the red reflectance starts increasing and it was maximum at 111 DAS. This sigmoid behaviour of graph confirm the hypothesis that red wavelength is absorbed more if chlorophyll concentration is more and as chlorophyll concentration decrease the red wavelength absorption also decreases. In the present study in early growth stage of crop exposed low leaf area, less chlorophyll concentration and high soil area and hence red absorption was less and reflectance was more. As leaf area and concentration of chlorophyll increased with advancement of crop growth, red reflectance was lowered down and absorption was increased. It is also noticed that all fertilizer levels treatments curves behaves in a similar way. However, there was relatively more absorption of red wavelength by treatment F_5 and less absorption of red wavelength by F_0. This might be because of F_5 treatment provided balanced supply of nutrients and F_0 was devoid of these nutrients. The maximum absorption was at 53 DAS. In a previous sub head 4.5, it is recorded that the chlorophyll concentration was increased upto 73 DAS and later on it was decreased which was responsible for behaviour of spectral reflectance curve.

Soil reflectance dominates the canopy reflectance

Canopy reflectance dominates the soil reflectance

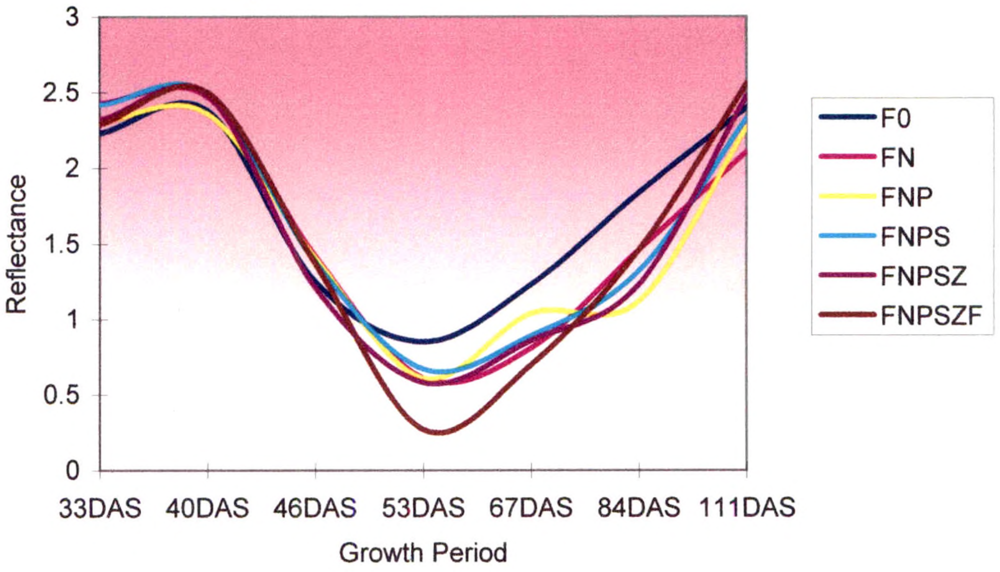


Fig. 4.4. Red reflectance as influenced by Fertility levels

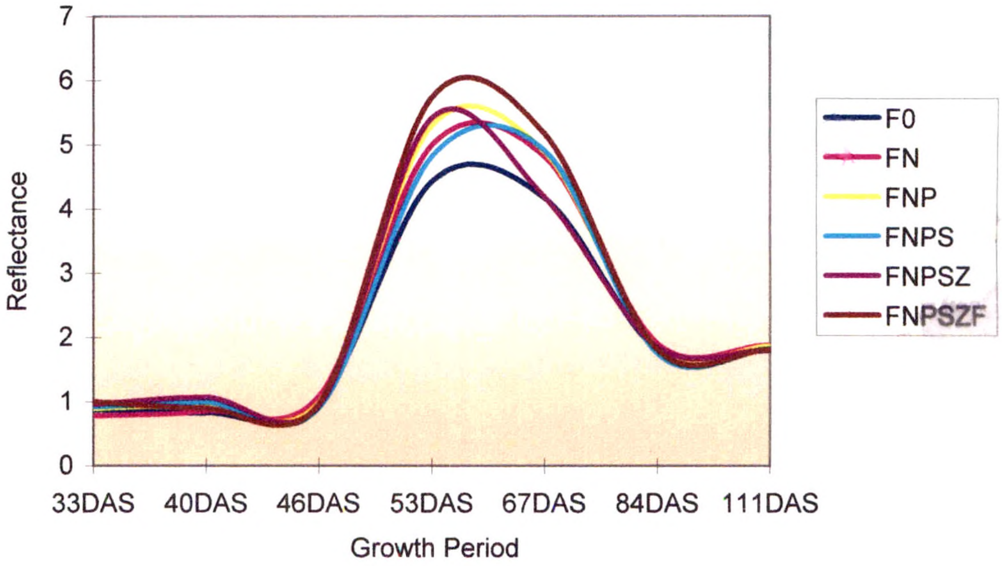


Fig. 4.5. NIR reflectance as influenced by Fertility levels

Figure 4.5 shows the NIR reflectance as influenced by various fertility levels during crop growth period from 33 DAS to 111 DAS. The NIR reflectance is almost a opposite mirror print of red reflectance. The NIR wavelength reflectance was lower upto 40 DAS and thereafter it was increased and peak at 53 days observation and remained there upto 67 DAS. The NIR reflectance decreased thereafter. This shows that the crop growth and leaf area attained maximum development rate during 53 to 67 days. Further the NIR reflectance was more in F_5 treatment between 53 to 67 DAS and it was low in F_0 treatment.

Thus, the result presented under subhead 4.7 showed that in early growth of maize (upto 46 DAS) the red reflectance was maximum and NIR reflectance by maize canopy was minimum. After 46 DAS the red reflectance was decreased because of increasing chlorophyll content while NIR reflectance was increased due to increased leaf area and vigour of crop. Further, in early stage of crop the spectral reflectance was dominated by soil and during grand growth period of maize leaf canopy dominates the spectral reflectance. It was inferred that fertility levels were differentiated by spectral measurements between 53 DAS to 67 DAS.

The results presented and discussed above are closely related with the observations drawn by Curran *et al.* (1995), Petkar (2004) and Patil *et al.* (2002) and Patil *et al.* (2007).

4.7.2 Effect of fertility levels of RVI, NDVI, SAVI and IPVI.

Effect of fertility levels on various spectral indices are depicted in figure 4.6 to 4.9.

The NIR and Red reflectance of maize canopy influenced by various nutrient addition treatments were collected to calculate spectral indices in order to find out the effect of chlorophyll and other parameters on spectral indices.

The simple NIR/R ratio (i.e. RVI) influenced by fertility levels is depicted in figure 4.6. As observed in a typical vegetation reflectance spectrum. Wavebands of red region were mostly absorbed by chlorophyll pigments. While, wavebands on NIR region were reflected significantly due to less absorption. So, the ratio NIR/R i.e. RVI gives idea about vigour or healthiness of crop. In the present research programme spectral index RVI show variations between 46 to 84 DAS. The differences among the treatments were very clear at 53 DAS (Figure 4.6). Further, the maize crop received no nutrient showed lowest RVI, while on the addition of nutrient to soil resulted in increase in RVI. The higher RVI RVI value was seen with the fertility level receiving recommended nitrogen, phosphorus, sulfur, zinc, through soil application and iron through foliar application (F_5), followed by F_4 treatment, same as that of F_5 except iron sprays. Further, the treatment F_0, F_1, F_4 and F_5 showed clear variations due to RVI. However, the effect due to addition of phosphorus was not recorded by RVI. This might be because of the fact that the role of phosphorus in chlorophyll synthesis is indirect, whereas clear discrimination due to nitrogen, sulfur and iron application in RVI was because of their direct involvement in chlorophyll synthesis. Role of nitrogen and sulfur in improvement of chlorophyll synthesis and increased RVI values were reported by Gole (2004), Kolte (2005) and Ashtikar (2006).

The Normalized Difference Vegetation Index shows the variations under various sets of treatments between 46 to 67 DAS (Figure 4.7). The Maize crop fertilized with N,P, S, Zn and Fe produced maximum NDVI followed by treatment received N, P, S and Zn. Similarly the figure 4.7 depicts clear differences between control plot and fertilized plots.

The Soil Adjusted Vegetation Index depicted in Figure 4.8 clearly shows the growth differences created by fertility levels and were distinctly discriminated by the SAVI ratio. In SAVI the errors due to soil background are minimized, because in SAVI determination coverage of soil by leaf area considered L factor (Where, L is a correction factor which ranged from 0 for very high vegetation cover to 1 for every low vegetation cover).factor L minimizes interference due to exposed soil surface. The most typically used value is 0.5 which is for intermediate vegetation cover. In early growth stage of crop soil reflectance is more as compared to vegetation cover reflectance and hence other spectral indices were not close to the reality. But in SAVI reflectance due to soil and leaves is considered and hence it results into real scenario. SAVI was able to discriminate the fertilized and non-fertilized crop even at early growth stage of maize (i.e. 33 to 46 DAS) as this ratio minimizes the errors occurred due to soil surface exposure.

The effect of fertility levels on growth of maize was not captured by IPVI. The relationship between infra red percentage vegetation index and crop growth was not established properly. Indicating thereby IPVI is not a suitable index for monitoring the growth and detection of chlorophyll pigment. The IPVI consider only photo-synthetically active radiation and hence this might be the reason of unsuitability of IPVI. Under such situations. Petkar (2004) reported that IPVI was unable to discriminate the stressed vs. normal crop. This might be the reason of not getting the relationship.

The RVI, NDVI and SAVI ratios depicted in Figure 4.6, 4.7 and 4.8 showed that all these ratios low in F_0 treatments (no nutrient application) whereas higher in F_5 treatments (i.e. application of N, P, K, S and Zn at rate of 150:50:50:30:20 kg/ha with two foliar sprays of 2 per cent iron at silking and cob development stage. The capability of discrimination of nutrient stress Vs nutrient applied crop was centralized between 46 to 67 DAS (grand growth period of maize). The grand growth period of maize is characterized by high chlorophyll concentration and leaf area. These two parameters influenced the spectral reflectance. Very clear distinction in spectral reflectance was noticed between nutrient applied and nutrient hungry maize. Because fertilizer nutrient application improved the influenced chlorophyll content of leaves and hence red radiation absorption was more by maize canopy. Under nutrient stress condition plants found to have lower NIR reflectance. Jackson and Pinter (1986) confirmed the result of present work. The results become clearer due to additional nutrients, wherein the maize crop grows vigorously and showed high NIR reflectance. This shows that amount and colour of reflected light is closely related to the concentration of chlorophyll in the leaves (Curren *et al.*, 1995). Deficiency of nitrogen (F_0 treatment) resulted in considerable chlorosis of leaves that in turn strongly altered to the leaf reflectance of control treatment. Changes in spectral characteristics of leaves were well

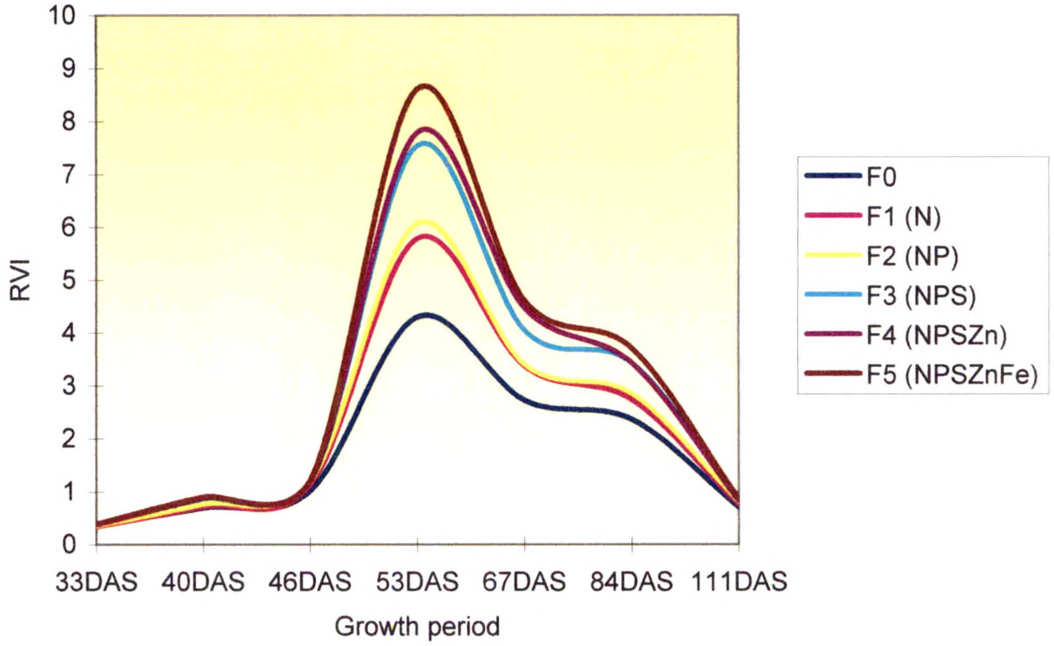


Fig. 4.6. RVI as influenced by fertility levels

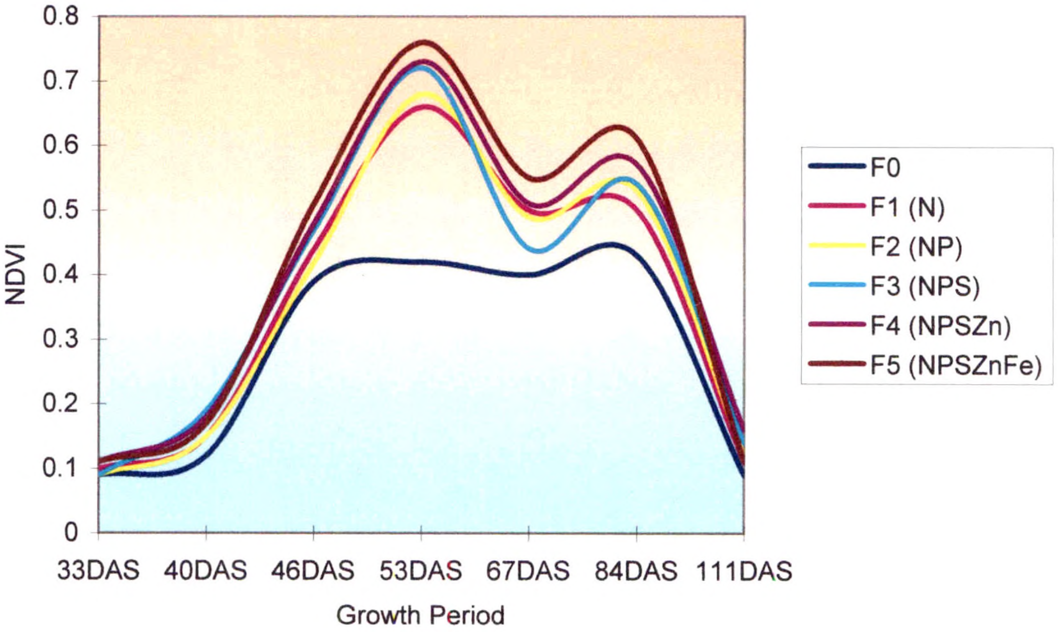


Fig. 4.7. NDVI as influenced by fertility levels

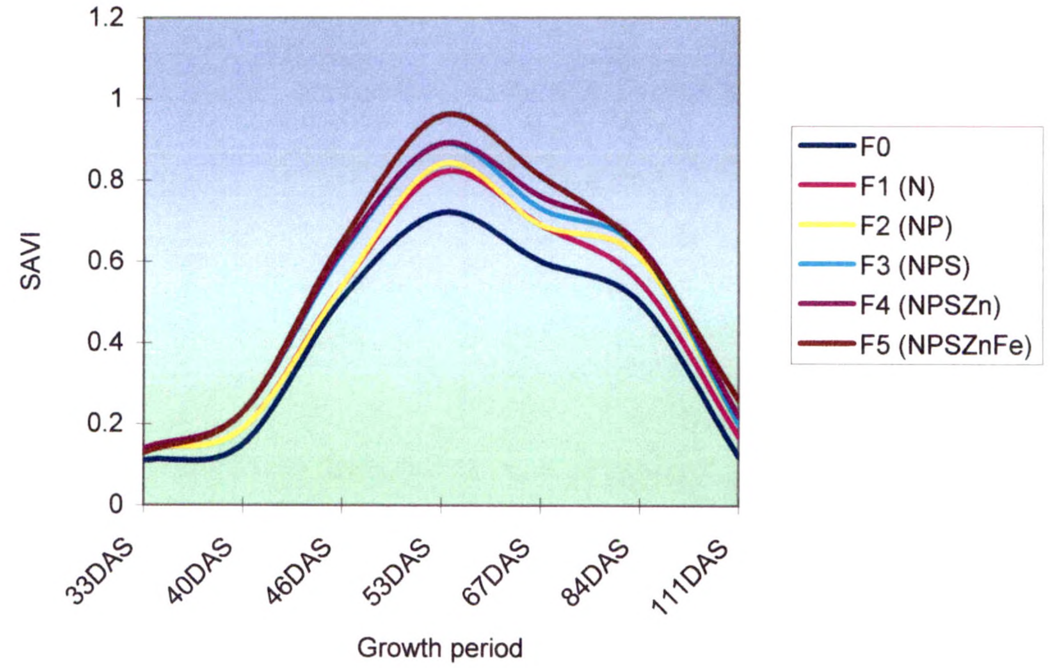


Fig. 4.8. SAVI as influenced by fertility levels

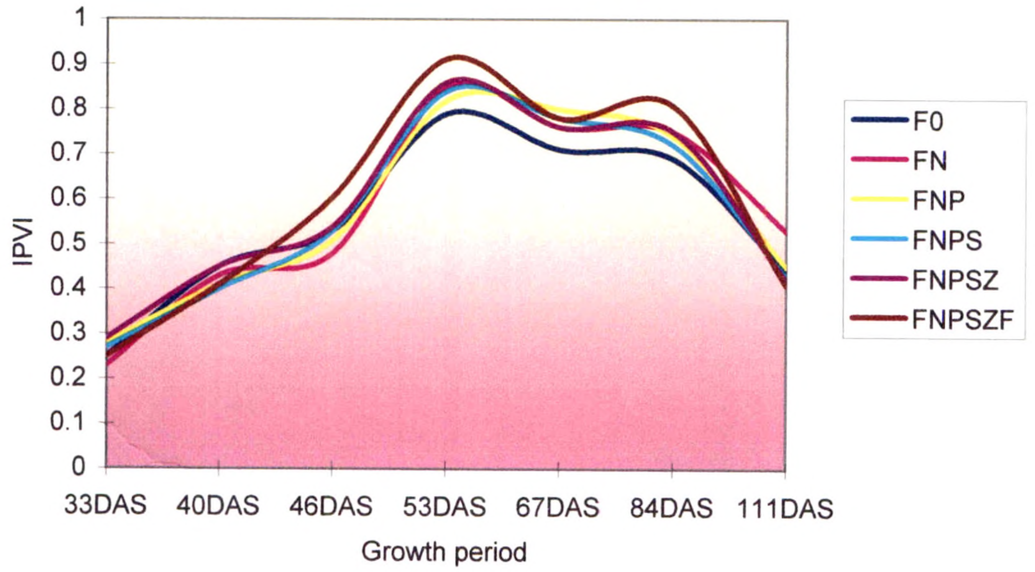


Fig. 4.9. IPVI as influenced by fertility levels

related to the level of chlorophyll and other pigments in the leaves (Vesk *et al.*, 1966). There are many spectral indices available for estimation of chlorophyll concentration of the leaves and vegetation vigour. In this study, RVI, NDVI SAVI and IPVI were tested as a yard stick for assessment of growth of maize. The result shown that except IPVI all other indices performed well. Bausch *et al.* (1996) was in agreement with the present investigation. The infrared percentage vegetation index given by Kriegler (1969) speaks mainly about PAR was unable to discriminate the fertilized and non-fertilized crop.

4.8 Influence of various plant species on spectral reflectance

4.8.1 Influence of various plant species in Red and NIR bands

In the present investigation efforts were made to find out the influence of specific plant species dominating in particular plant pigments on radiation reflectance. To serve this objective three plant species namely maize. Acalypha and Golden duranta which are dominating in chlorophyll, anthocyanin and carotenoid pigments, respectively were selected. The data regarding chlorophyll concentration varied due to nutrient application are discussed in previous chapter. In the following paragraphs influence of Acalypha and Golden duranta plant canopy on spectral reflectance in red and NIR bands are presented (Figure 4.10 and 4.11).

The figure 4.10 shows the red reflectance in Acalypha was lower as compared to Golden duranta. It is seen in previous chapter that the Acalypha had very high concentration of total chlorophyll (1.14 mg g^{-1}) as compared to Golden duranta (0.12 mg g^{-1}). Hence, because of low chlorophyll content in Golden duranta the absorption of red wavelength

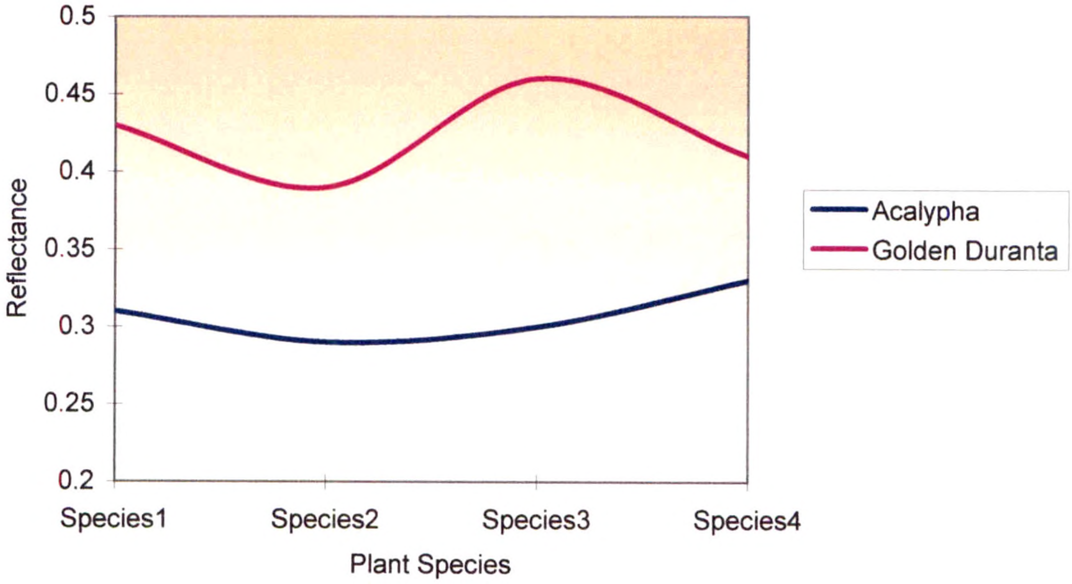


Fig. 4.10. Red reflectance by Acalypha and Golden Duranta

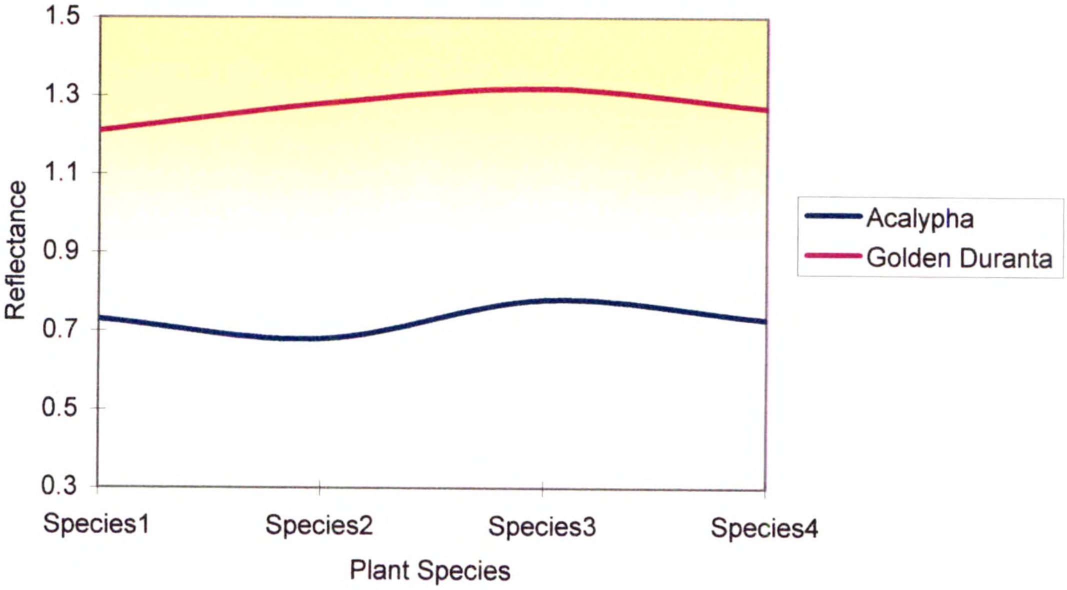


Fig. 4.11. NIR reflectance by Acalypha and Golden Duranta

was less whereas in Acalypha due to high chlorophyll concentration red wavelength absorption was more. Further the Figure 4.11 depicts the behaviour of Acalypha and Golden duranta in NIR region of EMR. The curve obtained in respect of Acalypha and Golden duranta behaves like figure 4.10. NIR reflectance in Golden duranta was higher than Acalypha. The high reflectance of Golden duranta might be because of low thickness of Golden duranta leaves as compared to highly thick leaves of Acalypha species.

4.8.2 Influence of various plant species on spectral indices

The spectral observations collected on Acalypha and Golden duranta in NIR and R bands were transformed into spectral ratios viz., RVI, NDVI, SAVI and IPVI and depicted in the form of figure 4.12 to figure 4.15. All the ratios studied in the present research programme do not set any pattern in respect of spectral indices. Such results obtained might be because of the unsuitability of spectral bands that covers the dominant pigments present in the plant species. The intentionally selected Acalypha and Golden duranta dominating in particular pigments masked the effect of chlorophyll. This shows that the pigments are specific in the wavelength absorption and the plants dominating in carotenoids, anthocyanin and lycopene cannot be monitored by the present ratios that use Red and NIR wavelength bands. Further, it can be stated that the pigments that masked the effect of chlorophyll must be taken into account in spectral reflectance studies. The stage of crop leaf maturity, are also important factors to be considered. Bhavnarayana (2006) reported that in early stage of crop growth chlorophyll concentration dominates and the chlorophyll leads the

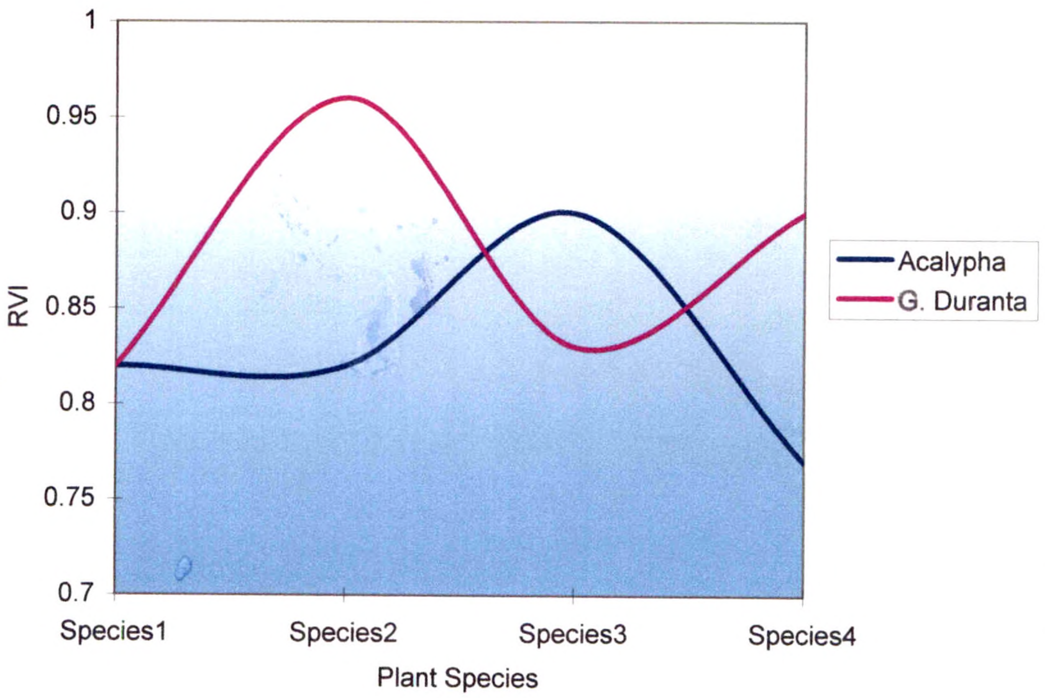


Fig. 4.12. RVI and Plant Species

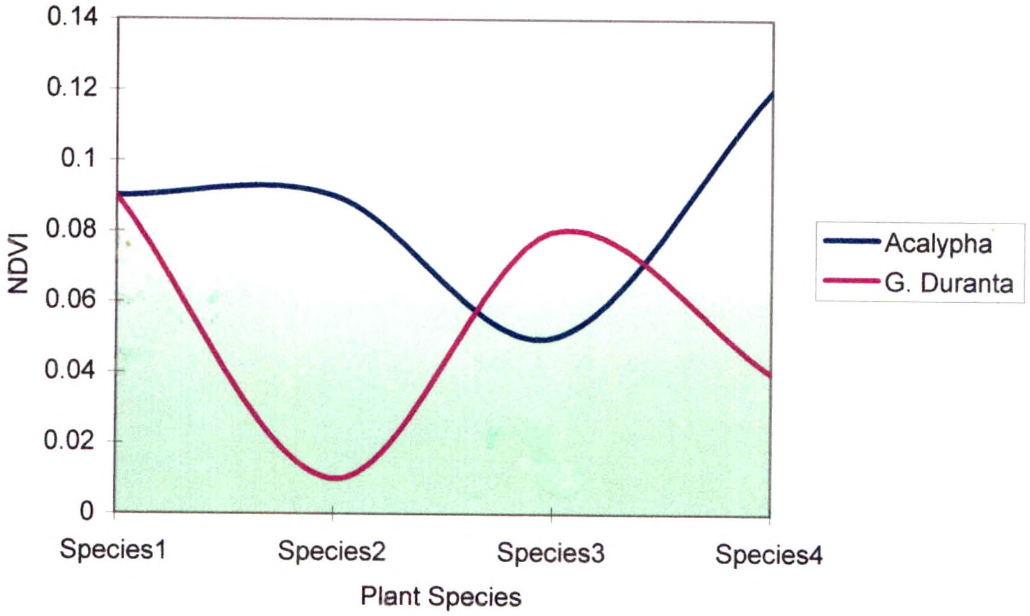


Fig. 4.13. NDVI and Plant Species

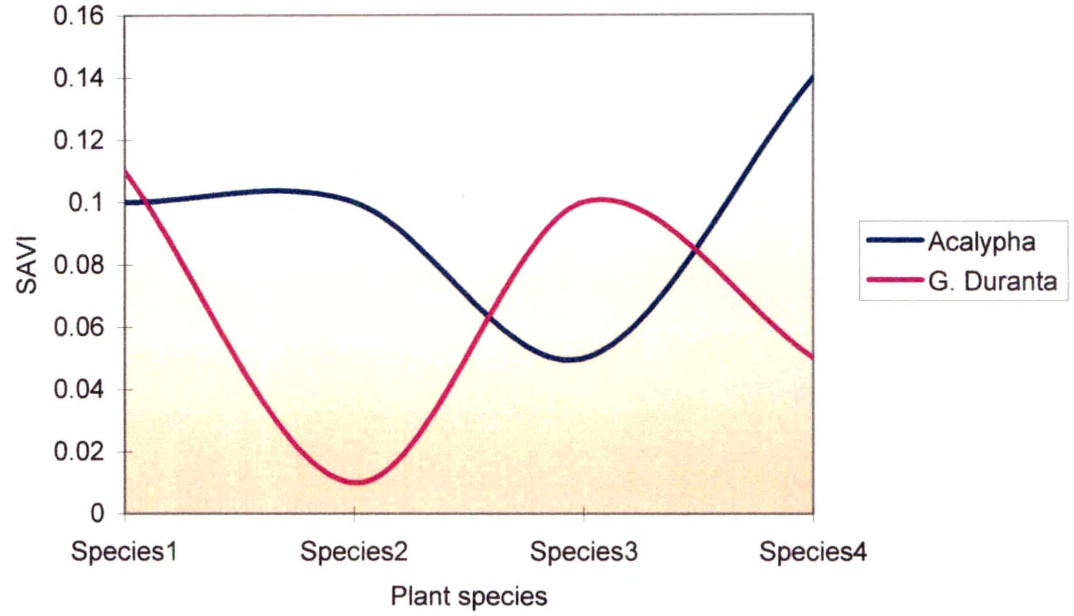


Fig. 4.14. SAVI and Plant species

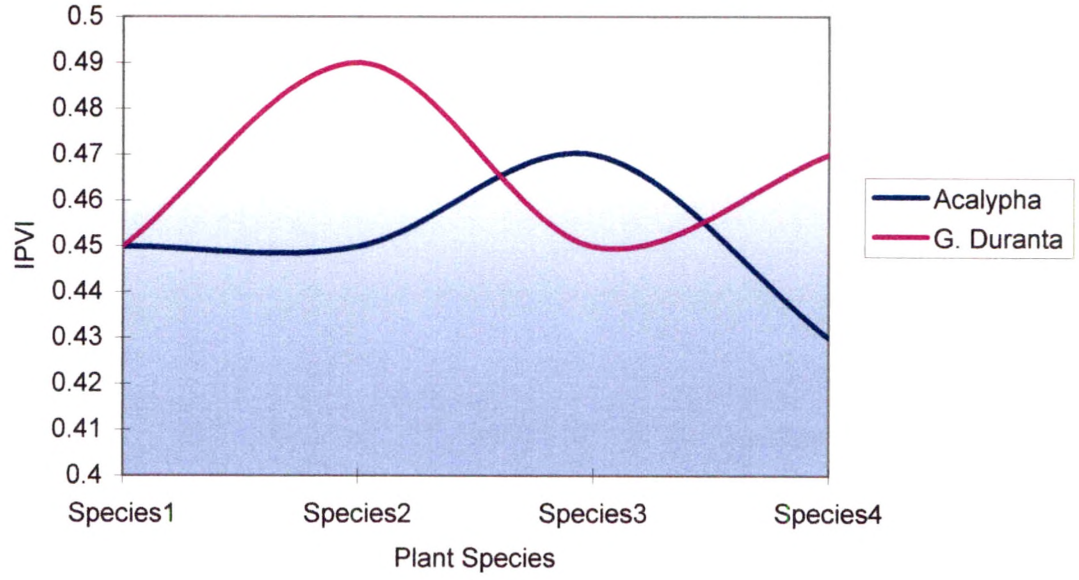


Fig. 4.15. IPVI and plant species

reflectance. This happens because of the carotenoid pigments are masked by chlorophyll and once chlorophyll breaks down later in the growth period, they become prominent and affect the light reflectance. However, as growth proceeds and plant ages other pigments (other than chlorophyll) dominate and contribute to the spectral reflectance. Under this situation the sensors that are sensitive to these pigments must be used. In the present research programme the bands available were Red and NIR. Therefore the contribution of the various pigments towards the spectral reflectance could not be quantified. For in-depth study use of hyper spectral data / narrow band width continuous spectrum instrument can be used. No doubt, this is totally a new area of investigation hence the specific hypothesis is difficult to set. The research programme to be conducted may be of trial and error basis in first phase of investigation. Samson *et al* (2000) suggested to use fluorescent light for studying the plant pigments.

4.9. Influence of fertility levels on thermal behaviour of maize crop canopy

Leaf canopy temperature of maize under various fertility treatments was measured by IR thermometer during growth cycle and depicted in Figure 4.16. It is observed that canopy temperature was not varied upto 73 days after sowing. However, thereafter the nutrient application shows distinguishable effect. Maximum temperature i.e. 31.49°C was recorded in nutrient stress canopy while minimum temperature of 27.63°C was recorded in nutrient sufficient crop canopy the temperature was found to increase with increase in age of the crop.

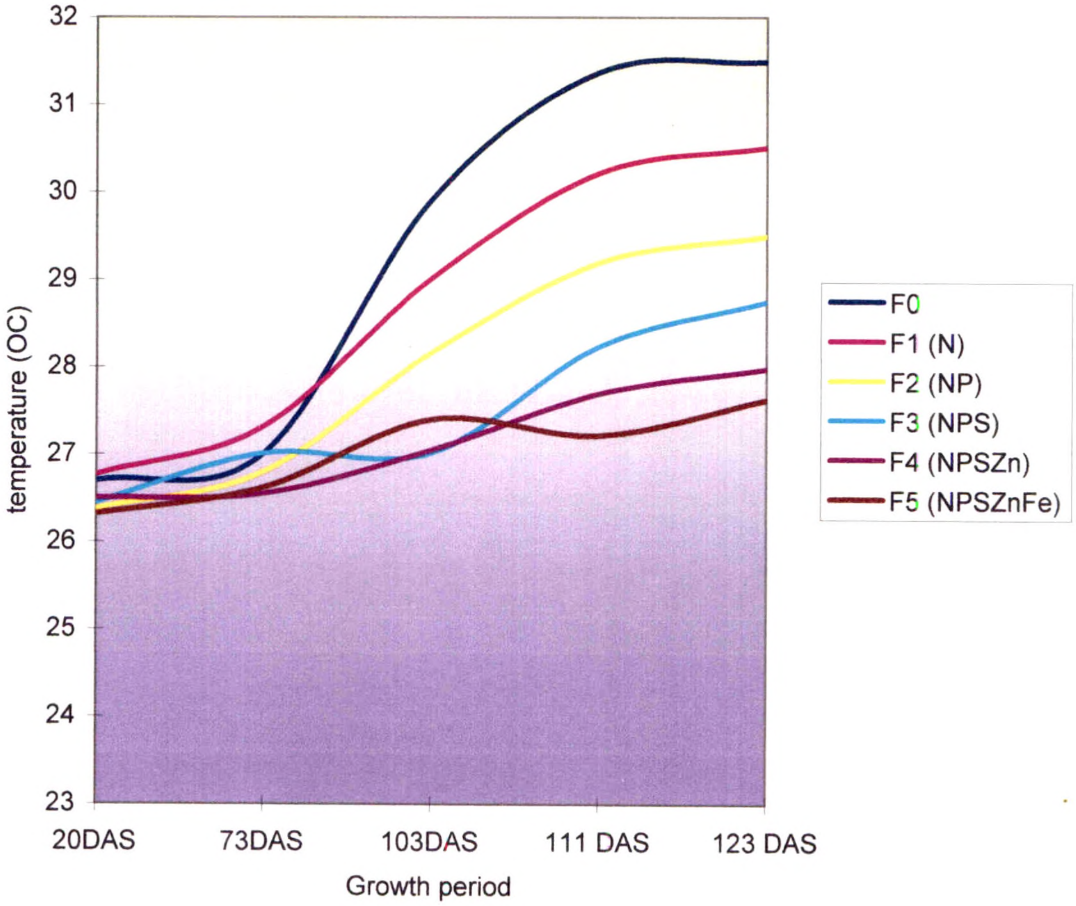


Fig. 4.16. Canopy temperature as influenced by fertility levels

Any undesirable change in a metabolism of a living organism tends to increases its body temperature. That means when stress and strain is observed by living body, body raise its temperature to cope up with undesirable change. Keeping this hypothesis in mind in the observations on canopy temperature was recorded in nutrient stress and nutrient sufficient crop. The results obtained were miraculous, showing high temperature in nutrient stress canopy and low temperature in nutrient sufficient crop canopy. This shows that measurement of canopy temperature can be a useful technique to support the observation in identification of stress of crop.

4.10 Relationship between physiological and spectral parameters in maize

Relationship between various spectral parameters with LAI and total chlorophyll was established and depicted through equations, R^2 value and trend line curves. (Figure 4.17 to 4.22).

4.10.1 Relationship between LAI and spectral indices in maize

The linear relationship between LAI and RVI (Figure 4.17) agree well by showing $R^2 = 0.8027$.The normalized difference vegetation index and LAI was linearly correlated with R^2 value 0.833. Further soil adjusted vegetation index and its relationship with LAI irrespective of growth stages establish linear relationship. R^2 value shows that SAVI established very good relationship with LAI ($R^2 = 0.7259$) and predicted 72 % LAI. The relationship between LAI and IPVI was very poor and hence trend line curve is not reported.

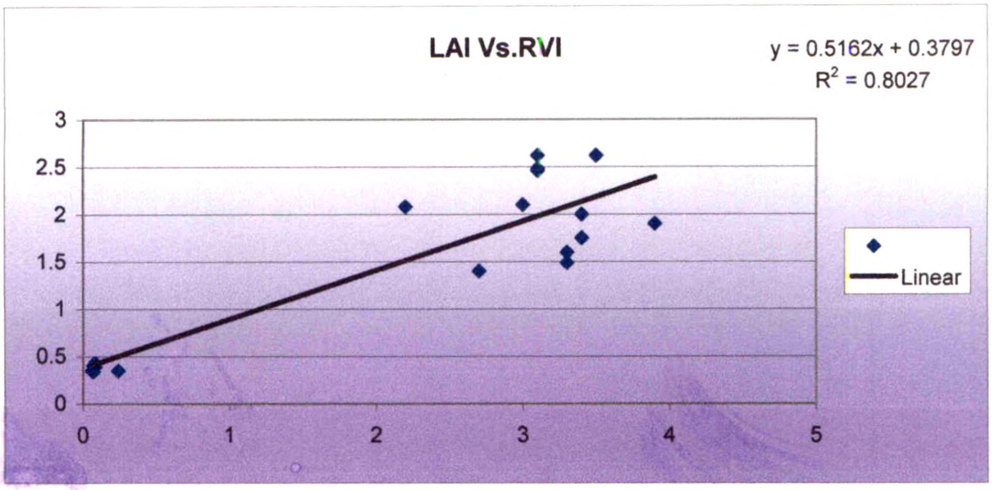


Fig. 4.17. Relationship of LAI with RVI in maize

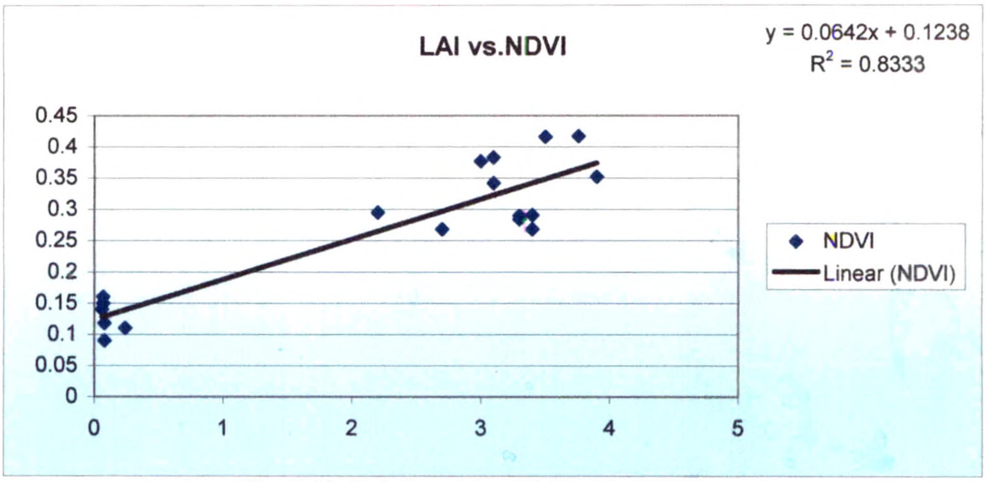


Fig. 4.18. Relationship of LAI with NDVI in maize

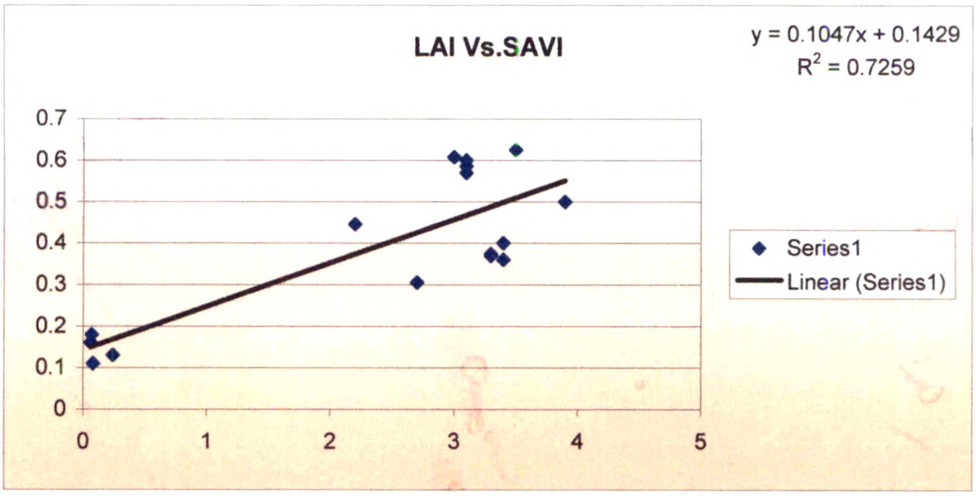


Fig. 4.19. Relationship of LAI with SAVI in maize

4.10.2 Relationship between total chlorophyll and spectral indices in maize

Figure 4.20 to 4.22 presents the total chlorophyll concentration of maize in context with spectral parameters. The total chlorophyll established linear relationship with RVI, NDVI and SAVI predicting 88 per cent, 81 per cent and 72 per cent total chlorophyll concentration, respectively. The equations obtained thereof are $Y = 1.6585 X - 0.2124$ (total chlorophyll Vs RVI), $Y = 0.3943 X - 0.0661$ (total chlorophyll Vs NDVI) and $Y = 0.5199 X - 0.1075$ (total chlorophyll Vs SAVI).

The results obtained from figure 4.17 to 4.22 on relationship between LAI and total chlorophyll with spectral parameters showed that leaf area index modified the spectral reflectance to the extent of 72 to 83 per cent. Filella *et al.* (1995) stated that leaf area index, total green biomass and chlorophyll concentration of leaf modified the spectral reflectance. LAI established significant relationship with RVI, NDVI and SAVI is because of the fact that as growth of crop proceeds LAI was increased and thereby there was increase in NIR reflectance. The chlorophyll pigments absorbs the radiation in 0.45 – 0.52 um (blue) wavelength and 0.62 – 0.68 um (Red) wavelength hence this pigments established significant relationship with spectral reflectance in red band of EMR. (Patil, 2007 and Patil, 2008).

The significant relationship of chlorophyll with spectral parameters is partly because of nutrient concentrations of leaves (Appendix IV –VI) influenced the chlorophyll synthesis and vigorous crop growth.

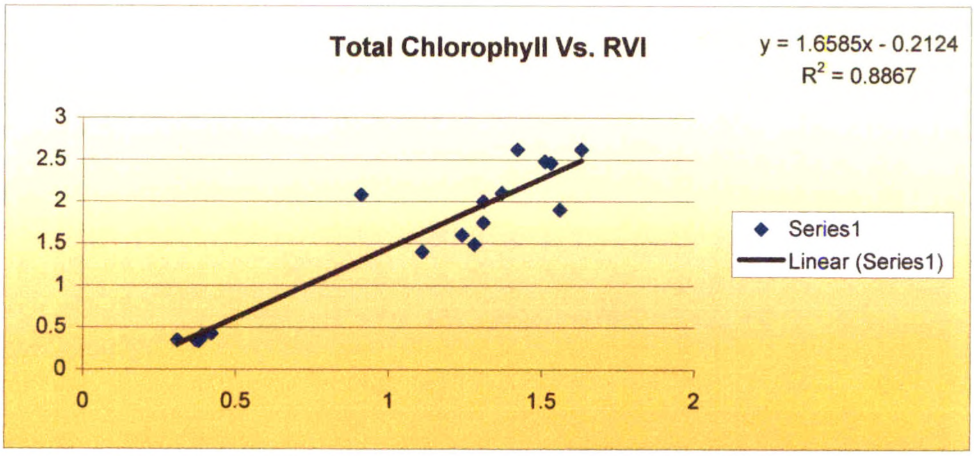


Fig. 4.20. Relationship of total chlorophyll with RVI in maize

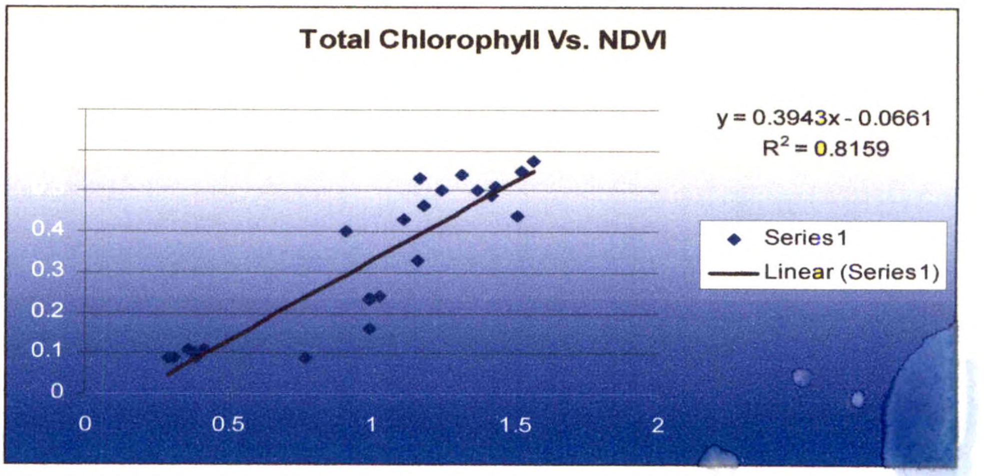


Fig. 4.21. Relationship of total chlorophyll with NDVI in maize

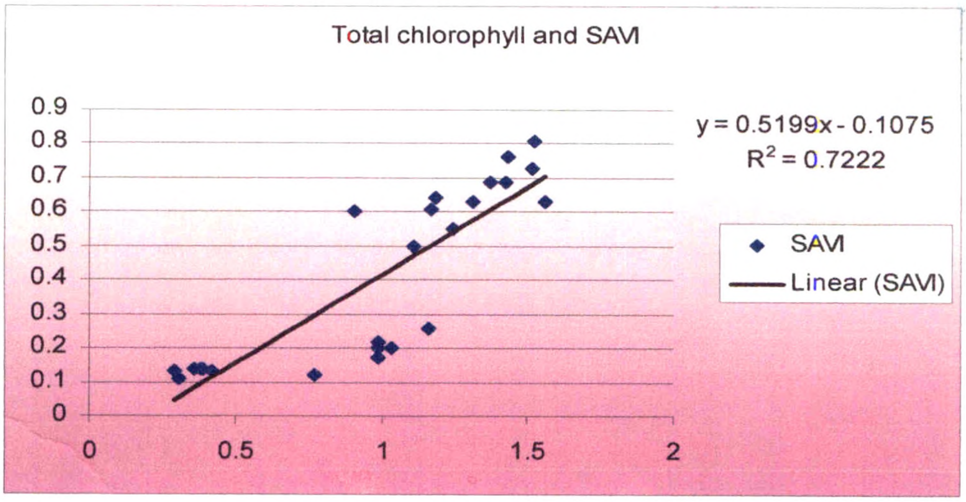


Fig. 4.22. Relationship of total chlorophyll with SAVI in maize

The Semebering *et al.* (1988) noticed that RVI and NDVI were good indices to predict the chlorophyll and leaf area index. In this study in addition to RVI and NDVI, SAVI also set close relationship with chlorophyll and LAI. This suggests that in general all the indices are better for assessment of chlorophyll. However, amongst all NDVI predicted highest R^2 and hence it is best amongst all.

4.10.3 Relationship between plant pigments and spectral indices in Acalypha

Table 4.12 represents the linear relationship equations and R^2 values of spectral parameters and plant pigments. The R^2 values presented in Table indicated that the spectral parameters viz., red wavelength interception, NIR wavelength interception, RVI, NDYI, SAVI and IPVI were poorly correlated with plant pigments except anthocyanin. These results reflect light on the morphology of Acalypha plant. The upper surface of Acalypha leaves is reddish in colour and hence red wavelength is not absorbed by Acalypha plant. Even though the chlorophyll a, b and total chlorophyll content was high (Table 4.11). The Acalypha leaves are thick and had high chlorophyll content but the upper surface of leaves is pink because of high anthocyanin. Whereas lower portion of leaf greenish in colour. The light (EMR) interacts with the above surface of leaf and hence the chlorophyll content of leaves and red wavelength absorption was not seen. Further the spectral indices RVI, NDVI, SAVI and IPVI considers red reflectance / absorption in calculation of ratios. Hence, they also represent very poor relationship with plant pigments except anthocyanin. Anthocyanin established relationship with Red and NIR giving R^2 = 0.2567

Table 12. Relationship between plant pigments and spectral parameters in Acalypha

Pigments	Red	NIR	RVI	NDVI	SAVI	IPVI
Chlorophyll 'a'	Y = 0.0051x + 0.03047	Y = 0.101x + 0.6749	Y = 0.1061x + 0.7697	Y = -0.0354x + 0.1068	Y = -0.0354x + 0.1168	Y = -1E-14x + 0.45
	R^2 =	**R^2 = 0.0202**	**R^2 = 0.0128**	**R^2 = 0.005**	**R^2 = 0.003**	**R^2 = 2E-27**
Chlorophyll 'b'	Y = -0.0073x + 0.3114	Y = 0.3893x + 0.5227	Y = 0.4404x + 0.593	Y = -0.2117x + 0.2002	Y = -0.2603x + 0.2361	Y = 0.0973x + 0.3982
	R^2 = 0.0006	**R^2 = 0.3114**	**R^2 = 0.2297**	**R^2 = 0.186**	**R^2 = 0.1709**	**R^2 = 0.1217**
Total chlorophyll	Y = 0.0032x + 0.3039	Y = 0.0702x + 0.6499	Y = 0.0705x + 0.747	Y = -0.0291x + 0.1208	Y = -0.0333x + 0.1356	Y = 0.0084x + 0.4404
	R^2 = 0.0008	**R^2 = 0.0702**	**R^2 = 0.0408**	**R^2 = 0.0244**	**R^2 = 0.0194**	**R^2 = 0.0063**
Anthocyanins	Y = -0.002x + 0.38	Y = -0.0087x + 1.0524	Y = -0.0042x + 0.9809	Y = 0.002x + 0.0148	Y = 0.0024x + 0.0087	Y = -0.0009x + 0.4823
	R^2 = 0.2567	**R^2 = 0.8893**	**R^2 = 0.116**	**R^2 = 0.0912**	**R^2 = 0.0828**	**R^2 = 0.0558**
Lycopene	Y = 0.0144x + 0.2866	Y = 0.0045x + 0.7235	Y = -0.0311x + 0.8725	Y = 0.0195x + 0.0593	Y = 0.0264x + 0.0593	Y = -0.0137x + 0.4699
	R^2 = 0.1596	**R^2 = 0.0027**	**R^2 = 0.0746**	**R^2 = 0.1031**	**R^2 = 0.1144**	**R^2 = 0.1578**
Carotenoids	Y = 0.0167x + 0.2919	Y = 0.009x + 0.7291	Y = -0.0405x + 0.8655	Y = 0.0248x + 0.0643	Y = 0.0332x + 0.0663	Y = -0.0169x + 0.4659
	R^2 = 0.1731	**R^2 = 9E-05**	**R^2 = 0.1028**	**R^2 = 0.1349**	**R^2 = 0.1474**	**R^2 = 0.1945**

and R^2 = 0.8893. This relationship might be because of Red and NIR interaction with surface of the Acalypha canopy. This shows spectral parameters are useful to studied the surface phenomenon and do not give idea about the internal chemical nature of leaves.

vegetation

4.10.4 Relationship between plant pigments and spectral indices in Golden duranta

The relationship between various plant pigments viz., chlorophyll 'a', chlorophyll 'b', total chlorophyll , anthocyanin, lycopene and carotenoid with Red / NIR spectral reflectance and RVI, NDVI, SAVI and IPVI spectral ratios are presented in Table 4.13. It was noticed ~~that the canopy of~~ Golden duranta ~~showed~~ little closer relationship ~~with~~ spectral parameters as compared with Acalypha canopy. The chlorophyll 'a', chlorophyll 'b' and total chlorophyll showed high R^2 values when correlated with NIR. ~~This might be~~ because of the fact that the leaves with ~~high~~ chlorophyll content were vigorous and with high leaf area which might have increased the NIR reflectance and established highly significant relationship. The R^2 values were 0.9751, 0.8446 and 0.866 just like Acalypha, Golden duranta also established highly significant linear relationship with red reflectance ~~RVI, N~~DVI, SAVI and IPVI showing R^2 0.9252, 0.6576, 0.6146, 0.4196 and 0.6703, respectively.

These results speak about the EMR reaction with Golden duranta canopy. As reported in Table 4.11, Golden duranta were high in carotenoid and lycopene pigments and relatively low in chlorophyll and anthocyanin pigments. However, the significant linear relationship with anthocyanin pigment might be because of the fact that Red wavelength had

Table 13. Relationship between plant pigments and spectral parameters in Golden duranta

Plant pigments	Red	NIR	RVI	NDVI	SAVI	IPVI
Chlorophyll 'a'	Y = 0.3143x + 0.3997	Y= 2.6286x + 1.0794	Y = 1.1714x + 0.7926	Y = -0.7429x + 0.1089	Y = -0.8857x + 0.1317	Y = 0.2857x + 0.4443
	R^2 = 0.0323	**R^2 = 0.9751**	**R^2 = 0.0933**	**R^2 = 0.1178**	**R^2 = 0.106**	R^2 = 0.0649
Chlorophyll 'b'	Y = -0.0909x + 0.4264	Y = 4.3636x + 1.0845	Y = 3.3636x + 0.7345	Y = -2x + 0.14	Y = -2.4545x + 0.1718	Y = 0.9091x + 0.4264
	R^2 = 0.0008	**R^2 = 0.8446**	**R 2 = 0.2417**	**R^2 = 0.2683**	**R^2 = 0.2559**	R^2 = 0.2066
Total chlorophyll	Y = -0.0746xz + 0.4316	Y = 1.791x + 1.0506	Y = 1.4478x + 0.7001	Y = -0.8657x + 0.161	Y = 1.0597x + 0.1973	Y = 0.3881x + 0.4175
	R^2 = 0.0035	**R^2 = 0.8666**	**R^2 = 0.2727**	**R^2 = 0.3062**	**R^2 = 0.2905**	R^2 = 0.2293
Anthocyanin	Y = 0.0118x + 0.3701	Y = 0.0057x + 1.2447	Y = -0.0218x + 0.9745	Y = 0.0122x + 0.0009	Y = 0.0154x – 0.0009	Y = -0.0064x + 0.4936
	R^2 = 0.117	**R^2 = 0.0930**	**R^2 = 0.6576**	**R^2 = 0.6416**	**R^2 = 0.6498**	R^2 = 0.6701
Lycopene	Y = -0.0158x + 0.4484	Y = 0.0343x + 1.2136	Y = 0.0551x + 0.7871	Y = -0.034x + 0.1108	Y = -0.041x + 0.1348	Y = 0.0141x + 0.4419
	R^2 = 0.117	**R^2 = 0.2386**	**R^2 = 0.2956**	**R^2 = 0.3534**	**R^2 = 0.3259**	R^2 = 0.2256
Carotenoids	Y = -0.0095x + 0.4342	Y = 0.042x + 1.2183	Y = 0.0456 + 0.8215	Y = -0.0294x + 0.0912	Y = -0.0348x + 0.1103	Y = 0.0108x + 0.4518
	R^2 = 0.0328	**R^2 = 0.2753**	**R^2 = 0.1557**	**R^2 = 0.2037**	**R^2 = 0.1806**	R^2 = 0.101

very strong relationship with anthocyanin ($R^2 = 0.9252$). These reflected in spectral indices, which also showed higher R^2 values.

From the above interpretation of results it can be concluded that the electromagnetic radiation interaction is controlled by pigment dominate in the surface canopy of the leaf. If the concentration of particular pigment is more in leaf it should be seen on surface of the leaf. Then only the present spectra indices and wavelengths used work to give a better relationship.

In the present study it was very clearly observed that the maize crop with high chlorophyll content was dark green colour and established strong and significant relationship with chlorophyll. Whereas the other two plant species that are rich in anthocyanin and carotenoid could not established the significant relationship with spectral parameters. Because the pigment concentration observed in the leaf did not appear on the surface of leaf. Further the specific wavelengths are sensitive to specific colour and the max is different for different colour.

Gausman *et al.* (1973) studied the chlorophyll and carotenoid interaction with spectral reflectance and showed that the chlorophyll concentration of leaves was unmasked the carotenoids pigments which affected the reflectance by carotenoids and anthocyanin.

Therefore, it can be concluded that the red and NIR reflectance and the spectral indices calculated from NIR and Red reflectance cannot be useful to study the plant pigments like anthocyanin, carotenoids lycopene. Further, the contribution of these pigments towards spectral reflectance was beyond the scope of present study.

In depth study between wavelength absorbed by plant pigments showed that lambda-max for chlorophyll 'a' is lies between 430 and 662 nm, lambda-max for chlorophyll 'b' is lies between 453 and 642 nm, lambda-max for carotenoids is lies between 450 and 470 nm and lambda-max xanthophylls lies between 450 and 540 nm and the red band of GTMR used in present research programme also between 0.62 – 0.68 um, which does not covered the lambda-max of all pigments. This might be the reason of getting poor correlation with spectra parameters.

4.11 Visual nutrient deficiency symptoms, plant nutrient concentration and nutrient uptake by maize

The amount and form of plant nutrient are to important parameters of nutrient management. Deficiency of specific nutrient produces specific deficiency symptoms. In the research programme carried out as a part of partial fulfillment of degree maintained six fertility levels. The plots where nitrogen, sulfur, zinc and iron were not applied showed the deficiency symptoms by maize crop. The plot F_0 i.e. divide of all nutrients showed chlorosis (yellowing) of the leaves shortened the internodes and leaf area. The nitrogen deficient plot showed yellowing of older leaves while sulfur deficient plot showed yellowing of younger leaves. Interveinal chlorosis was noticed in the plants which had not received iron and zinc.

The nutrient concentration in the leaves at three growth stages i.e. at early vegetative, silking and grain ripening stage were determined and presented in Appendix IV,V and VI for ready reference.

Summary and Conclusions

CHAPTER 5

SUMMARY AND CONCLUSIONS

Use of optical sensors for measuring the green colour reflectance of plant canopy to assess the health of vegetation is operational. However the exact diagnosis of plant nutrient concentration is still visionary. Moreover, in addition to chlorophyll other plant pigments like carotenoids, anthocyanin, lycopene, and xanthophylls contribute towards spectral reflectance and influence the spectral parameters. There are ample references quoting use of various spectral indices for identification and quantification of chlorophyll. However, work is strewed on identification and quantification of other plant pigments that contribute and influence nearly 30 per cent of spectral reflectance. Once this is identified, it will be a key in diagnosis of plant nutrient deficiencies of crop plant in a nondestructive way. Initial step in this direction is the present research on "Relationship between chlorophyll and other pigments and spectral reflectance in various plant species". The results interpreted and discussed in previous chapter are summarized in this chapter.

The experiment was conducted at Parbhani which comes under semi-arid tropics with annual rainfall ranging from 700 to 900 mm. Except few days in monsoon most of the year Parbhani receives bright sunshine. The experimental soil was clayey, alkaline in reaction, calcareous in nature and low in organic matter available, nitrogen, sulfur and zinc. Because of calcareous nature of soil iron availability reduces down to a greater extent and become a limiting factor in crop production.

A field experiment on maize comprising of six fertility levels viz., F_0 No fertilizer application, F_1 :Only N 150 kg N ha^{-1}, F_2 :N + P (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1}), F_3 :N + P + S (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1} + 30 kg S ha^{-1}), F_4 :N + P + S + Zn (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1} + 30 kg S ha^{-1} + 20 kg $ZnSO_4$ ha^{-1}) and F_5 :N + P+ S + Zn + Fe (150 kg N ha^{-1} + 50 kg P_2O_5 ha^{-1} + 30 kg S ha^{-1} + 20 kg $ZnSO_4$ ha^{-1} + 2 % Fe foliar spray at 2 stages) replicated four times. The observation on plant height, number of leaves, LAI, biomass and cob yield production and spectral reflectance were recorded. Chlorophyll 'a', 'b', total chlorophyll, leaf nutrient concentration was determined. The special plant species Acalypha and Golden duranta were selected to study the behaviour of other pigments towards spectral reflectance. These species are dominant in anthocyanin and carotenoid pigments. The results showed that various growth attributes of maize viz., height of plant, number of leaves, leaf area index, and total biomass per plant and cob yield were improved due to application fertility levels.

The chlorophyll concentration of the leaf was increased with the advancement of growth of maize and with various fertility levels. Each additional nutrient found superior in increasing the leaf chlorophyll content except phosphorus.

In Acalypha species posses high concentration of chlorophyll and anthocyanin while Golden duranta had high concentration of lycopene and carotenoids. Acalypha being dominant in red hue showed higher anthocyanin and the yellowish hue of Golden duranta support the higher concentration of carotenoids pigments.

Very clear distinction in spectral behaviour of maize canopy was noticed between 53 to 67 DAS (Grand Growth period). While under nutrient stress condition, plants were found to have lower NIR reflectance and high red reflectance compared to nutrient supplied crop.

Among the spectral indices viz., RVI (Relative Vegetation Index), NDVI (Normalized Difference Vegetation Index), IPVI (Infra Red Percentage Vegetation Index) and SAVI (Soil Adjusted Vegetation Index) used as a yard stick for assessment of growth and diagnosis of effect of nutrient deficiency of maize. The RVI, NDVI and SAVI relies showed that all these relies low in F_0 treatments (no nutrient application), whereas high in F_5 treatments (i.e. application of N, P, K, S and Zn at rate of 150:50:50:30:20 kg/ha with two foliar sprays of 2 % iron at silking stage and cob development stage. The capability of discrimination of nutrient stress vs. nutrient applied crop was centralized between 46 to 67 DAS (grand growth period of maize). The IPVI and crop growth was not established. Indicating there by IPVI is not a suitable index for monitoring the growth and detection of chlorophyll pigment.

In Acalypha and Golden duranta RVI, NDVI, IPVI and SAVI do not set any pattern in respect of spectral indices because of unsuitability of spectral bands.

Leaf canopy temperature measured by IR thermometer during growth cycle showed distinguishable effect in canopy temperature due to nutrient application the maximum temperature i.e. 31.49 °C was recorded in nutrient stress and minimum temperature of 27.63 °C was recorded in nutrient sufficient crop canopy The canopy temperature was increase with the age of crop.

LAI with RVI and NDVI was linearly correlated (R^2 = 0.8027 and 0.833 respectively) similarly SAVI established good relationship with LAI (R^2 = 0.7259) suggesting that RVI, and NDVI and SAVI are better indicators for assessment of growth of maize crop. Total chlorophyll established linear relationship with RVI, NDVI and SAVI predicting 88, 81 and 72 per cent total chlorophyll content in maize plant.

The relationship between plant pigments and spectral parameters in Acalypha and Golden duranta could not establish. The Red and NIR reflectance and spectral indices calculated from NIR and R bands were unable to predict the behaviour of plant pigments except chlorophyll.

Contribution of plant pigments towards the spectral reflectance was beyond the scope of the instrument used in the study because GTMR does not cover the lambda-max of all pigments.

CONCLUSIONS

From the summarized results following conclusions are drawn:

- The various growth parameters of maize viz., height, LAI, total biomass, chlorophyll 'a', chlorophyll 'b', total chlorophyll and plant nutrient concentration were improved significantly due to application of 150kgN, 50kg P_2O_5, 50kg K_2O, 30 kg S, 20kg $ZnSO_4$ per hectare and two foliar spraying of 2% iron.

- RVI, NDVI and SAVI can be used as a yard stick to identify the crop condition i.e. fertilizer fed crop vs. non-fertilized / stressed crop and
- RVI found better index for assessment of growth of maize and NDVI performed as a better index for assessment of chlorophyll with established significant correlation with LAI and total chlorophyll.
- Chlorophyll was the most important independent factor affecting spectral reflectance
- Acalypha species were dominant in anthocyanin pigments and Golden duranta species were dominant in carotenoids and lycopene pigment
- The predominance of chlorophyll in the plant tissue compared with the other pigments and the strong positive relationship between chlorophyll a and chlorophyll b resulting in concurrent increases of both pigments suggests that the chlorophylls had a greater influence on spectral reflectance than the accessory pigments tested. The Red (0.62-0.52um) and NIR band (0.77-0.86um) failed to establish relationship with accessory plant pigments viz. carotenoids, anthocyanin and lycopene.

Future Research Needs

To study the relationship and contribution of other accessory pigments (other than chlorophyll) towards spectral reflectance, absorption and transmission, continuous spectrum hyper spectral radiometers will be useful. So, the data collected from continuous spectrum hyper spectral narrow bandwidth radiometer will give insight on behaviour of accessory plant pigment with radiation reaction.

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Annexure

APPENDIX I

Meteorological data from October 2007 to February 2008 recorded at Meteorological Observatory, M A. U, Parbhani.

MW	RF (mm)	Temp °C		Humidity %		EVP (mm)	BSS (Hrs.)
		Max.	Min.	AM	PM		
October							
40	0.0	32.6	18.4	79	44	5.5	9.1
41	0.0	33.5	15.3	77	44	5.8	9.7
42	0.0	33.1	13.8	75	33	4.4	10.0
43	0.0	32.3	10.4	76	38	4.6	10.4
November							
44	16.2	31.2	16.2	81	48	4.3	6.7
45	0.0	33.1	13.7	77	36	4.8	10.1
46	0.0	31.5	9.8	78	29	4.4	10.0
47	0.0	29.4	7.6	77	30	4.1	10.5
December							
48	0.0	29.6	8.6	73	32	4.1	9.1
49	0.0	28.1	9.9	77	31	4.0	7.1
50	0.0	29.9	12.7	68	38	4.4	8.7
51	0.0	30.0	12.6	69	33	4.8	9.9
52	0.0	28.7	12.1	62	27	4.1	8.6
January							
01	2.0	30.5	13.0	76	36	4.1	8.9
02	0.0	30.8	10.7	77	31	3.9	9.6
03	0.0	31.7	10.9	74	28	4.4	10.3
04	0.0	28.9	9.6	71	27	3.9	9.8
February							
05	0.0	29.5	8.2	70	28	4.8	10.5
06	0.0	27.2	12.0	71	29	4.8	7.9
07	0.0	31.8	15.7	670	34	5.9	9.3
08	0.0	34.5	13.8	63	27	6.9	10.6

APPENDIX II

Weather parameters prevailed on dates of observation

Dates	Temperature (^{o}C)		Soil temperature (0^{o}C)				Relative humidity (%)		Rainfall (mm)	Wind speed (km/hr)
	Max.	Min.	A.M.		P.M.		A.M.	P.M.		
			5 cm	10 cm	5 cm	10 cm				
12 Novembe, 2007	32.5	10.0	20.5	21.0	38.5	36.5	81	40	0.0	1.7
19 November, 2007	29.0	10.5	19.0	20.5	35.5	31.0	70	37	0.0	2.4
25 November, 2007	29.0	6.5	15.0	16.5	35.5	30.0	83	32	0.0	2.2
2 December, 2007	27.5	11.0	18.5	22.0	35.5	31.0	76	26	0.0	2.0
16 December, 2007	29.3	12.5	18.5	21.5	35.0	29.0	71	33	0.0	4.0
2 January, 2008	32.0	16.5	20.5	22.5	37.8	32.0	63	38	0.0	4.0
29 January, 2008	28.5	7.9	14.0	18.0	40.0	31.5	69	18	0.0	3.2

APPENDIX-III

WORK DONE IN FIELD EXPERIMENTATION

Date	Work done
7 October, 2007	Preparatory tillage
8 October,2007	Layout of field experimentation
9 October, 2007	Application of basal dose, sowing of maize, raising of ridges of plots
10 October, 2007	First irrigation
17 October, 2007	Thinning and gap filling
18 October, 2007	Second irrigation
13 November, 2007	First weeding
9 November, 2007	Top dressing (75 kg Nha^{-1})
19 November, 2007	Raising of ridges of plots
20 November, 2007	Third irrigation
25 November ,2007	Hoeing and earthling-up
1 December, 2007	First 2 % foliar spraying at silking stage
3 December, 2007	Second Weeding
13 December, 2007	Fourth irrigation
23 December, 2007	Second 2 % foliar spraying at cob development stage
4 January, 2008	Third weeding
20 January, 2008	Fifth irrigation
13 February, 2008	Harvesting of maize

APPENDIX IV

Nitrogen, phosphorus and Potassium concentrations in Maize

Treatments	Nutrient concentration (%)								
	Nitrogen			Phosphorus			Potassium		
	20 DAS	**73 DAS**	**103 DAS**	**20 DAS**	**73 DAS**	**103 DAS**	**20 DAS**	**73 DAS**	**103 DAS**
F0	1.87	1.19	0.51	0.58	0.93	0.45	3.83	1.62	1.18
F1 (N)	2.37	1.44	0.99	0.68	0.93	0.64	4.20	2.07	1.33
F2 (NP)	2.77	1.59	1.03	0.68	0.95	0.65	4.22	2.00	1.36
F3 (NPS)	3.12	1.52	1.16	0.73	1.00	0.66	4.25	2.62	1.13
F4 (NPSZn)	3.41	2.26	1.09	0.70	0.82	0.68	4.26	3.17	1.10
F5 (NPSZnFe)	3.61	2.02	1.35	0.86	1.23	0.77	3.73	2.30	1.26
Mean	2.86	1.67	1.02	0.70	0.98	0.64	4.08	2.30	1.23
SEm±	0.374	0.177	0.166	0.081	0.238	0.089	0.209	0.491	0.064
CD at 5%	1.127	0.535	0.499	0.245	0.717	0.269	0.629	1.478	0.193

APPENDIX V

Sulfur, Zinc and Iron concentrations in Maize

Treatments	Nutrient concentration (mg kg^{-1})								
	Sulphur			Zinc			Iron		
	20 DAS	73 DAS	103 DAS	20 DAS	73 DAS	103 DAS	20 DAS	73 DAS	103 DAS
F0	0.17	0.14	0.71	67.1	19.8	17.4	290.0	95.6	72.0
F1 (N)	0.20	0.13	0.76	73.0	27.0	17.5	295.6	111.7	78.7
F2 (NP)	0.23	0.15	0.78	74.4	27.1	17.8	301.0	114.5	87.7
F3 (NPS)	0.24	0.15	0.79	78.9	24.7	17.8	302.1	117.3	88.2
F4 (NPSZn)	0.20	0.15	0.79	83.5	27.8	17.8	439.1	133.0	95.0
F5 (NPSZnFe)	0.23	0.17	0.76	88.9	28.5	18.0	466.1	140.3	120.0
Mean	0.215	0.151	0.770	77.63	25.85	17.77	34.90	118.7	90.29
SEm±	0.029	0.016	0.021	8.12	3.83	0.203	35.27	16.82	20.08
CD at 5%	0.088	0.050	0.065	25.34	11.53	0.613	106.13	50.63	60.44

APPENDIX VI

Macro and micronutrient Uptake by Maize at Harvest stage

Treatments	Nitrogen (Kg ha^{-1})	Phosphorus (Kg ha^{-1})	Potassium (Kg ha^{-1})	Sulfur (Kg ha^{-1})	Zinc (g ha^{-1})	Iron (g ha^{-1})
F0	94.69	87.4	168.3	101.2	29.00	128.4
F1 (N)	147.10	107.1	248.5	142.6	30.10	144.6
F2 (NP)	193.40	121.2	249.8	144.3	32.16	168.2
F3 (NPS)	208.50	121.9	217.2	151.3	33.20	189.6
F4 (NPSZn)	216.50	122.9	211.0	151.3	41.50	223.1
F5 (NPSZnFe)	259.90	132.5	245.2	152.0	41.80	233.8
Mean	186.72	115.5	223.3	137.1	34.62	181.2
SEm±	29.46	0.162	12.65	7.449	0.60	33.56
CD at 5%	88.64	0.489	38.06	22.507	1.80	100.9