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**Physiological studies in relation to nodulation and
nitrogen fixation, yield and yield attributes in
Groundnut using Plant Growth Regulators**

*A Thesis submitted to the
Orissa University of Agriculture and Technology
in Partial fulfilment of the Requirement for the degree of
Master of Sciences in Agriculture
(Plant Physiology)*

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PALLAVI NAYAK
Adm. No. 08/PP/15



**DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR**

2017



ORISSA UNIVERSITY OF AGRICULTURE & TECHNOLOGY
DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
BHUBANESWAR

Mrs. R. K. Bhol
Assistant Professor

Bhubaneswar
Date:

CERTIFICATE - I

This is to certify that the thesis entitled “**Physiological studies in relation to nodulation and nitrogen fixation, yield and yield attributes in Groundnut using Plant Growth Regulators**” submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PHYSIOLOGY)** to the Orissa University of Agriculture and Technology is a faithful record of *bonafide* and original research work carried out by **Pallavi Nayak (Adm No 08PP/15)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

R. K. Bhol
3/7/17

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DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
BHUBANESWAR

CERTIFICATE II

This is to certify that the thesis entitled “**Physiological studies in relation to nodulation and nitrogen fixation, yield and yield attributes in Groundnut using Plant Growth Regulators**” submitted by **Pallavi Nayak (Adm No.08PP/15)** to the Orissa University of Agriculture and Technology, Bhubaneswar, in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Plant Physiology) has been approved by the student’s advisory Committee and the external examiner.

Advisory Committee

Chairman

Mr. R. K. Bhol
Assistant Professor,
Dept. of Plant Physiology

R. K. Bhol
10/7/17

Members

Dr. G.R. Rout
Professor & Head
Department of Plant Physiology

G.R. Rout
10/7/17

Dr. R.R. Dash
Associate professor
Department of Agronomy

R.R. Dash
10/7/17

Dr. K.C. Samal
Associate Professor
Department of Agricultural Biotechnology

K.C. Samal

External Examiner

B. K. Ghosh
10.7.2017

(Name and Designation)

Dr. B. K. Ghosh
Retd. Prof & Head
Pl. Phys. OUAT

ACKNOWLEDGMENT

I avail the unique opportunity to express my immeasurable gratitude and reverence to Mrs R. K. Bhol Assistant Professor, Department Of Plant Physiology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar and Chairman of the my Advisory Committee, for valuable guidance, constant supervision, sustained interest, dynamic thought, constructive criticism, steady encouragement, whole-hearted co-operation and painstaking efforts during the entire period of investigation leading to my path to achieve the destination during the entire move and the successful completion of my dissertation.

I would also like to express sincere thanks Dr. G. R. Rout Professor and Head, Department of Agricultural Biotechnology OUAT, Bhubaneswar and member of them advisory committee.

I express my heartfelt gratitude to Dr. R. R. Dash Associate Professor, Department of Agronomy, and Dr. K. C. Samal, Associate Professor, Department of Agricultural Biotechnology OUAT Bhubaneswar and member of the my Advisory committee for providing the necessary facilities for the present investigation and preparation of this manuscript.

I am also indebted to all the Professors of Department of Plant Physiology for giving their valuable and constructive suggestions for timely completion of the thesis work.

I am imbedded with unboundful pleasure in acknowledging ardency towards my best friend Nutan Moharana for supporting me through my bad times.

My heartiest thanks to all my friends and well-wishers for their sincere co-operation, especially to Pragati Dash, Rahul, Abhilash Sonali didi, Jyotiprakash for their inspiration, assistance, moral support and affection during my thesis work.

Lastly I deeply acknowledge my beloved father and mother, my husband and in-laws for being there for me in all good and bad times with constant support, encouragement and blessings to build my career. I would remain ever indebted to them.

Bhubaneswar

Date-10.07.2017



Pallavi Nayak

LIST OF ABBREVIATIONS

%	:	Percentage
Kg	:	Kilogram
Cm	:	Centimetre
i.e.	:	That is
e.g.	:	For example
@	:	At the rate of
DAS	:	Days after sowing
RGR	:	Relative growth Rate
NAR	:	Net Assimilation Ratio
LAI	:	Leaf Area Index
NR	:	Nitrate Reductase
CV	:	Coefficient of variation
SED	:	Standard error of difference
SEM	:	Standard error of mean
CD	:	Critical difference
Ha	:	Hectare
OD	:	Optical Density
Fig	:	Figure
FYM	:	Farm Yard Manure
m	:	Meter
IAA	:	Indole-3-acetic acid
GA	:	Gibberellic acid
CCC	:	Cycocel

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ABSTRACT

Field experiments conducted in Central research station, OUAT, Bhubaneswar during Rabi 2016-17 to study the effect of growth regulators on physiological studies in relation to nodulation and nitrogen fixation, yield and yield attributing characters in groundnut Cultivar Devi was taken for the study of different morphological, physiological and biochemical parameters. It was revealed from the result that there was an enhancement of plant height in all the treatments during all the stages of the growth period except the application of growth retardant. The maximum increase in height (22.9 %) was found in 20 ppm NAA whereas the minimum of 18.4% was observed in 20 ppm CCC. No. of leaves per plant, no. of branches per plant showed more increase in 10 ppm GA and 20 ppm GA. Leaf area per plant also showed more increase in higher concentrations of NAA. This increase is due to triggering of elasticity of the cell walls by promoting the intake of water, stimulating the secretion of hydrogen ions from the cell walls and lowering of the pH of the cell wall. The root dry weight had a minimal effect on the effect of growth regulators. The maximum root: shoot ratio was found in 20 ppm IAA. The root volume was found to be maximum in 20 ppm IAA. Chlorophyll content increased upto 33.33 % in 20 ppm NAA. Nitrate reductase activity increased to 68.3 % in 20 ppm NAA and Dehydrogenase activity increased to the tune of 43.24% in 20 ppm GA. The lowest protein content was found in 10 ppm CCC. Number of nodules per plant, Pod length and pod diameter was increased in 20 ppm GA. Highest grain yield was obtained in 20 ppm GA followed by 10 ppm GA. Basing on the above facts the putative traits like leaf number, dehydrogenase activity, number of nodules per plant, pod length and pod diameter were enhanced significantly due to application of higher dose of GA which can be used as a future thrust for the farmers.

CHAPTER – I

INTRODUCTION

INTRODUCTION

India is one of the largest producers of groundnut (*Arachis hypogea*) in the world and occupies an important position in Indian agricultural economy. It is the 13th most important food crop and 4th most important oilseed crop. Groundnut is the most important cash crop of our country. Major groundnut producing states in India are Gujrat, A.P., Tamil Nadu and Karnataka. Groundnut is grown mainly in the tropics and its cultivation is extended to subtropics. Groundnut is best suited sandy loam and loamy soils. Nearly 66% of groundnut produced in the country are crushed for oil. Groundnut seeds (kernels) contain 40-50% oil, 20-50% protein and 10-20% carbohydrate.

Worldwide production of groundnut is approx.37.19 mt. India being second in production produces nearly 4.96 mt and total productivity of groundnut is 18042 kg/ha and area under groundnut cultivation is 4.77mha.

Nutritional value of groundnut: Amount per 100 gm., calories-567, proteins - 26 gms, calcium-9%, vit C-15%, total fat-49 gms, sodium-18 gms, carbohydrate - 16gms.

Groundnut seeds are nutritionally rich in vitamin E, niacin, calcium, phosphorous and magnesium, zinc, iron, riboflavin, thiamine and potassium. And their calorific value is 349 per 100 gram of seed.

Groundnut kernels are consumed directly as raw, roasted or boiled or oil is extracted for culinary purpose.it is also used as animal feed. It is an important source of protein supplement to cattle and poultry feed. Groundnut shells are used to manufacture coarse boards, cork substitutes. These multiple uses of groundnut plant makes it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries. The crop is suitable for cultivation in tropical regions. It prospers well in a light, sandy loam soil. However, it is also known for its ability to survive in less favourable agro climatic conditions. The pods need 3-4 months to ripen depending upon varieties.

According to Food and Agriculture Policy Research Institute (FAPRI), world groundnut production is projected to increase by 12% over the outlook period of 2010-2011 to 2025-2026. In terms of production, Odisha is the sixth largest in groundnut producer in India. The exercise was conducted at six districts namely Puri, Jajpur, Kendrapada, Jagatsinghpur, Baleswar, Bhadrak, where the crop was grown in nearly 2,50,000 hectare (ha) areas. It was grown primarily in Kalahandi and Boudh districts. In Odisha total area of production of groundnut was 263.41 thousand ha in 2012-13 and 267.78 thousand ha in 2013-14, productivity was 70.4 kg/ha and 70.87 kg/ha in 2012-2013 and 2013-14. The total production in the state was 460.30 thousand metric tonnes and 478.33 thousand metric tonnes in 2012-13 and 2013-14.

Hormones play a vital role in various Physiological, Biochemical and Morphological processes of groundnut. Leguminous plants form root nodules, in which symbiotic rhizobia fix atmospheric nitrogen. The nodulation process in the legume-rhizobium symbiosis consists of a series of events. Phytohormones are signal molecules involved in most plant physiological activities, they are likely to positively or negatively regulate nodulation and nitrogen fixation in the legume-rhizobium symbiosis.

Auxins were the first class of plant hormones discovered, and play a central role on the regulation of germination, plant growth, flower bud formation and flowering, and other developmental processes. In addition, auxins are involved in responses to environmental stimuli such as temperature, light and gravity. The most important member of the auxin family is indole-3-acetic acid (IAA), a native auxin in plants. The highest auxin levels are found in the cells undergoing cell division, elongation, differentiation and vascular bundle formation. Therefore, auxin may play a significant role in nodulation. The possible involvement of auxins in nodule formation was first reported by Thimann in 1936. It suggests that auxins play an important role in the development of nodule vasculature.

Cycocel are growth retardants and are used in present study in order to improve the partitioning of assimilates, productivity of groundnut and their effects of germinability of groundnut. The term growth retardant is used for all chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects. These are new group of quaternary

ammonium growth regulators. It is now extensively used as a retardant known as cycocel. It is highly hygroscopic, crystalline solid having a molecular weight of 158 and is completely soluble in water and persistent in soil upto 3 weeks, its spray application is also effective.

The response of groundnut plants to ccc was investigated by applying concentrations of 0,500,1000 and 1500 ppm three times as foliar spray. CCC at 500ppm gave more kernels and heavier yield of pods, kernels and oils than 1000 or 1500 ppm or the control treatment. Application of CCC resulted in a greater percentage of shell and of moisture in the whole pod than in the con

Gibberellic Acids (GAs) are another important family of growth regulators in higher plants. GAs are also involved in root nodule symbiosis.

Legume plants are capable of developing an association with nitrogen fixing bacteria generally known as rhizobia to form a nodule. A nodule is an unique organ commonly found in a plant where nitrogen fixing occur. Nodules provide a suitable environment for nitrogenase enzyme complex of bacteria to convert nitrogen gas from atmosphere to ammonia which will be assimilated by the plant.

Different concentrations of Phytohormones affect groundnut crop at various stages affecting plant height, leaf area, pod number, pod size and overall yield.

Therefore, the present research work ransacks Physiological studies in relation to nodulation and nitrogen fixation, yield and yield attributes in groundnut using plant growth regulators with the following objectives;

- 1- Effect of growth regulators on plant growth and other physiological parameters.
- 2- To establish the enzyme activity (auxin, gibberlin, cycocel) during the growth and development of plant.
- 3- Comparison of yield performance with regards to nitrogen fixation and growth regulation effect.

CHAPTER – II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Groundnut (*Arachis hypogaea*) is called the “king” of oilseeds. Groundnut is by far the most important cash crop of our country. It is cultivated in large areas both in kharif and rabi season in well drained light texture soils. Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in the cellular metabolism of plants. Various literature support the fact that application of plant growth regulators affect overall growth, development of a plant that increases its yield and productivity. Plant growth regulators like Indole-3-acetic acid (IAA) Gibberellic acid (GA) and Cycocel (CCC) affect various morphological, biochemical and physiological processes of plant.

Indole-3-acetic acid (IAA)

Indole-3-acetic acid (IAA, 3-IAA) is the most common, naturally occurring, plant hormone of the auxin class. It is the best known of the auxins, and has been the subject of extensive studies by plant physiologists. IAA is a derivative of indole, containing a carboxymethyl substituent. It is a colorless solid that is soluble in polar organic solvents.

IUPAC Name: 2-(1H-indol-3-yl) acetic acid

Systematic IUPAC name: 2-(1H-indol-3-yl) ethanoic acid

Other names: Indole-3-acetic acid, indolylacetic acid, 1H-Indole-3-acetic acid, indoleacetic acid, heteroauxin, IAA

Chemical formula: $C_{10}H_9NO_2$

Molar mass: $175.19 \text{ g}\cdot\text{mol}^{-1}$

Appearance: White solid

Melting point: 168 to 170 °C (334 to 338 °F; 441 to 443 K)

Solubility in water: Moderate

IAA is predominantly produced in cells of the apex (bud) and very young leaves of a plant. Plants can synthesize IAA by several independent biosynthetic pathways. Four of them start from tryptophan, but there is also a biosynthetic pathway independent of tryptophan. Plants mainly produce IAA from tryptophan through indole-3-pyruvic acid. IAA is also produced from tryptophan through indole-3-acetaldoxime. As all auxins, IAA has many different effects, such as inducing cell elongation and cell division with all subsequent results for plant growth and development. On a larger scale, IAA serves as signaling molecule necessary for development of plant organs and coordination of growth.

Gibberellic Acid (GA)

Gibberellic acid (also called Gibberellin A3, GA, and GA3) is a hormone found in plants and fungi. Its chemical formula is C₁₉H₂₂O₆. When purified, it is a white to pale-yellow solid.

IUPAC Name: (3S,3aS,4S,4aS,7S,9aR,9bR,12S)-7,12-dihydroxy-3-methyl-6-methylene-2-oxoperhydro-4a, 7-methano-9b, 3-propenoazuleno [1,2-b] furan-4-carboxylic acid

Chemical Formula: C₁₉H₂₂O₆

Molar mass: 346.37 g/mol

Melting point: 233 to 235 °C (451 to 455 °F; 506 to 508 K) (decomposition)

Solubility in water: 5 g/l (20 °C)

Plants in their normal state produce low amounts of GA₃. It is possible to produce the hormone industrially using microorganisms. Nowadays, it is produced by submerge fermentation, but this process presented low yield with high production costs and hence higher sale value. One alternative process to reduce costs of the GA₃ production is Solid-State Fermentation (SSF) that allows the use of agro-industrial residues. Gibberellic acid is a simple gibberellin, a pentacyclic diterpene acid promoting growth and elongation of cells. It affects decomposition of plants and helps plants grow if used in small amounts, but eventually plants develop tolerance to it. GA stimulates the cells of germinating seeds to produce mRNA molecules that code for hydrolytic enzymes. Gibberellic acid is a very potent hormone whose natural occurrence in plants controls their development. Since GA regulates growth, applications of very low concentrations can have a profound effect while too much will have the opposite effect. It is usually used in concentrations between 0.01 and 10 mg/L.

GA was first identified in Japan in 1926, as a metabolic by-product of the plant pathogen *Gibberella fujikuroi* (thus the name), which afflicts rice plants; *fujikuroi*-infected plants develop bakanae ("foolish seedling"), which causes them to grow so much taller than normal that they die from no longer being sturdy enough to support their own weight.

Gibberellins have a number of effects on plant development. They can stimulate rapid stem and root growth, induce mitotic division in the leaves of some plants, and increase seed germination rate.

Chlormequat Chloride (CCC)

Chlormequat is an organic compound that is used as a plant growth regulator. It is typically sold as the chloride salt, chlormequat chloride, a colorless hygroscopic

crystalline substance that is soluble in water and ethanol. It is an alkylating agent and a quaternary ammonium salt.

IUPAC Name: 2-Chloro-N, N, N-trimethylethanaminium

Other names: Chlorocholine; Chlorcholine

Chemical formula: $C_5H_{13}ClN$

Molar mass: $122.62 \text{ g}\cdot\text{mol}^{-1}$

Chlormequat has been called the "most important inhibitor of gibberellin biosynthesis." As such, it inhibits cell elongation, resulting in thicker stalks, which are sturdier, facilitating harvesting of cereal crops.

2. Effect of Plant growth regulators on various Parameters

2.1 Plant height

In field trials with hemp (Kvaswikov, 1967); growth, plant height, fresh and dry weight and yield of fibre were increased by GA at 25mg/l solution sprayed alone or with ammonium nitrate (0.2%) or superphosphate (1.0%).

Gruodiene and Sipaviciene (1971) reported that foliar applications of GA at 40 or 50 mg/l increased plant growth in bean.

In trials with GA and MH spraying 3 or 4 weeks old pea plant with 50-400 ppm GA increased plant height, number and length of laterals. Also GA increased internodes length and adversely affected stem strength. (Patel and Srivastava, 1971)

Chakravati and Raghvaram (1973) reported that GA delayed senescence in pea plants by delaying maturity by 3 weeks, during which the number of internodes

increased from 16 to 23. The 14th and 15th internodes of GA –treated plants became measurable by 1 week earlier than those of untreated plants indicating that GA accelerated both cell division and elongation .

Spraying the plants of French bean (*Phaseolus vulgaris* L.) with 50-200 pp, GA increased plant height and number of leaves (Deka and Das ,1975).

Plants growth substances can modify plant growth and development raising the productivity level of crop on proper application. (Kene *et al.*, 1991)

GA are associated with various plant growth and development process such as seed germination, stem and hypocotyls elongation; leaf expansion and fruit development (Matusurka 2003, Hooley 1994) had proposed that the types of responses of plant cells and tissues to GAs can be classified into 3 categories cell growth in vegetative tissues, seed reserve mobilisation and fruit development.

Auxin in plants also mediates the tropism, response to gravity and light (M. Kelen *et al.*, 2004). Higher concentrations of auxin increased the plant height.

This was the first group of plant hormones discovered. The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (El-Tarabily, 2008)

Auxin is a general name for a group of hormones that are involved with growth responses (i.e., elongate cells, stimulate cell division in callus). The term "auxin" is derived from the Greek word means "to increase or grow" (Dr. Stephen G. *et al.*, 2009).

Auxins, a class of essential plant hormones, orchestrate almost every aspect of plant growth and development (Woodward and Bartel 2005; Vanneste and Friml 2009).

2.2 Number of leaves

The plant growth retardant cycocel (2-chloroethyl) trimethyl ammonium chloride (CCC) has received a great attention, especially in connection with dwarfening properties, to the plant (HUMPHRIES 1963, 1968; CATHEY 1964; LOWE and CARTER 1971; and others).

GA are associated with various plant growth and development process such as seed germination, stem and hypocotyls elongation; leaf expansion and fruit development (Matusurka 2003, Hooley 1994) had proposed that the types of responses of plant cells and tissues to GAs can be classified into 3 categories cell growth in vegetative tissues, seed reserve mobilisation and fruit development.

Abdelmula et al. (2007) observed a significant positive correlation between the no of leaves and the Leaf area index

Among PGRs, auxin and gibberellin play vital role in regulating developmental processes within plant bodies (Gou et al. 2010)

2.3 Leaf area and leaf area index (LAI)

Leaf area ratio indicates the size of assimilatory surface area in relation to total dry matter accumulation. The LAR was more during early stages of crop growth and decreased towards maturity. LAI is an important determinant of dry matter accumulation and grain yield and it can be adjusted by agronomic practises. The LAI varies with environmental conditions, cultural practises and stage of the crop growth stage when comparing different crop species or cultivars within species.

The range of maximum photosynthesis in groundnut is reported to be 16 to 65 mg CO₂ sq dm per hour (Kerting et al 1982). Crop growth rate is influenced by LAI, Photosynthetic rate and leaf angle. The specific leaf weight (SLW) is an index of leaf thickness and is increased from 30 to 60 DAP

Decrease in leaf area due to cycocel application was reported by HUMPHRIES and DYSON (1967) and HUMPHRIES and BOND (1969).

The results of the research conducted by Gosh and Basu (1999) showed that the effect of auxin hormone on grain yield, harvest index, and 100-grain weight was statistically significant at 1% level.

Briant (1974) studied the effects of four consecutive daily sprays of GA on the growth of leaves of young tomato plants and noted that total leaf weight and area were increased by GA and the percentage of changes were larger in the younger leaves than in the older leaves but also absolute increases of the middle leaves accounted for the most of the total response. Palisade cell length and Palisade cell number per unit section length were reduced by GA in the oldest leaves and increased in the youngest. There were larger intercellular spaces in the mesophyll and a larger cross-sectional area of midribs in the oldest and 2 youngest leaves of GA – treated plants. The surface areas of epidermal cells were also increased by GA treatment. leaf fresh weight per unit area was only a true index of lamina thickness in the 2 oldest leaves.

Maximum LAI values ranges from 3.3 to 7 have been reported for groundnut (Saxena et al 1983). The LAI reaches a maximum value 65-70 DAS in cultivars with 115 to 125 days duration. The LAI may continue to increase around 7 in some cultivars

Growth retardants reduced the leaf area significantly at all concentrations. Synthesis of some plant hormones is adversely affected by environmental factors,

which causes restriction on physiological processes of the plant and ultimately, limits their growth potential (Copur et al. 2010).

2.4. Root dry weight, shoot dry weight and Root:Shoot ratio

Many of the genes involved in the synthesis of auxin are expressed in roots and that root-generated auxin contributes to the maintenance of the gradients and maxima required for normal root development. (Ljung et al. 2005; Ikeda et al. 2009; Petersson et al. 2009).

Cycocel increased the seed yield mainly due to increased root growth and more drought resistance under water deficit conditions (De et al., 1982). Hambris et al. (1960) reported that any yield increase by CCC was related to the enhanced dry matter accumulation.

N. Moalemi and M. Chehrazi, reported that with different levels of Auxin hormone caused increase in getting rooting percent, number and dry weight of root.

As such, CCC inhibits cell elongation, resulting is thicker stalks, which are sturdier, facilitating harvesting of cereal crops, Gowariker, Vasant; Kalyani Paranjape; Sudha Gowariker (2013).

Hambris et al. (1960) reported that any yield increase by CCC was related to the enhanced dry matter accumulation. Growth retardants such as CCC affect the plant height, increase the shoots diameter and standing, reduce seed yield loss and hence may go to increased seed yield per plant and plot (Emam and Karimi,1996; Emam and Moaied, 2000).

Treating the seeds of egg plant with 10,30 or 100 ppm GA; Das and Prusty (1972) observed that GA enhanced plant height,internodal length ,leaf number and

reduced leaf thickness. The fresh and dry weights of roots and shoots were increased considerably.

2.5. Number of pods.

The elevated ABA content in the crop reproductive structures had been thought to be involved in controlling kernel pod abortion, presumably *via* inhibition of cell division in the young ovaries (Setter & Ammgam, 2001; Lie *et al.*, 2003).

In addition, exogenous application of ABA to developed plant ovaries inhibits cell division in the embryo and endosperm, and this effect is probably due to a depression cell-cycle gene expression by high levels of ABA (Setter & Ammgam, 2001).

In addition, in soybean (*Glycine max*) the application of growth regulators (e.g. Ethrel) resulted in higher vegetative growth and pod yield when applied at 40 and 60 days after sowing (Devi *et al.*, 2011)

Previous studies of large-fruited cultivars have reported that the application of growth retardants, e.g. chlormequat chloride (CCC) (Zayed *et al.*, 1985) or paclobutrazol (Syed *et al.*, 1997) may restrict plant height and even increase the number of pods produced per plant (Bhatt & Srinivasa Rao, 2009), perhaps by changing the level of hormones involved in floral induction and differentiation (Jiang *et al.*, 2010).

Bottger (1974) indicated that IAA had a strong promotive effect on initiation of lateral roots in peas where GA had no significant effect

Hammerton (1975) stated that spraying pigeon pea (*Cajanus cajan*) CV.17 twice with 25 ppm IAA solutions, increased fresh pod number/plant.

200 ppm of GA applied to the tomato (CV. Mingel porelra) plants at four true leaf stage had no significant effect on total fruit weight and number of fruits /plants; and reduced the average fruit weight (Castro and Malavolta.1976),

In field trials with *Cicor ariotinum* L. (Khan and Chowdhry,1976) applications of GA at 50 ,100 or 150 ppm. Alar at 500 ,1000 or 1500 ppm or Planofix (NAA) at 10,20 or 30 ppm as foliar sprays improved seed setting, resulting in an increase in number of pods/plant. All concentrations of GA and 1000 ppm Alar significantly increased the number of flowers per plant.Planofix at 10 ppm and all concentrations of GA also significantly increased the seed yield and maximum increase was obtained with 50 ppm GA.

2.6. Shelling percentage

Groundnut is cultivated mostly in the arid and semiarid regions of the world facing soil moisture deficit. Groundnut yield is a function of many plant and environmental factors, which are often inter-related. Several workers reported yield reduction with water stress induced at different stages of growth in groundnut (Balasubramanyam & Yayock,)

The shelling percentage of groundnut is around 80 % for early maturing bunch types compared with 60-70% for spreading cultivars (Saxena et al 1983). Under stress many pods are partly empty and assimilates are mostly transferred to the shell, hence maintaining nearly constant pod weight while reducing shelling percentage i.e.,compensation of pod weight with shelling percentage.Kernel size itself seems to be slightly reduced under some conditions of stress.

2.7 Number of root nodules and lateral roots

The effects of the phytohormone abscisic acid (ABA) on plant growth and root nodule formation were analyzed in *Trifolium repines* (white clover) and *Lotus japonicus*, which form indeterminate and determinate nodules.

In *L. japonicus*, the number of nodules was also reduced by ABA treatment. Thus, exogenous ABA inhibits root nodule formation after inoculation with rhizobia. Observation of root hair deformation revealed that ABA blocked the step between root hair swelling and curling. The nodulation capability is thought to have favored the success and radiation of the Leguminosae. Indeed, the Leguminosae (or Fabaceae) is the third largest family of flowering plants and most of its 20,000 species are able to nodule. Nodulation implies the formation of a new organ, the nodule, that hosts soil bacteria, called rhizobia, in conditions optimal for nitrogen fixation. To some extent, nodules resemble lateral roots and they originate from the same root cell layers. Two types of nodules can be discriminated depending on the persistence or not of their meristematic activity. Hence, most temperate legumes, like *Medicago* ssp., *Pisum sativum* (pea) and *Trifolium* sp. (clover), produce indeterminate nodules whose meristems persist over the entire nodule lifespan while other legumes, like *Lotus japonicus* (Lotus) or *Glycine max* (soybean), produce determinate nodules, whose meristematic activity stops early on during nodule development giving rise to small, round shaped nodules. In legume species, both the onset of nodulation and rhizobial entry into the root are dependent on a specific molecular dialog involving the production by the rhizobia of Nodulation Factors (NF) which also control notably host specificity. In Leguminosae, many phytohormones control both root and nodule development.

Interestingly, Liang and Harrishave studied the ability of various legumes and non-legumes plants to respond to the phytohormone abscisic acid (ABA) by increasing their lateral root (LR) density and hypothesized that the common predisposition of plants to form nodules may be linked to a difference in ABA sensitivity.

2.8 Pod size

The successful fertilization of the ovule is followed by cell division and cell expansion resulting in the growth of the fruit. Gibberellins are known to influence both cell division and cell enlargement (Adams et al., 1975; Kamijima, 1981).

In many species, GA₃ produced by developing seeds stimulates the growth and maturation of that fruit. Studies on endogenous GAs and GA metabolism in developing and mature seeds (Pharis and King, 1985)

2.9 Root length

The role of auxin in nodulation is closely linked to the development of other root structures like lateral root as these structures undergo a similar development program which involved cell division and differentiation. Both lateral roots and nodules are regulated by auxin-to-cytokinin ratio but in opposite way. An increase in auxin stimulated lateral root formation while an increase of cytokinin concentration or inhibiting auxin transport induced the development of pseudo-nodules. Analysis of ethylene responses on nodulation reflects a similar 'inverse' regulation of nodule and lateral root ontogeny. One of the earliest indications was the observation from exogenous cytokinins application to roots induced formation of pseudo-nodule structures of legumes (garden pea and alfalfa) and even non-legumes (tobacco). These physiological studies revealed a role for this hormone for the control of root architecture and nodule development. Exogenous application of cytokinins on legume root induced responses similar to Nod factors. This includes cortical cell divisions, amyloplast deposition and induction of early nodulin gene expression.

The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (El-Tarabily, 2008).

N. Moalemi and M. Chehrazi, reported that with different levels of Auxin hormone caused increase in getting rooting percent, number and dry weight of root. Application of higher quantities of NAA and IAA increased the root length and root volume

2.10 Nitrate Reductase Activity

Nitrate reductase is known to be a substrate –inducible enzyme (Candela, Fisher & Hewit,1957).

Developmental changes in plants affect the level of nitrate reductase activity and this decreases with age (Zieserl, Riverbark & Hangman,1963).

Knylp and Ferguson (1975) have reported that NR activity is induced by hepes buffer, ascorbate, aspartate glutamate, oxalate, succinate and tartarate and pH 3.0.

Plants usually reduce nitrate in their leaves where energy and reducing power arise (Beevers and Hageman, 1980).

Experiments show that the nitrate flux to the leaves from the roots plays a much larger regulatory role than the leaf nitrate content in controlling the level of Nitrate Reductase Activity in intact plants. (This work was supported by University of Illinois fellowships to D.L. S. and National Science Foundation Grant GB 41314.)

Nitrate reduction in roots depends on the import of photosynthates which provide carbon skeletons, energy and reductants (Huppe and Turpin, 1994).

Glucose induces Nitrate reductase activity in carbohydrate depleted green *Hordeum vulgare* leaves in light and in dark. Nitrate from the soil is the main

nitrogen source for higher plants, but it cannot be assimilated directly into ammonium ions in order to generate the organic N-compounds. Plants assimilate most of the nitrate absorbed by their roots into organic compounds by two step process. The availability of nitrogen and carbon particularly affects nitrate reductase which is inducible by nitrate, whereas ammonium and amino acids can inhibit its activity (Solomonson and Barber, 1990; Li et al, 1995; Sivansankar and Oaks, 1995). Carbon metabolism also regulates nitrate reductase and, in leaves, the light effect on transcription of NR-mRNA can be mimicked by sucrose (Cheng et al., 1992; Vincentz et al., 1993). Moreover, light controls nitrate reductase activity in leaves by phosphorylation-dephosphorylation reactions (Glaab and Kaiser, 1995; Huber et al., 1992; Kojima et al., 1995). In roots, light modulates nitrate reductase probably indirectly by photoassimilates (Merlo et al.,1994). These regulatory processes involving both carbon and nitrogen metabolism, assign to nitrate reductase an essential function in regulating the carbon: nitrogen ratio and shoot: root allocation (Beck, 1994)

Concentration of cytokinins within roots and xylem responds to the nitrogen availability in terms of either total nitrate availability (Samuelson and Larsson, 1993) or of differential distribution of nitrate along the lateral roots (Samuelson et al., 1995). Their transport is submitted to fluctuations of evapotranspiration and hence to foliar activity (Beck, 1994). They are known to regulate NR activity in response to light (Schmerder and Borris, 1986). or nitrate induction (Lu et al., 1990, 1992; Samuelson et al., 1995). In *Agrostemma githago* embryos, cytokinins induced NRA only in the presence of ethylene.

Nitrate reductase is a highly regulated enzyme. It has a short half life of some hours. In higher plant nitrate reductase exists in two forms: NADH: NR and NAD (P) H: NR. The bi specific NAD (P): NR is found in the non-green tissues of monocots and in both green and non-green tissues of legumes. Plant NR is a~100KDa polypeptide which binds 1 equivalent each of flavin adenine dinucleotide (FAD),

heme-Fe and molybdenum-molybdopterin (Mo-MPT). Nitrate reductase in higher plants is under influence of growth regulators Plant hormones are specific in nature and affect plant metabolism, subsequently to coordinate the growth and development of tissue.

Indole acetic acid also increases NR activity and it has been established that the effect of growth substances on the enzyme was not due to stimulation of general protein synthesis. IAA did not influence the nitrate reductase activity in *Hordeum vulgare*.

Auxin synergistically cytokinin activity on cell division and organogenesis. In a legume tree (*Parkia javanika*) showed significant increase in the nitrate reductase activity up to 12 days after treatment for IAA.

In pea roots, auxin compounds such as naphthalene acetic acid, indole propionic acid, butyric acid and indole acetic acid also increased GDH activity NAA (10⁻⁶M) enhanced seven times in vivo nitrate reductase in vitro NRA was increased 2.5 times in chicory roots. NAA may control nitrate reductase by a phosphorylation mechanism underscored by higher sensitivity of in vitro NRA to magnesium. The auxins increases ammonium assimilating potential of maize.

Gibberellin has no effect on the induction of NR activity in *H. Vulgare* aleurone layers GA in combination with a cytokine in increase NR A in leaves Foliar application of GA₃ showed significant increase in the NR activity in *Perkia* and *Nigella sativa* The optimum concentration was found to be 10 ppm for GA₃.

ABA stimulates NRA in many plant tissues such as in cucumber cotyledons of *Phaseolus aconitifolium* and cotton cotyledons. In nitrogen free medium, the enzyme activity in cotyledons of *Leucaena leucocephala* was decreased slightly with 1 μM to 100 μM ABA but in the presence of KNO₃ it increased slightly.

Cytokinins are regulators of many plant processes including cell division, apical dominance, photosynthesis leaf senescence and sink source relationships. Cytokinin induced enhancement of nitrate reductase has been first demonstrated in *Agrostemma githago*. The effect of cytokinins is independent of nitrate as has been reported in *Agrostemma githago* Embryos. The induction of nitrate reductase activity by cytokinins is negligible in the absence of nitrate. NR activity found maximum in the presence of light and cytokinins in fenugreek cotyledons. In excised cotyledons of cucumber 6-benzyl adenine induced nitrate reductase activity even under dark condition.

Cytokinins were shown to enhance NR-protein and NR-mRNA in the roots of the plant.

2.11 Effect on protein content

Cotton plants grown under water stress conditions showed significant decreases in yield, though only slight decreases in protein N as well as in chlorophyll content were detected. Chlormequat (CCC) application minimized the decreases in yield and caused significant increases in both protein N and chlorophyll contents. It is concluded that changes induced by CCC in cotton yield are independent from those occurred in chlorophyll and protein N contents. (Mohamed M. El-Fouly, Hassan A. El-Hamawi and Ahmad F. A. Fawzi Plant August 1971)

Cell growth refers to the increase in size of proliferating cells, is part of the cell cycle and coincides with an increase in total nucleic acid and protein content and no vacuolization due to application of NAA & IAA. (Trewavas AJ, Cleland RE 1983.)

2.12 Effect on Dehydrogenase activity

In nodule, nitrogenase activity was reduced by about 84 % under stress as compared with the control set. Glutamine synthetase activity was reduced by about 31, 16 and 23 %, glutamate oxoglutarate aminotransferase activity was reduced by 78, 57 and 42 % and glutamate dehydrogenase activity was reduced by 9, 8 and 42 % in leaf, root and nodule, respectively, under salt stress.

2.13 Chlorophyll Content

CCC at growth inhibiting concentrations decreased apparent photosynthesis while GA increased it (Marcelle and Oben,1973).

The growth regulators CCC and GA not only affect plant growth but they also influence leaf contents in chlorophyll, carotenoids, protein free amino acids, soluble sugars and starch (Marcelle et al,1974).

Four consecutive daily sprays of GA to young tomato plants reduced the chlorophyll content, both total and per unit weight, in the older leaves and increased in the younger leaves (Briant,1974).

The fact that cytokinins are so effective in delaying chlorophyll breakdown indicates that these growth regulators are somehow involved in maintaining the photosynthetic apparatus of plant organs. Cytokinin treatment can stimulate photosynthesis but also inhibit it in some tissues. (Ministry of science, Republic of Serbia.)

Phytohormones like IAA and kinetin inhibit chlorophyll loss during aging of chloroplasts during in vivo and in vitro. GA, on the other hand, stimulates the pigment degradation during aging of attached leaves in contrast to its senescence inhibiting action in detached leaves and isolated chloroplasts. (A.N.Misra, U.C.Biswal 1980).

Leaves treated with abscisic acid showed a longer lag phase and a lower rate of accumulation of chlorophyll as compared to the control than did leaves treated with gibberellic acid and kinetin. (Curt Bengston 1988)

2.14 Harvest index

The harvest index of groundnut varies from 0.35 to 0.50 (Bunting and Elston 1980).

Sequentially branched forms in which plant structure is smaller and formed over a shorter period of time and in which rate of leaf growth to total growth decline earlier tend to have a higher harvest index than the longer lived and more freely branched alternate forms in which vegetative growth is not arrested so soon by the internal regulation of partitioning.

2.15 Yield

CCC results in smaller grains has been shown by PRIMOST (1964) and BOND (1969). Groundnut kernel yield is the product of pod number, number of kernels per pod and weight of individual kernel.

Kernels per pod vary from 2-6, pods per plant from 50-105 and 100 kernel weight from 28 gm-62gm. (ICRISAT 1987). The variation is related to cultivar, spacing, fertiliser and climate. Yield components are mostly sensitive to environment stress during flowering and kernel growth stages. There is high positive correlation between the number of mature pods and pod yield. In general, pod number per plant decreases with increase in plant density. Wider spacing increases the number of branches per plant and number of mature pods per plant. Literature on pod yield indicates maximum contribution to pod yield to from the number of pods per plant followed by 100 kernel weight

Marri et al. found that harvest index was negatively correlated with plant height, but positively correlated with grain number/panicle, grain number/plant, percentage spikelet fertility, test grain weight and yield/plant in rice.

Sabouri et al. verified the negative correlation of harvest index with plant height and positive correlation with spikelet number and grain weight per panicle, and reported the impact of some flag leaf characteristics on harvest index in rice. In maize, harvest index is negatively correlated with plant height, but positively correlated with grain yield both phenotypically and genotypically

Cycocel foliar application increased cytokine in translocation from roots to shoots, leading to prolonged above ground parts life-span and hence increased yield (Omidi et al., 2005).

Major plant growth regulators (PGRs) significantly enhanced fiber yield in cotton (Copur et al. 2010), protein content in pea (Bora and Sarma 2006), chemical constituents in Croton (Soad et al. 2010), fruit size in Molina (Vwioko and Longe 2009), seed germination rate in black gram and horse gram (Chauhan et al. 2009), floral buds in Jojoba (Prat et al. 2008) and other growth parameters in different plants.

CHAPTER – III

MATERIALS AND METHODS

MATERIALS AND METHODS

A field experiment was conducted on “**Physiological studies in relation to nodulation and nitrogen fixation, yield and yield attributes in Groundnut using Plant Growth Regulators**” during *Rabi*’season 2016-17. The details of the experiment, materials used and methods and procedures followed during the conduction of experiment were briefly explained in this chapter.

3.1 Experimental details

A field experiment was conducted on Groundnut (*Arachis hypogaeae*) var Devi in Central Research Station, OUAT, Bhubaneswar which is situated at 85 degrees 52’ E Longitude and 20 Degrees 15’ N Latitude with an elevation of 25.5 meters above sea level. The Experimental field of Central Research Station is, Bhubaneswar is well drained and sandy loam in nature. Composite soil sample to a depth of 0.15 cm was collected before sowing from experimental site and was analyzed for important physico- chemical characters. The details of which are given below.

3.2 Soil Characteristics

Table 1. Soil characteristics

Factors	Value	Soil status	Method of analysis
pH (Soil:Water) (1:2)	5.2	Acidic	Digital pH meter. Model-ELICO-L-110
CEC m mole/cm ² (1:2)	0.13	Normal	Conductivity meter, model-Systronix
Organic carbon (%)	0.524	Medium	Walkley and Black
Available P ₂ O ₅ (kg/ha)	32.2	High	Olsen’s method
Available K ₂ O (kg/ha)	217.0	Medium	Flame Photometer Method
Available N (kg/ha)	559	Medium	Kjeldahal’s digestion and distillation method
Soil structure	Sandy loam		
Sand	76.4%		Bouyoucos Hydrometer method (Piper, 1950)
Silt	13.2%		
Clay	9.8%		

3.3 Weather Data:

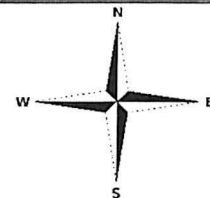
The mean monthly meteorological data of the cropping year 2016-2017 collected from “B” class observatory located near the experimental site are presented in the Table

Table 2. weather Data

Week No	Month	Date	temperature		Relative humidity		Rain fall (mm)
			Max(°C)	Min(°C)	% Mornin g	% After noon	
48	Dec 2016	29-5 th Dec	31.4	20.6	94.3	48	0
49		6-12	31.3	19.8	88.6	54	0
50		13-19	31.5	18.1	92.4	52.4	5.1
51		20-26	26.9	18.4	81.5	63.8	9.7
52		27-2 nd Jan	27.6	14.3	83.7	45.8	0
1	Jan 2017	3-9	29.3	16.3	96	44.9	0
2		10-16	30.4	14.1	93.3	32.3	0
3		17-23	31	16.9	91.1	35.1	0.6
4		24-30	28.1	14.5	85.1	44.1	0
5		31-6 th Feb	32.8	20.5	93	36.3	0
6	Feb 2017	7-13	32.5	19.7	93	39.3	0
7		14-20	35.1	21.3	87.1	40	0
8		21-27	37.3	22.8	85.7	38.7	0
9		28-5 th Mar	34	21.3	89.1	50.4	3
10	Mar 2017	6-12	10	22.1	87.1	33.6	0
11		13-19	11	23.2	86.4	42.9	1.3

Table 3. Lay out of the experiment

Replication 1	Replication 2	Replication 3
T-1	T-5	T-7
T-5	T-3	T-9
T-3	T-9	T-1
T-9	T-8	T-5
T-7	T-4	T-8
T-6	T-7	T-2
T-2	T-6	T-4
T-4	T-1	T-3
T-8	T-2	T-6



3.4.1 Experiment details

1. **Design** : Randomized block design
2. **Replication** : Three
3. **Variety** : Devi
4. **Treatments** : T-1= Control, T-2= IAA-10 ppm, T-3= IAA-20 ppm,
T-4= NAA-10 ppm, T-5= NAA-20 ppm, T-6= GA-10 ppm
T-7= GA-20 ppm, T-8= Cycocel 10 ppm, T-9 Cycocel-20
ppm
5. **Spacing** : 25 X 10cm
6. **Total Plot Area** : 108 sq meters
7. **Foliar application of hormones done at:** 30 DAS & 45 DAS

3.4.2 Calendar of Operation

- 1. Date of sowing:**07/12/2016
- 2. Irrigation dates:** 6/01/2017, 17/02/2017, 07/03/2017
- 3. Fertilizer application:** Fertilizers are applied as per recommended dosage and in 2 splits –i) Basal
ii) At 37 DAS (14/01/2017)

Rhizobium culture treatment was done @ 20gm/kg of seed. Bavistin was applied @2 gm/kg of seed.

FYM was applied @10 tons/ha. Urea, Single Super phosphate(SSP) and Muriate of Potash(MOP) were applied in the ratio 20:40:40 kg/ha. In 2nd dose of fertiliser application 20:40:20 kg/ha of Urea, SSP and MOP were applied.

3.4.3 Weeding: weeding was done thrice and was done manually.

1st weeding – 24/12/2016

2nd weeding- 4/01/2017

3rd weeding-02/02/2017

3.4.4 Plant protection

No herbicides or insecticides were applied in the field. Weeding was done manually thrice.

3.4.5 Date of Harvesting: 04/4/2017

3.5 Morphological parameters

3.5.1 Plant Height

Height of the main shoot was measured from the base of the main shoot up to the tip of the top most leaf at all the stages, averaged out and expressed in cm.

3.5.2 Number of leaves per plant

Number of leaves per plant of 5 randomly selected plants of a plot was counted and leaf number was averaged out.

3.5.3 Leaf area

Leaf area of each plant is measured by non-destructive method of measuring by measuring the leaf length and maximum width of the 4th leaf was recorded and the area was calculated by multiplying the length and width with k value. (MUTanko et al 2016) The leaf area obtained in (cm²/plant).

3.5.4. Root length

The plants are uprooted from the field and brought to the lab as such by tagging and the roots were cleaned under running tap water. Precautions were taken that the roots are not damaged by inundating the plant taken to facilitate easy uprooting. Then the length of the root was measured with the help of a scale

3.5.5 Root volume

Due to size factor measurement of groundnut roots is possible with the help of a 250/500 ml measuring cylinder. The water was filled upto 400 cc and roots immersed in it. The volume of water risen was measured in cc.

3.5.6 Number of branches per plant

Total number of primary branches arising from the main stem was counted which are collected from 5 different site of a treated subplot and their mean was taken as the number of leaves per plant.

3.5.7 Number of nodules per plant.

Five plants were chosen at random and uprooted with intact roots. The root portions were washed thoroughly and the number of nodules of each plant were counted under magnifying lens.

3.5.8 Total biomass

5 plants were uprooted randomly from different site of a treated plot. Root portion and pods of all five plants separated and washed it thoroughly, dried at 80 degree Celsius in hot air oven for 48 hours. After drying weighed separately then total biomass of a plant was calculated.

3.5.9 Root: Shoot Ratio

Root: Shoot ratio is measured by dividing root dry weight by shoot dry weight.

3.6 Physiological parameters

3.6.1 Chlorophyll content

The chlorophyll content of leaves was measured using chlorophyll meter was designed by Spectrum Technologies, Inc. The measurements were made at the middle of top leaves of three randomly selected plants and averaged to make an approximate estimate of the whole and express in SPAD values. The observations were recorded at different growth stages viz at vegetative stage and at flowering stage

$$\text{Chlorophyll (mg/sq cm)} = 0.0996(X) - 0.152$$

3.6.2 Leaf Area Index (TN Carlson et al)

LAI is expressed as the ratio of the leaf area (only one side) to the ground area (P) occupied by the crop. Formula adopted for determining leaf area index is furnished below:

$$\text{LAI} = \frac{\text{Total Leaf Area (CM}^2\text{)}}{\text{Ground Area (P)}}$$

3.6.3 Net Assimilation Rate (H. Pooter et al 1990)

NAR is defined as dry matter increment per unit leaf area per unit time and expressed as mg/cm²/day.

$$\text{NAR} = \frac{(W_2 - W_1) \times (\ln L_2 - \ln L_1)}{(T_2 - T_1) (L_2 - L_1)}$$

Where,

W_1 = dry weight of whole plant at time t_1

W_2 = dry weight of whole plant at time t_2

L_1 = leaf area at t_1

L_2 = leaf area at t_2

$t_2 - t_1$ = time interval in days between initial and final observation.

3.6.4 Relative Growth Rate (Leopold and Kriedemann 1975)

Relative growth rate (RGR) is defined as the total plant dry weight increase in a time interval in relation to the initial weight or dry matter increment per unit biomass per unit time or milligram of dry weight increase per gram of dry weight increase per gram of original weight per unit time and expressed as unit dry weight / unit dry weight / unit time (mg / g / day).

$$\text{RGR} = \frac{\text{Ln } W_2 - \text{Ln } W_1}{T_2 - T_1}$$

Where,

W_1 = whole plant dry weight at t_1

W_2 = whole plant dry weight at t_2

$T_2 - T_1$ = time interval in days

3.7 Biochemical parameters

3.7.1 Estimation of Nitrate reductase content

200 mg of fresh leaf after washing and soaking in blotting paper were weighed and then cut into small pieces. The cut pieces were taken in a 50 ml test tube containing 2 ml phosphate buffer, 1.6 ml 25% n-propanol, 3.0 ml 0.2M KNO_3 , 1.0 ml EDTA and 2.4 ml water. Leaf discs were submerged in reaction mixture and incubated in dark for 1 hour at 27 degree Celsius and shaken frequently. The reaction was stopped by adding 4 ml sulfanamide and 4 ml 0.2% NED solution. After 20 mins without removing the plant tissue or leaf discs the O.D. value of samples and standard were taken at 540 nm. The activity of Nitrate Reductase was calculated by NO_2 formed per gram fresh weight per hour by using standard curve. Standards were of following concentrations;

Table 4. Standard values

50 ml testube	ml of NaNO_2
1	0
2	0.2(0.92 μg)
3	0.4(1.84 μg)
4	0.6(2.76 μg)
5	0.8(3.68 μg)
6	1.0(4.60 μg)
7	1.2(5.52 μg)

3.7.2 Estimation of protein content

1 gm fresh sample was macerated in pestle and mortar in 10 ml TCA (10%) solution. The content was transferred to centrifuge at 5000 rpm for 10 minutes. After centrifugation the supernatant was discarded and 10 ml of NAOH was added in the tubes and mixed well and again centrifuged at 10000 rpm for 10 minutes. The supernatant was collected for true protein estimation. For estimation 0, 0.4, 0.8, 1.2, 1.6, 2 was pipette out into series of test tubes. 0.2 ml of sample was extracted. Volume make up was done by adding 2 ml distilled water. 10 ml of alkaline copper solution was added to each testube including blank. The contents were mixed and allowed to stand for 10 mins. Then 1 ml of 1N Folin-Ciocalteau was added mixed well and kept in dark for 30 minutes till blue colour develops. The O.D. value was then taken at 660 nm. Standard curve was drawn and protein content in sample was expressed in mg/gm of sample.

3.7.3 Estimation of Dehydrogenase Activity in seed

1 gm of seed of same size were taken and soaked in water for 12-13 hours. After soaking the seed coats were removed and the seeds were kept in 0.05% tetrazolium solution (volume=20ml) for 4 hours. After soaking the seed the tetrazolium solution was decanted seeds were washed thoroughly. 20 ml of methanol was added and seeds were soaked in it till completely discoloured. Then the spectrophotometer reading was taken at 480nm using methanol as blank.

Standard curve was prepared by preparing stock solution using 10 mg formazone in 100 cc methanol (concentration 100ppm). Then 1 ml of stock solution was added to 19 ml of methanol so that the final volume becomes 20ml and corresponding concentration is 5ppm. Similarly, for preparing 10ppm solution 2ml of stock solution and 18ml of methanol was used. For preparing 15ppm solution 3 ml of stock solution and 17 ml of methanol was used and for preparing 20ppm solution 4ml of stock solution and 16 ml methanol was used. O.D. value was noted down and standard curve was prepared.

3.8 Post-Harvest observations

3.8.1 Length of the pod (cm)

Length of the pods of ten labeled plants were measured from tip of the pod to the base of the pod and expressed in centimeter (cm). The average of five randomly selected pods was worked out.

3.8.2 Diameter of pod (cm)

Diameter of pod is measured and averaged to get the mean diameter

3.8.3 Number of pods

The total number of pods present in each plant from a particular treatment. The average number of pods of five randomly selected plants were worked out.

3.8.3 100 kernels weight

100 kernels were chosen from the sample plants at random and were weighed in gram after three consecutive sun drying for 4 hours a day under paper cover.

3.8.4 Harvest index

Harvest index is defined as the ration of economic yield to total biological yield (Donald, 1962) and expressed in percentage. The harvest index for maize was worked out as indicated below

$$\text{Harvest Index (HI)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

3.9 Statistical Analysis

The experimental design adopted for this research work was simple randomized block design (RBD). The pre and postharvest observations were statistically analysed following RBD as outlined by Panse and Sukhatme (1985).

3.9.1 Test of significance of correlation coefficient

The degree of correlation between different plant characters were measured in terms of correlation coefficient values. These correlation coefficients were estimated by using software packages.

The observed value of correlation coefficient is compared with the tabulated value for (n-2) degree of freedom. If observed value is more than tabulated value, the correlation coefficient is said to be significant.

3.9.2 Estimation of coefficient of variation (CV)

A measure of variation which is independent of unit of measurement and is therefore useful for comparison between different populations is provided by standard deviation expressed as percentage of mean. This measure is known as coefficient of variation given by equation

$$CV = (\delta/\mu) \times 100$$

Where δ = Standard deviation

μ = Mean of the observation

Comparative analysis of various treatments in field





Sowing of seeds

First foliar application of plant growth regulators at 30 DAS



Second foliar application of plant growth regulators at 45 DAS



Watering of plants



Manual weeding



Harvesting of the crop



CHAPTER – IV

EXPERIMENTAL FINDINGS

EXPERIMENTAL FINDINGS

The present experiment entitled “Physiological studies in relation to nodulation and nitrogen fixation, yield and yield attributes in Groundnut using Plant Growth Regulators” was conducted in the Central farm of OUAT, Bhubaneswar during Rabi season 2016-2017 to assess the improvement in yield, nitrogen fixation ability and nodulation in groundnut using plant growth regulators. The morphological, biochemical and yield characters are reflected in following heads and sub-heads.

4.1 Effect of plant growth regulators on plant height at different stages of the plant

The plant height was measured at 45 DAS, 60 DAS and 90 DAS revealed that maximum height was observed in 20 ppm NAA recording a value of 10.5cm, 12.3cm and 22 cm respectively followed by 20 ppm GA. The minimum height was observed in 10 ppm Cycocoeel recording a value of (5.5, 7, 15.1) cm. The percent increase in plant height was maximum in 90 DAS in 20 ppm NAA. (22.9%). The plant height was reduced due to the application of growth retardant 20 ppm CCC to the tune of 18.4 % at 90 DAS.

4.2 Effect of plant growth regulators on the number of leaves per plant

The number of leaves per plant increased in both the concentrations of GA, IAA & NAA. Maximum increase in number of leaves was noted in 20 ppm GA at 60 DAS. With the Application of growth retardants there was increase in leaf number at 10 ppm CCC but the leaf number reduced significantly at 30 & 60 DAS respectively at 20 ppm CCC.

4.3 Effect of plant growth regulators on leaf area per plant and leaf area index per plant

The leaf area observations showed that there was a significant increase in leaf area at 45 DAS, 60 DAS, and 90 DAS. The maximum leaf area was observed in 20 ppm NAA of 81.6, 93.8 square cm at 60 DAS and 90 DAS. The minimum leaf area was.

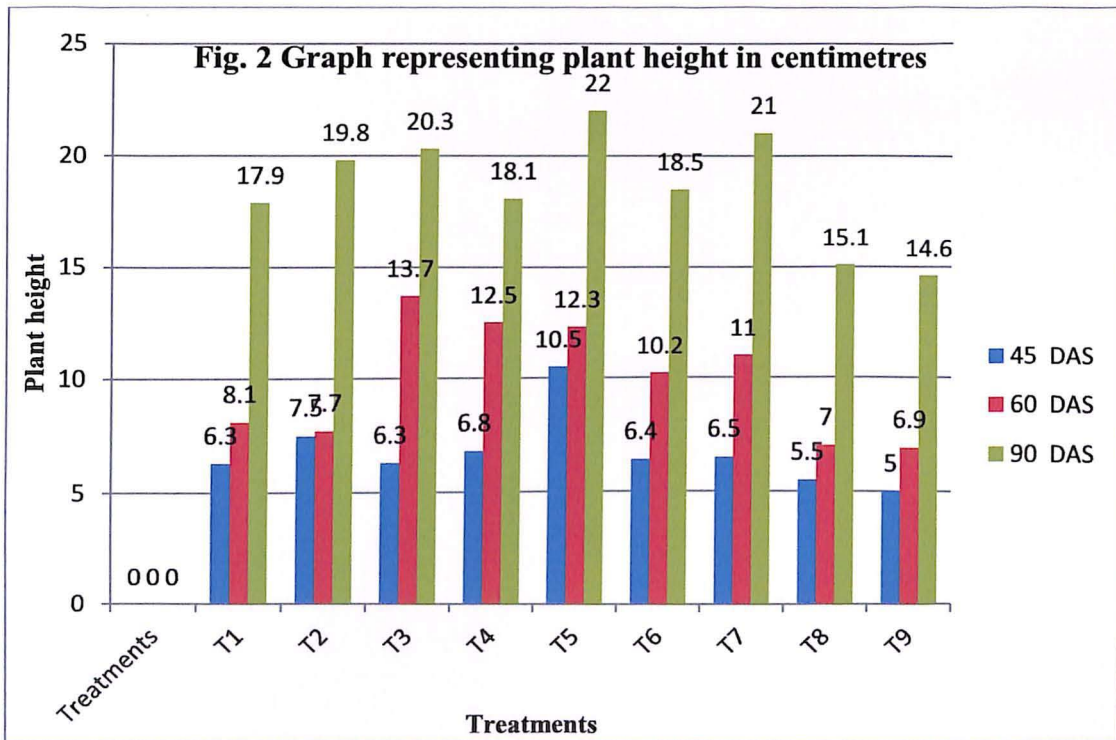
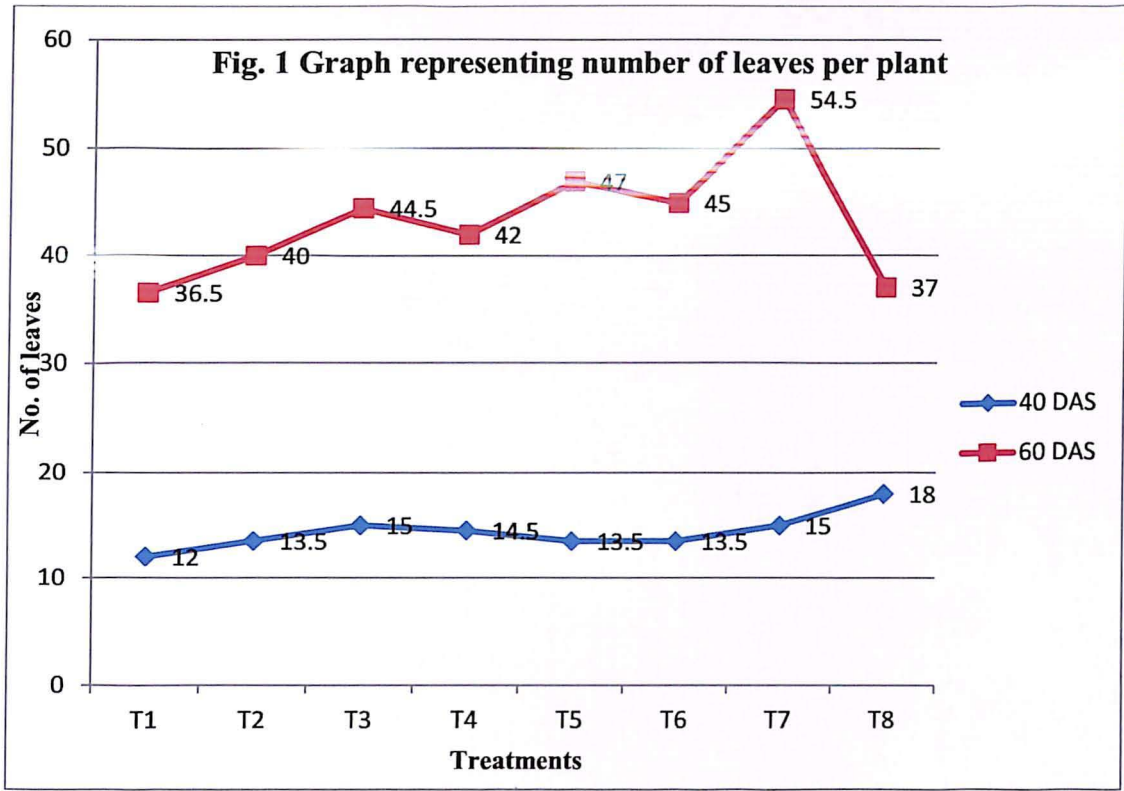


Fig. 3 Leaf area in square cm

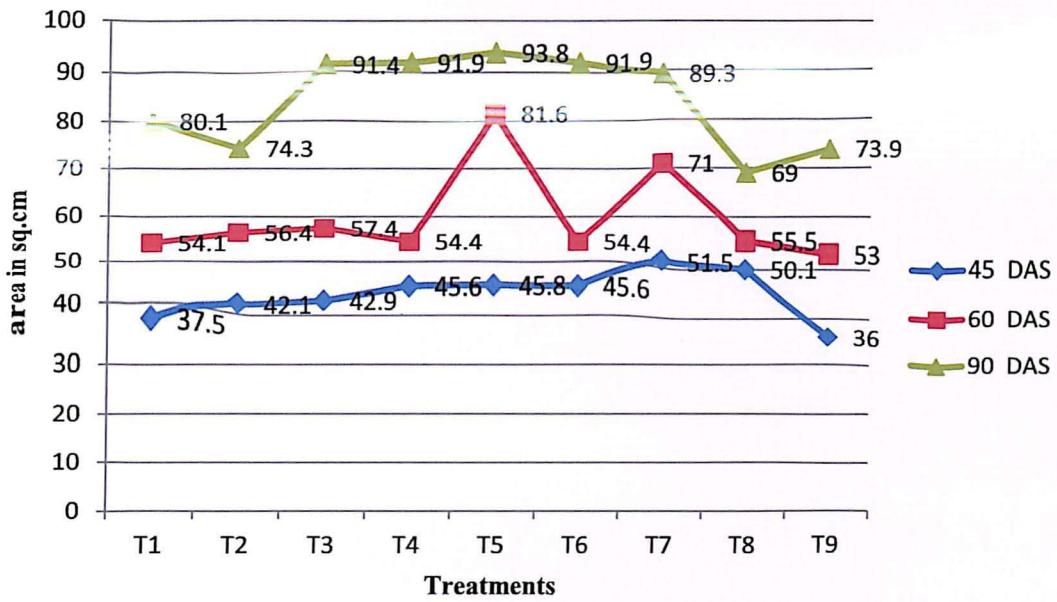


Fig.4 Graph representing number of branches per plant

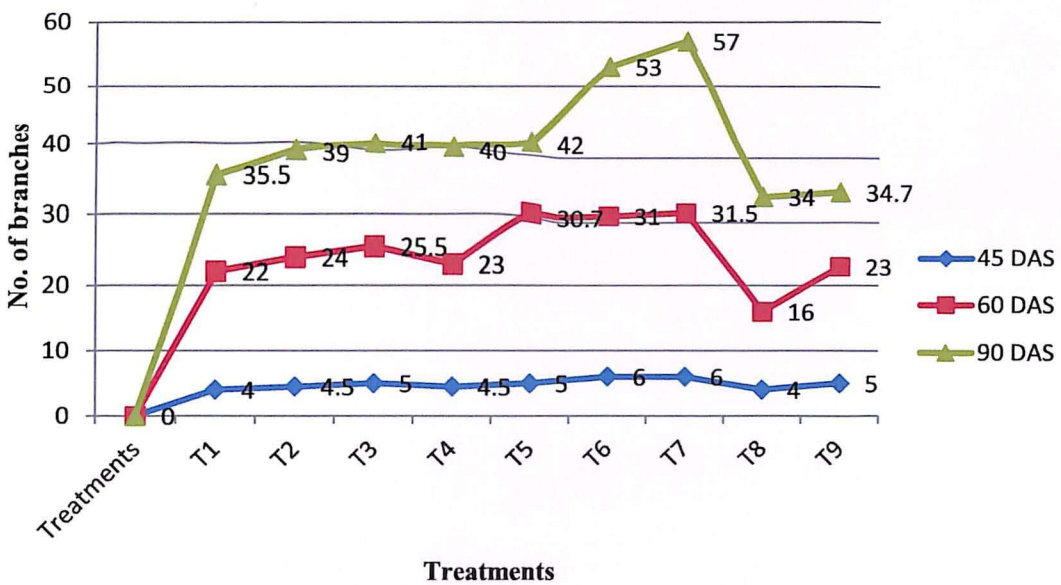


Fig. 3 Leaf area in square cm

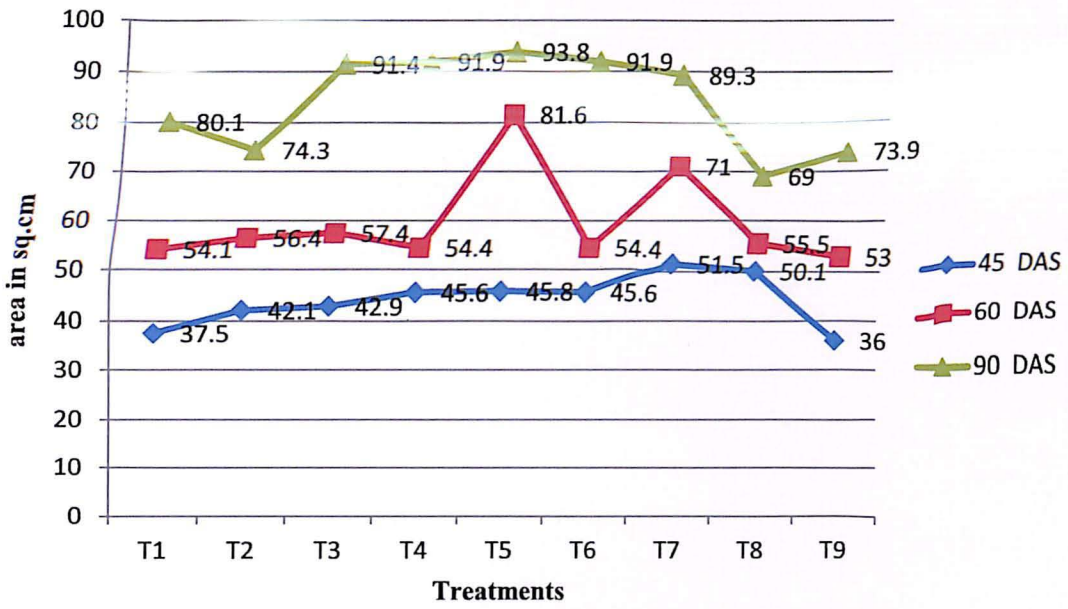
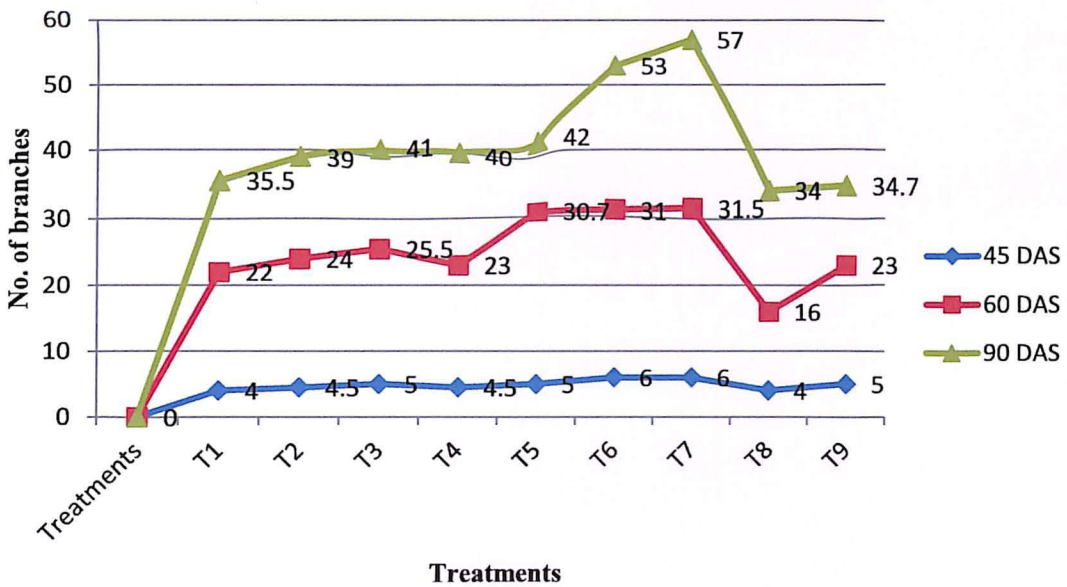


Fig.4 Graph representing number of branches per plant



recorded in 10ppm CCC at 90DAS. There was a significant reduction in leaf area by 14% at 90 DAS

Table 5. Leaf area index

TREATMENTS	DAS	
	45 DAS	65 DAS
T ₁	3.3	3.8
T ₂	4.0	4.2
T ₃	4.4	4.7
T ₄	4.0	4.3
T ₅	5.4	6.0
T ₆	4.3	4.8
T ₇	4.7	5.1
T ₈	3.1	3.6
T ₉	3.1	3.4
SEM	0.2435	0.2009
SED	0.3444	0.284
C.D.	0.73	0.6022

The leaf area index increased significantly over control in all the treatments except the application of growth retardants. Maximum leaf area index value was recorded in 20 ppm NAA recording a value of 5.4 and 6.0 at 45 DAS & 60 DAS and the minimum leaf area index of 3.1 was recorded in 20ppm CCC.

4.4 Effect of plant growth regulators on Number of branches per plant

The number of branches per plant increased in both the concentrations of GA. Among the treatments, 20ppm NAA resulted in increase in number of branches followed by 20ppm IAA at the stages. CCC resulted in the increase in number of branches at all the stages.

Table 6. Effect of plant growth regulators on Root dry weight, Shoot dry weight and Root: Shoot Ratio

Treatments	Shoot dry weight(gm)		Root dry weight(gm)		Root : Shoot	
	45 DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS
T 1	1.228	5.89	0.020	1.55	0.016	0.26
T 2	1.343	6.97	0.030	2.40	0.1977	0.344
T 3	1.517	7.0	0.117	2.48	0.0760	0.356
T 4	1.457	6.0	0.182	2.90	0.125	0.483
T 5	1.538	7.04	0.50	3.06	0.312	0.439
T 6	1.220	6.5	0.104	2.60	0.085	0.40
T 7	1.302	6.89	0.166	2.8	0.127	0.43
T 8	1.229	5.90	0.05	1.66	0.048	0.29
T 9	1.335	5.91	0.08	1.94	0.059	0.33
SEM	0.0016	1.3856	0.0008	0.2067	0.0201	0.27
SED	0.0011	1.9592	0.0011	0.2922	0.0284	0.29
C.D.	0.0033	4.1535	0.0024	0.6195	0.0602	0.0027

The effect of growth regulators on shoot dry weight, root dry weight and root: shoot ratio revealed that 20 ppm NAA had the maximum shoot dry weight(1.538 gm) at 45 DAS and maximum shoot dry weight (7.04 gm) at 90 DAS. 10ppm CCC recorded increase in the shoot dry weight at 45 DAS (1.229 gm) & 90 DAS (5.90 gm) followed by 20 ppm CCC which recorded a value of (1.335 gm and 5.91 gm at 45 and 90 DAS respectively. The maximum root: shoot ratio is found in 20 ppm NAA (0.312 & 0.439) at 45 DAS & 90 DAS respectively. The lowest root: shoot ratio was found in Control 0.26 gm

Table 7. Effect of plant growth regulators on Root length, Root Volume and Number of nodules.

Treatments	Root length in cm			Root volume (cc)	Number of nodules
	45 DAS	60 DAS	90 DAS	60 DAS	60 DAS
T 1	5.0	5.5	6.7	7.5	43.0
T 2	6.3	5.6	6.9	10.8	52.0
T 3	6.3	6.5	7.8	12.5	65.0
T 4	5.3	5.5	8.8	9.3	56.0
T 5	6.0	8.8	11.8	15.8	60.0
T 6	5.4	5.5	8.6	8	66.0
T 7	3.5	5.5	9.0	10	70.0
T 8	4.2	4.9	6.2	7.5	38.5
T 9	4.1	4.5	5.6	6	35
SEM	0.4333	0.4093	0.1052	2.0955	2.5
SED	0.6127	0.5787	0.1488	2.9631	3.535
C.D.	1.299	1.2269	0.3154	6.2817	7.4942

The effect of growth regulators on Root length, root volume and number of nodules revealed that 20 ppm NAA had the highest root length at 45 DAS, 60 DAS & 90 DAS (6,8.8,11.8) cm and the lowest root length was recorded by 20 ppm CCC at 45 DAS (4.1 cm). Maximum root volume was recorded by 20 ppm NAA (15.8 cc) followed by 10 ppm NAA (12.5 cc), the minimum root volume was recorded in 20 ppm CCC. Maximum number of nodules was seen in 20 ppm GA (70) followed by 10 ppm CCC. Maximum number of nodules was seen in 20 ppm GA (70) followed by 10 ppm GA (66) the lowest number of nodules were seen in 20 ppm CCC (35) followed by 10 ppm CCC (38.5) at 60 DAS.

Table 8. Effect of plant growth regulators on chlorophyll content

TREATMENTS	Chlorophyll content in mg per square cm of leaf	
	45 DAS	60 DAS
T 1	1.2	1.3
T 2	1.3	1.5
T 3	1.4	1.6
T 4	1.1	1.2
T 5	1.4	1.8
T 6	1.1	1.4
T 7	1	1.4
T 8	0.8	1.0
T 9	1.0	1.1
SEM	0.1407	0.0622
SED	0.1989	0.088
C.D	0.4216	0.1866

The effect of plant growth regulators on chlorophyll content of leaves showed that highest chlorophyll content was recorded in 20 ppm NAA (1.4 mg/sq cm of leaf) and (1.8 mg/sq cm of leaf) at 45 and 60 DAS respectively. The lowest chlorophyll content was recorded by 10 ppm CCC (1.0 mg/sq cm of leaf) followed by 20 ppm CCC at 60 DAS. The percent of reduction ranged from minimum of 8.3 % in 10 ppm CCC to maximum of 33.3 % in 20 ppm NAA & IAA AT 45 DAS.

Table 9. Effect of Plant Growth regulators on Relative Growth Rate and Net Assimilation Rate

Treatments	NAR (mg/dm ² /day)	RGR(mg/g/day)
T 1	0.112	0.105
T 2	0.202	0.116
T 3	0.315	0.221
T 4	0.205	0.121
T 5	0.323	0.257
T 6	0.301	0.183
T 7	0.300	0.187
T 8	0.102	0.101
T 9	0.114	0.110
SEM	0.0012	0.0663
SED	0.0017	0.0937
C.D.	0.0036	0.1986

4.5 Effect of plant growth regulators on Net Assimilation Rate

The effect of plant growth regulators on Net assimilation Rate (NAR) showed that there is increase in NAR for all the treatments during the periods of growth. During 45-60 DAS of growth, significant increases were observed at both the concentrations of NAA, 10ppm GA and 20ppm IAA. The highest NAR value was observed in 20 ppm NAA (0.323 mg/dm²/day) followed by 20 ppm IAA (0.315 mg/dm²/day). The lowest NAR value was recorded by 10 ppm CCC (0.102 mg/dm²/day).

4.6 Effect of plant growth regulators on Relative Growth rate

The effect of plant growth regulators on RGR showed that there is marginal increase in RGR for all the treatments during the periods of growth.

Highest RGR value was recorded in 20 ppm NAA (0.257 mg/gm/day) followed by 20 ppm IAA(0.221mg/gm/day) and lowest RGR was recorded by 10 ppm CCC (0.101 mg/gm/day).

Table 10. Effect of plant growth regulators on Nitrate Reductase Activity

Treatments	N0 ₂ Formed per gm fresh weight per hour
	60 DAS
T 1	0.672
T 2	0.674
T 3	0.832
T 4	0.755
T 5	1.131
T 6	0.647
T 7	0.713
T 8	0.486
T 9	0.690
SEM	0.0011
SED	0.0015
C.D.	0.0032

The effect of plant growth regulators on Nitrate reductase (NR) activity indicates that maximum NR activity was recorded by 20 ppm NAA followed by 20 ppm IAA at 60 DAS. The minimum NR activity was recorded by 10 ppm CCC. The

percent increase in NR activity was found to be (68.3%) in 20 ppm NAA. The percent reduction in NR activity was found to be 27.6 % in 10 ppm CCC.

Table 11. Effect of plant growth regulators on Protein content

Treatments	Protein content in mg/gm fresh weight of leaf
	60 DAS
T 1	3.57
T 2	4.19
T 3	4.48
T 4	4.07
T 5	4.64
T 6	4.12
T 7	4.18
T 8	3.51
T 9	3.80
SEM	0.0906
SED	0.1281
C.D.	0.2715

The effect of plant growth regulators on protein content of leaves indicated that the highest protein content was found in 20 ppm NAA (4.64 mg/gm fresh weight) followed by 20 ppm IAA (4.48 mg/gm fresh weight) at 60 DAS. The lowest protein content was recorded in 10 ppm CCC (3.51 mg/gm fresh weight at 45DAS .and 20 ppm CCC (3.80 mg/gm fresh weight) at 60 DAS.

Table 12. Effect of plant growth regulators on dehydrogenase activity of seed

Treatments	Formazone content in µg per hour
	120 DAS
T 1	18.5
T 2	19
T 3	13.9
T 4	15
T 5	13.5
T 6	23.5
T 7	26.5
T 8	12.5
T 9	13.5
SEM	1.6933
SED	2.3944
C.D.	5.0761

Table 13. Yield and yield attributing characters of groundnut

Treatments	Number of pods per plant	Pod diameter (cm)	Pod length (cm)	100 Kernel weight(grams)	Harvest Index	Yield (kg per sub plot)
T 1	14	1.2	2.1	58.4	0.15	2.2
T 2	17	1.3	2.3	63.9	0.26	2.3
T 3	19	1.5	2.5	67.3	0.30	2.4
T 4	20	1.4	2.3	64.1	0.27	2.2
T 5	22	1.6	2.6	67.5	0.33	2.4
T 6	24	1.7	2.7	68.4	0.50	2.5
T 7	28	2.1	3.0	70	0.53	2.7
T 8	8	0.5	2.0	53.4	0.10	2.0
T 9	10	1.0	2.2	62.5	0.20	2.1
SEM	0.2222	0.0556	0.0511	0.5011	0.0034	0.0056
SED	0.3142	0.0786	0.0723	0.7086	0.0047	0.0079
C.D.	0.6662	0.1665	0.1532	1.5021	0.0101	0.0167

The effect of plant growth regulators on Dehydrogenase activity of seeds indicated that highest accumulation of formazone occurred in 20 ppm GA (26.5 $\mu\text{g}/\text{hour}$) followed by 10 ppm GA (23.5 $\mu\text{g}/\text{hour}$) at 120 DAS. The lowest accumulation of was observed in 10 ppm CCC (12.5 $\mu\text{g}/\text{hour}$) followed by 20 ppm CCC (13.5 $\mu\text{g}/\text{hour}$) at 120 DAS. The percentage increase in formazone content in seeds was 43.24% in 20 ppm GA and 27 % in 10 ppm GA. The percent reduction of formazone content in seeds was 32.4% in 10 ppm CCC at 120 DAS.

4.7 Effect of plant growth regulators on number of pods

The effect of plant growth regulators on number of pods indicated that maximum number of pods occurred in 20 ppm GA (28) followed by 10 ppm GA (24). The percent increase in number of pods was near about 100 % in 20 ppm GA and 71.42 % in 10 ppm GA. The minimum number of pods occurred in 10 ppm CCC (8) followed by 20 ppm CCC (10). The percent reduction in number of pods in 10 ppm CCC was 42.8% over the control.

4.8 Effect of plant growth regulators on pod diameter

The effect of plant growth regulators on pod diameter indicated that maximum pod diameter occurred in 20 ppm GA (2.1 cm) followed by 10 ppm GA (1.7 cm). The percent increase in pod diameter was 75 % in 20 ppm GA and 41.66% in 10 ppm GA. The minimum pod diameter occurred in 10 ppm CCC (0.5 cm) followed by 20 ppm CCC. The percent reduction in pod diameter in 10 ppm CCC was 58.33% over the control.

4.9 Effect of plant growth regulators on pod length

The effect of plant growth regulators on pod length indicated that pod length was maximum in 20 ppm GA (3 cm) followed by 10 ppm GA (2.7 cm). The percent increase in pod length was 42.85% in 20 ppm GA and 28.57 % in 10 ppm GA. The minimum pod length occurred in 10 ppm CCC (2 cm). The percent reduction in pod length in 10 ppm CCC was 4.76 % over the control.

4.10 Effect of plant growth regulators on 100 kernel weight

The effect of plant growth regulators on 100 kernel weight indicated that maximum kernel weight occurred in 20 ppm GA (70 gm) followed by 10 ppm GA

(68.4 gm). The percent increase in kernel weight was 19.86 % in 20 ppm GA and 17.12 % in 10 ppm GA. The minimum kernel weight occurred in 10 ppm CCC (53.4 gm). The percent reduction in kernel weight in 10 ppm CCC was 8.56 % over the control.

4.11 Effect of plant growth regulators on harvest index

The effect of plant growth regulators on harvest index indicated that highest harvest index occurred in 20 ppm GA (0.53) followed by 10 ppm GA (0.50). The percent increase in harvest index in 20 ppm GA was 23 % and in 10 ppm GA the percent increase in harvest index was 21 %. The lowest harvest index occurred in 10 ppm CCC (0.10). The percent reduction in harvest index was 33.33% in 10 ppm CCC.

4.12 Effect of plant growth regulators on yield

The effect of plant growth regulators on yield indicated that highest yield was obtained by 20 ppm GAA (2.7 kg) followed by 10 ppm GA (2.5 kg). The percent increase in yield in 20 ppm GA was 22.72 % and 13.63 % in 10 ppm CCC. Lowest yield was obtained in 10 ppm CCC (2 kg) followed by 20 ppm CCC (2.1 kg). The percent reduction in yield in 10 ppm CCC was 9 % and 5 % in 20 ppm CCC.

CHAPTER – V

DISCUSSIONS

DISCUSSION

A field experiment was conducted during 2016-2017 (Rabi season) in central farm, OUAT Bhubaneswar to assess the effect of plant growth regulations on nodulation, Nitrogen fixation yield & yield attributes in groundnut. Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in the cellular metabolism of plants. Results of the present experiment have been presented in chapter 4 along with tables. This chapter indicates to interpret the findings obtained into supporting evidences & literature without any prejudice under the following heads & sub heads. Available literature in the regard in available and attempted has been made to correlate the fact and figures in present experiment. This finding should provide a strong support to farming committees to increase the yield of groundnut per unit land area, along with reduction in cost of cultivation.

5.1 Plant height

The maximum plant height was observed in highest concentrations of NAA followed by highest concentrations of IAA. The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (El-Tarabily, 2008). There was significant response in relation to plant height by the application of plant growth regulations. There was considerable reduction in height due to application of growth retardants at both low and high concentration of CCC. Plant height was decreased by the Cycocel application (Mushtaq Ahmad Bhat, Inayatullah Tahir, Waseem Shahri and Sheikh Tajamul Islam, 2011) The percentage increase in plant height was maximum at 90DAS in 20 ppm NAA (22.9%). The plant height was reduced due to application of CCC to the tune of 18.9% at 90 DAS. CCC interacts with GA preventing its synthesis that leads to subsequent loss in vegetative growth.

5.2 Number of leaves

The number of leaves per plant increased in both the concentration of GA, IAA and NAA. Maximum increase in number of leaves was noted in 20ppm GA at 60 DAS. There was increase in leaf number at 10ppm CCC but leaf number reduced significantly at 30DAS and 60DAS respectively at 20 ppm CCC. CCC being a growth retardant. The plant growth retardant cycocel (2-chloroethyl) trimethyl ammonium chloride (CCC) has received a great attention, especially in connection with dwarfening properties, to the plant (HUMPHRIES 1963, 1968; CATHEY 1964; LOWE and CARTER 1971; and others).

5.3 Leaf Area

There was significant increase in leaf area at 45 DAS, 60DAS and 90DAS. The maximum leaf area was observed in 20 ppm NAA at 60DAS and 90 DAS. NAA helps in growth and elongation of cells so maximum leaf area was seen in 20 ppm NAA followed by 20 ppm IAA. The minimum leaf area was found in 10 ppm CCC. Growth retardants reduced the leaf area significantly at all concentrations. Synthesis of some plant hormones is adversely affected by environmental factors, which causes restriction on physiological processes of the plant and ultimately, limits their growth potential (Copur et al. 2010). Leaf area decreased to an extent of 14% at 90DAS. Leaves will remain small if circumstances are unfavourable, or they will expand a large surface area when the necessary nutrients are available (Chapin 1991). Decrease in leaf area due to cycocel application was reported by HUMPHRIES and DYSON (1967) and HUMPHRIES and BOND (1969).

5.4 Leaf Area Index

The leaf area increased significantly over all treatments except the application of growth retardants and growth retardants reduced the leaf area. The results of the research conducted by Gosh and Basu (1999) showed that the effect of auxin hormone on grain yield, harvest index, and 100-grain weight was statistically significant at 1% level. Growth retardants CCC hindered cell growth and elongation decreasing leaf area and leaf area index. Maximum leaf area was recorded in 20 ppm NAA at both 45 DAS and 60 DAS and minimum leaf area was found in 20 ppm CCC followed by 10 ppm CCC. Decrease in leaf area due to cycocel application was reported by HUMPHRIES and DYSON (1967) and HUMPHRIES and BOND (1969).

5.5 Number of branches

The number of branches per plant increased in both the concentrations of GA (10 ppm and 20 ppm). A higher concentration of gibberellins increases plant growth (Bora and Sarma 2006) while higher concentration of auxin inhibits it (Hussain et al. 2010). Mckenzie and Deyholos (2011) reported that treatment of GA causes stem elongation, expansion and proliferation and cell wall thickening in bast fiber of linseed. The findings from the experiment indicated a similar trend as sited in literature. GA increases shoot formation in plants increasing the number of leaves and number of branches. CCC resulted in the increase in number of branches at all the stages.

5.6 Root dry weight, Shoot dry weight and Root:Shoot ratio

Higher NAA concentration promotes rapid cell division resulting in increase in growth and biomass of a plant. The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root

initiation, seed germination and seedling growth (El-Tarabily, 2008). N. Moalemi and M. Chehrazi, reported that with different levels of Auxin hormone caused increase in getting rooting percent, number and dry weight of root. Application of higher quantities of NAA and IAA increased the root length and root volume resulting in an overall increase in root dry weight. Application of growth retardant CCC showed an increase in root dry weight and shoot dry weight over the control. Cycocel increased the seed yield mainly due to increased root growth and more drought resistance under water deficit conditions (De et al., 1982). " As such, CCC inhibits cell elongation, resulting is thicker stalks, which are sturdier, facilitating harvesting of cereal crops, Gowariker, Vasant; Kalyani Paranjape; Sudha Gowariker (2013); V. N. Krishnamurthy (2013). Hambris et al. (1960) reported that any yield increase by CCC was related to the enhanced dry matter accumulation. Growth retardants such as CCC affect the plant height, increase the shoots diameter and standing, reduce seed yield loss and hence may go to increased seed yield per plant and plot (Emam and Karimi,1996; Emam and Moaied, 2000).

5.7 Root length and root volume

Higher NAA concentration promotes rapid cell division resulting in increase in growth and biomass of a plant. The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (El-Tarabily, 2008). N. Moalemi and M. Chehrazi, reported that with different levels of Auxin hormone caused increase in getting rooting percent, number and dry weight of root. Application of higher quantities of NAA and IAA increased the root length and root volume

The root volume decreased with increase in application of CCC. Root-growth potential was decreased at high doses of CCC while shoot growth potential was not affected. (Aphalo, P. J. and Lehto, T. 1997). Higher doses of CCC resulted in more

significant decrease in root volume and root length. Maximum root length and root volume was seen in higher doses of NAA & IAA because auxins promote root elongation and root growth. In roots, the most well-characterized auxin-associated phenotypes are the dose-dependent increase in the length of epidermal-derived root hairs, the bimodal effect of auxin concentration on primary root length, the dose-dependent increase in number of lateral root primordia, and the response to gravity (Pitts et al. 1998; Rahman et al. 2002; Ishida et al. 2008; Peret et al. 2009). Many of the genes involved in the synthesis of auxin are expressed in roots and that root-generated auxin contributes to the maintenance of the gradients and maxima required for normal root development. (Ljung et al. 2005; Ikeda et al. 2009; Petersson et al. 2009).

5.8 Chlorophyll content

The chlorophyll content was seen to increase in higher concentrations of NAA followed by IAA. NAA & IAA in higher concentrations increased the leaf area favouring more chlorophyll accumulation and more photosynthesis. Auxins evidently delayed senescence and increased chlorophyll contents in excised stems (Arru L., Rognoni S. Poggi A. 2008). Chlorophyll content increased slightly with increase in application of growth retardants CCC. The percent of reduction ranged from minimum of 8.3 % in 10 ppm CCC to maximum of 33.3 % in 20 ppm NAA & IAA AT 45 DAS. Effect of CCC and ethrel modify the crop canopy and initiates luxurious vegetative growth that may be beneficial for flower initiation and fruiting points. Singh et al. (1993). CCC at growth inhibiting concentrations decreased apparent photosynthesis while GA increased it (Marcelle and Oben,1973).

5.9 Net Assimilation Rate(NAR)

NAR increased for all the treatments during the periods of growth. During 45-60 DAS of growth, significant increases were observed at both the concentrations of NAA, 10ppm GA and 20ppm IAA. The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (El-Tarabily, 2008). A higher concentration of gibberellins increases plant growth (Bora and Sarma 2006) while higher concentration of auxin inhibits it (Hussain et al. 2010). Mckenzie and Deyholos (2011) reported that treatment of GA causes stem elongation, expansion and proliferation. Higher concentrations of NAA had higher NAR values. Application of CCC resulted in reduction of the NAR due to reduction in plant growth and reduced development. Decrease in leaf area due to cycocel application was reported by HUMPHRIES and DYSON (1967) and HUMPHRIES and BOND (1969).

5.10 Relative Growth Rate(RGR)

The effect of plant growth regulators on RGR showed that there is marginal increase in RGR for all the treatments during the periods of growth. The auxin are a group of indole ring compounds which have the ability to improve plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth (El-Tarabily, 2008). A higher concentration of gibberellins increases plant growth (Bora and Sarma 2006) while higher concentration of auxin inhibits it (Hussain et al. 2010). Mckenzie and Deyholos (2011) reported that treatment of GA causes stem elongation, expansion and proliferation. Higher concentrations of NAA had higher RGR values. Application of CCC resulted in reduction of the RGR due to reduction in plant growth and reduced development. Decrease in leaf area due to cycocel application was reported by HUMPHRIES and DYSON (1967) and HUMPHRIES and BOND (1969).

5.11 Nitrate Reductase Activity (NR activity)

Maximum NR activity was recorded by 20 ppm NAA followed by 20 ppm IAA at 60 DAS. The NR activity increased with the increase of dose of NAA and IAA application. It was demonstrated that IBA induced Nitrate generation in root primordia, (Wilson ID, Neill SJ, Hancock JT,2008). Nitrate reduction occurs mainly in roots until they differentiate tubers. Thereafter, nitrate reductase activity is localized in leaves (Dorchies and Rambour, 1985). However, a relationship between the development of NR Activity and the rhizogenic potential of IAA and IBA on rooting of pea cuttings has once been reported (Ahmad, 1988). Findings showed positive correlation between NR activity and increased dose of NAA and IAA application. The percent increase in NR activity was found to be (68.3%) in 20 ppm NAA. There was considerable decrease in NR activity with the application of CCC. The percent reduction in NR activity was found to be 27.6 % in 10 ppm CCC.

5.12 Protein Content

Protein content of leaves increased with increased dose of NAA application followed by IAA application. 2,4-D prevents protein loss in autumn leaves by maintaining protein synthesis. Auxin promotes cell division and meristem maintenance and also plays an important role in the establishment of cellular patterning. Plant development thus integrates the regulation of cell proliferation and growth with further cell expansion and differentiation. NAA & IAA increases cell growth and cell expansion. Cell growth refers to the increase in size of proliferating cells, is part of the cell cycle and coincides with an increase in total nucleic acid and protein content and no vacuolization due to application of NAA & IAA. (Trewavas AJ, Cleland RE 1983.) The lowest protein content was recorded in 10 ppm CCC. Protein content increased slightly with the increase in CCC application.

5.13 Dehydrogenase content

The effect of plant growth regulators on Dehydrogenase activity of seeds indicated that highest accumulation of formazone occurred in 20 ppm GA. Higher concentrations of GA induced more accumulation of formazone hence showed an increase in activity of dehydrogenase enzyme. Plants showing an increase in dehydrogenase activity have greater Reactive Oxygen Species scavenging mechanism hence show better results when subjected to stress condition. Hence it can be said that if we apply higher quantities of GA the plant will perform better in stress conditions. The lowest accumulation of formazone was observed in 10 ppm CCC (12.5 µg/hour) followed by 20 ppm CCC (13.5 µg/hour) at 120 DAS. The percentage increase in formazone content in seeds was 43.24% in 20 ppm GA and 27 % in 10 ppm GA. The percent reduction of formazone content in seeds was 32.4% in 10 ppm CCC.

5.14 Number of pods

The effect of plant growth regulators on number of pods indicated that maximum number of pods occurred in 20 ppm GA followed by 10 ppm GA. Number of pods increased with the increase in dose of application of GA. The percent increase in number of pods was near about 100 % in 20 ppm GA and 71.42 % in 10 ppm GA. The minimum number of pods occurred in 10 ppm CCC followed by 20 ppm CCC. There was slight increase in number of pods with the increase in dose of application of CCC. The percent reduction in number of pods in 10 ppm CCC was 42.8% over the control. There is a slight increase in number of pods with increase in dose of application of CCC. In soybean (*Glycine max*) the application of growth regulators (e.g. Ethrel) resulted in higher vegetative growth and pod yield when applied at 40 and 60 days after sowing (Devi et al., 2011). Previous studies of large-fruited cultivars have reported that the application of growth retardants, e.g. chlormequat chloride (CCC) (Zayed et al., 1985) or paclobutrazol (Syed et al., 1997) may restrict

plant height and even increase the number of pods produced per plant (Bhatt & Srinivasa Rao, 2009), perhaps by changing the level of hormones involved in floral induction and differentiation (Jiang et al., 2010).

5.15 Pod diameter

The effect of plant growth regulators on pod diameter indicated that maximum pod diameter occurred in 20 ppm GA followed by 10 ppm GA. Pod diameter increased with the increase in dose of application of GA. The successful fertilization of the ovule is followed by cell division and cell expansion resulting in the growth of the fruit. Gibberellins are known to influence both cell division and cell enlargement (Adams et al., 1975; Kamijima, 1981). In many species, GA₃ produced by developing seeds stimulates the growth and maturation of that fruit. Studies on endogenous GAs and GA metabolism in developing and mature seeds (Pharis and King, 1985). The percent increase in pod diameter was 75 % in 20 ppm GA and 41.66% in 10 ppm GA. The minimum pod diameter occurred in 10 ppm CCC followed by 20 ppm CCC. The percent reduction in pod diameter in 10 ppm CCC was over the control.

5.16 Pod length

The effect of plant growth regulators on pod length indicated that pod length was maximum in 20 ppm GA followed by 10 ppm GA. The percent increase in pod length was 42.85% in 20 ppm GA and 28.57 % in 10 ppm GA. The minimum pod length occurred in 10 ppm CCC. The percent reduction in pod length in 10 ppm CCC was 4.76 % over the control.

5.17 100 kernel weight

The effect of plant growth regulators on 100 kernel weight indicated that maximum kernel weight occurred in 20 ppm GA (70 gm) followed by 10 ppm GA

(68.4 gm). The percent increase in kernel weight was 19.86 % in 20 ppm GA and 17.12 % in 10 ppm GA. The minimum kernel weight occurred in 10 ppm CCC (53.4 gm). The percent reduction in kernel weight in 10 ppm CCC was 8.56 % over the control.

5.18 Harvest index

The effect of plant growth regulators on harvest index indicated that highest harvest index occurred in 20 ppm GA (0.53) followed by 10 ppm GA (0.50). Plants having less height show greater harvest index. Plants applied with GA showed reduction in height over plants applied with NAA & IAA but showed an increase in number of pods and other yield characters. Marri et al. [9] found that harvest index was negatively correlated with plant height, but positively correlated with grain number/panicle, grain number/plant, percentage spikelet fertility, test grain weight and yield/plant in rice. Sabouri et al. [10] verified the negative correlation of harvest index with plant height and positive correlation with spikelet number and grain weight. The percent increase in harvest index in 20 ppm GA was 23 % and in 10 ppm GA the percent increase in harvest index was 21 %. The lowest harvest index occurred in 10 ppm CCC (0.10). The percent reduction in harvest index was 33.33% in 10 ppm CCC.

5.19 Yield

The effect of plant growth regulators on yield indicated that highest yield was obtained by 20 ppm GAA followed by 10 ppm GA. Plants applied with GA showed reduction in height over plants applied with NAA & IAA but showed an increase in number of pods and other yield characters. Marri et al. found that harvest index was negatively correlated with plant height, but positively correlated with grain number/panicle, grain number/plant, percentage spikelet fertility, test grain weight

and yield/plant in rice. Sabouri et al. verified the negative correlation of harvest index with plant height and positive correlation with spikelet number and grain weight. The percent increase in yield in 20 ppm GA was 22.72 % and 13.63 % in 10 ppm CCC. Lowest yield was obtained in 10 ppm CCC followed by 20 ppm CCC. The percent reduction in yield in 10 ppm CCC was 9 % and 5 % in 20 ppm CCC. There was slight increase in yield in plants applied with 20 ppm CCC. Than in plants applied with 10 ppm CCC. Increase in yield was seen with increase in CCC dose. Previous studies of large-fruited cultivars have reported that the application of growth retardants, e.g. chlormequat chloride (CCC) (Zayed et al.,1985) or paclobutrazol (Syed et al., 1997) may restrict plant height and even increase the number of pods produced per plant (Bhatt & Srinivasa Rao, 2009), perhaps by changing the level of hormones involved in floral induction and differentiation (Jiang et al., 2010).

CHAPTER – VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in the cellular metabolism of plants. Application of plant growth regulators to plants result in substantial increase in yield and yield attributing characters. Plant growth regulators affect various physiological and biochemical processes in plants that cause alteration in cellular metabolism resulting in increased yield and productivity. Keeping view of the above facts , field trial was conducted in Central farm, OUAT, taking plant growth regulator namely IAA, NAA & GA in order to study **“Physiological studies in relation to nodulation and nitrogen fixation,yield and yield attributes in Groundnut using Plant Growth Regulators”**. Of groundnut variety Devi under Randomised Block Design(RBD) with 3 replications.

1. Due to application of plant growth regulators maximum height was observed in 20 ppm NAA recording a value of 10.5cm,12.3cm and 22 cm respectively followed by 20 ppm GA. The minimum height was observed in 10 ppm Cycocoel recording a value of (5.5,7,15.1) cm. The percent increase in plant height was maximum in 90 DAS in 20 ppm NAA. (22.9%). The plant height was reduced due to the application of growth retardant 20 ppm CCC to the tune of 18.4 % at 90 DAS.(Figure 2)
2. Due to application of plant growth regulators the number of leaves per plant increased in both the concentrations of GA, IAA & NAA. Maximum increase in number of leaves was noted in 20 ppm GA at 60 DAS. With the Application of growth retardants there was increase in leaf number at 10 ppm CCC but the leaf number reduced significantly at 30 & 60 DAS respectively at 20 ppm CCC.(Figure 1)
3. Due to application of plant growth regulators the maximum leaf area was observed in 20 ppm NAA of 81.6,93.8 square cm at 60 DAS and 90 DAS.

The minimum leaf area was recorded in 10ppm CCC at 90DAS. There was a significant reduction in leaf area by 14% at 90DAS. (Figure 3)

4. Due to application of plant growth regulators the leaf area index increased significantly over control in all the treatments except the application of growth retardants. Maximum leaf area index value was recorded in 20 ppm NAA recording a value of 5.4 and 6.0 at 45 DAS & 60 DAS and the minimum leaf area index of 3.1 was recorded in 20ppm CCC. (Table 5)
5. The number of branches per plant increased in both the concentrations of GA. Among the treatments, 20ppm NAA resulted in increase in number of branches followed by 20ppm IAA at the stages. CCC resulted in the increase in number of branches at all the stages. (Figure 4)
6. Due to application of growth regulators 20 ppm NAA had the maximum shoot dry weight, root dry weight and root:shoot ratio. 10ppm CCC recorded increase in the shoot dry weight at 45 DAS (1.229 gm) & 90 DAS (5.90 gm) followed by 20 ppm CCC which recorded a value of (1.335 gm and 5.91 gm at 45 and 90 DAS respectively. The maximum root : shoot ratio is found in 20 ppm NAA (0.312 & 0.439) at 45 DAS & 90 DAS respectively. The lowest root:shoot ratio was found in Control 0.26 gm (Table 6)
7. Due to application of growth regulators Root length, root volume and number of nodules increased significantly in 20 ppm NAA 20 ppm NAA had the highest root length at 45 DAS, 60 DAS & 90 DAS (6,8.8,11.8) cm and the lowest root length was recorded by 20 ppm CCC at 45 DAS (4.1 cm). Maximum root volume was recorded by 20 ppm NAA (15.8 cc) followed by 10 ppm NAA (12.5 cc), the minimum root volume was recorded in 20 ppm CCC. Maximum number of nodules was seen in 20 ppm GA(70) followed by 10 ppm GA (66) the lowest number of nodules were seen in 20 ppm CCC (35) followed by 10 ppm CCC (38.5) at 60 DAS (Table 7)

8. Due to application of plant growth regulators highest chlorophyll content was recorded in 20 ppm NAA (1.4 mg/sq cm of leaf) and (1.8 mg/sq cm of leaf) at 45 and 60 DAS respectively. The lowest chlorophyll content was recorded by 10 ppm CCC (1.0 mg/sq cm of leaf) followed by 20 ppm CCC at 60 DAS. The percent of reduction ranged from minimum of 8.3 % in 10 ppm CCC to maximum of 33.3 % in 20 ppm NAA & IAA AT 45 DAS. (Table 8)
9. Due to application of plant growth regulators Net assimilation Rate (NAR) increased in all the treatments during the periods of growth. During 45-60 DAS of growth, significant increases were observed at both the concentrations of NAA, 10ppm GA and 20ppm IAA. The highest NAR value was observed in 20 ppm NAA (0.323 mg/dm²/day) followed by 20 ppm IAA (0.315 mg/dm²/day). The lowest NAR value was recorded by 10 ppm CCC (0.102 mg/dm²/day). (Table 9)
10. Due to application of plant growth regulators RGR marginal increase in all the treatments during the periods of growth. Highest RGR value was recorded in 20 ppm NAA (0.257 mg/gm/day) followed by 20 ppm IAA(0.221mg/gm/day) and lowest RGR was recorded by 10 ppm CCC (0.101 mg/gm/day). (Table 9)
11. Due to application of plant growth regulators on Nitrate reductase (NR) activity maximum NR activity was recorded by 20 ppm NAA followed by 20 ppm IAA at 60 DAS. The minimum NR activity was recorded by 10 ppm CCC .The percent increase in NR activity was found to be (68.3%) in 20 ppm NAA .The percent reduction in NR activity was found to be 27.6 % in 10 ppm CCC (Table 10)
12. Due to application of plant growth regulators protein content of leaves showed significant increase. Highest protein content was found in 20 ppm NAA (4.64 mg/gm fresh weight) followed by 20 ppm IAA (4.48 mg/gm fresh

weight) at 60 DAS. The lowest protein content was recorded in 10 ppm CCC (3.51 mg/gm fresh weight at 45DAS .and 20 ppm CCC (3.80 mg/gm fresh weight) at 60 DAS. (Table 11)

13. Due to application of plant growth regulators Dehydrogenase activity of seeds increased. Highest accumulation of formazone occurred in 20 ppm GA (26.5 $\mu\text{g}/\text{hour}$) followed by 10 ppm GA (23.5 $\mu\text{g}/\text{hour}$) at 120 DAS. The lowest accumulation of was observed in 10 ppm CCC (12.5 $\mu\text{g}/\text{hour}$) followed by 20 ppm CCC (13.5 $\mu\text{g}/\text{hour}$) at 120 DAS. The percentage increase in formazone content in seeds was 43.24% in 20 ppm GA and 27 % in 10 ppm GA. The percent reduction of formazone content in seeds was 32.4% in 10 ppm CCC at 120 DAS (Table 12)

14. The effect of plant growth regulators on number of pods indicated that maximum number of pods occurred in 20 ppm GA (28) followed by 10 ppm GA (24). The percent increase in number of pods was near about 100 % in 20 ppm GA and 71.42 % in 10 ppm GA. The minimum number of pods occurred in 10 ppm CCC (8) followed by 20 ppm CCC (10). The percent reduction in number of pods in 10 ppm CCC was 42.8% over the control. (Table 13)

15. The effect of plant growth regulators on pod diameter indicated that maximum pod diameter occurred in 20 ppm GA (2.1 cm) followed by 10 ppm GA (1.7 cm). The percent increase in pod diameter was 75 % in 20 ppm GA and 41.66% in 10 ppm GA. The minimum pod diameter occurred in 10 ppm CCC (0.5 cm) followed by 20 ppm CCC. The percent reduction in pod diameter in 10 ppm CCC was 58.33% over the control.(Table 13)

16. The effect of plant growth regulators on pod length indicated that pod length was maximum in 20 ppm GA (3 cm) followed by 10 ppm GA (2.7 cm). The percent increase in pod length was 42.85% in 20 ppm GA and 28.57 % in 10 ppm GA. The minimum pod length occurred in 10 ppm CCC (2 cm). The

percent reduction in pod length in 10 ppm CCC was 4.76 % over the control. (Table 13)

17. Due to application of plant growth regulators 100 kernel weight increased Maximum kernel weight occurred in 20 ppm GA (70 gm) followed by 10 ppm GA (68.4 gm). The percent increase in kernel weight was 19.86 % in 20 ppm GA and 17.12 % in 10 ppm GA. The minimum kernel weight occurred in 10 ppm CCC (53.4 gm). The percent reduction in kernel weight in 10 ppm CCC was 8.56 % over the control. (Table 13)

18. Due to application of plant growth regulators highest harvest index occurred in 20 ppm GA (0.53) followed by 10 ppm GA (0.50). The percent increase in harvest index in 20 ppm GA was 23 % and in 10 ppm GA the percent increase in harvest index was 21 %. The lowest harvest index occurred in 10 ppm CCC (0.10). The percent reduction in harvest index was 33.33% in 10 ppm CCC. (Table 13)

19. Due to application of plant growth regulators highest yield was obtained by 20 ppm GAA (2.7 kg) followed by 10 ppm GA (2.5 kg). The percent increase in yield in 20 ppm GA was 22.72 % and 13.63 % in 10 ppm CCC. Lowest yield was obtained in 10 ppm CCC (2 kg) followed by 20 ppm CCC (2.1 kg). The percent reduction in yield in 10 ppm CCC was 9 % and 5 % in 20 ppm CCC. (Table 13)

Conclusion

From the above experiment it was concluded that application of plant growth regulators can alter various parameters in a plant in context to yield. Application of plant growth regulators effectively increased the root nodule number, plant height, leaf area, number of branches, root: shoot ratio and other enzymatic activity of plant. Among the plant growth regulators GA gave outstanding results.

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