

**Effect of different fertilizers on growth and biochemical
parameters of *Oryza sativa* L. var. IR 20**

**Anusuya, S.
(13PBO001)**

**Thesis submitted to
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore – 641 043**

**In Partial Fulfilment of the Requirements for the
Degree of Master of Science in Botany**

March 2015

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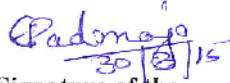
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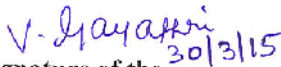
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Signature of the
Head of the Department


Signature of the
Supervisor

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CONTENTS

CHAPTER NO	TITLE	PAGE NO.
	LIST OF TABLES	
	LIST OF FIGURES	
	LIST OF PLATES	
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	5
III	MATERIALS AND METHODS	13
IV	RESULTS AND DISCUSSION	21
V	SUMMARY AND CONCLUSION	41
	REFERENCES	

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Shoot length and root length of Rice (<i>Oryza sativa</i> L.) seedlings (cm)	25
2	Fresh weight and Dry weight of Rice (<i>Oryza sativa</i> L.) seedlings (cm)	27
3	Chlorophyll “a” chlorophyll “b” and “Total” chlorophyll content of Rice (<i>Oryza sativa</i> L.) seedlings (mg/g)	31
4	Protein content of Rice (<i>Oryza sativa</i> L.) seedlings (mg/g)	36
5	Carbohydrate content of Rice (<i>Oryza sativa</i> L.) seedlings (mg/g)	38

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1	Root length of Rice Seedlings on 30 th and 45 th day	26
2	Shoot length of Rice Seedlings on 30 th and 45 th day	26
3	Fresh Weigth of Rice Seedlings on 30 th and 45 th day	28
4	Dry Weigth of Rice Seedlings on 30 th and 45 th day	28
5	Chlorophyll 'a' content of Rice Seedlings on 30 th and 45 th day	32
6	Chlorophyll 'b' content of Rice Seedlings on 30 th day 45 th day	33
7	Total chlorophyll concent of Rice Seedlings on 30 th and 45 th day.	34
8	Protein content of Rice Seedlings	37
9	Carbohydrate content of Rice Seedlings	39

LIST OF PLATES

PLATE NO	TITLE	PAGE NO.
1	Paddy seeds sown in petriplates	22
2	Germination of paddy seeds	22
3 & 4	Paddy seedlings transferred to cups	23
5	Growth of paddy seedlings after transfer into pots	30

CHAPTER - I

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food of more than three billion people in the world; most of them live in Asia, (Dwivedi *et al.*, 2014). Rice, the most important food crop in terms of direct consumption, is cultivated under a wide range of environment (Mohammed and Tarpley, 2009) and chemical fertilizer is the most important input required for rice cultivation. In the red soil regions, paddy is the main cereal crop, contributing 19% and 29% of the world rice area and rice production, respectively (Sun and Huang, 2011) . In recent years, due to the rapid population growth and a continuous decline in the amount of cultivated land area, the rate of fertilizer application keeps on rising in order to obtain high crop production (Wang *et al.*, 2010) .

Non – leguminous plants like rice, maize and wheat belonging to the poaceae family form staple food for approximately 6.5 billion people around the world. An exponential rise in the world population indicates the need for increased crop production. This rise in production has been a result of the indiscriminate use of chemical fertilizers (NPK) in combination with advanced technology. Nitrogen fertilization of non-leguminous crops is one of the most expensive inputs in agriculture (Bhattacharjee *et al.*, 2008).

The high-yielding rice variety has resulted in an increase in rice production but requires large amounts of chemical fertilizers, leading to health hazards and environmental pollution. Recently, there is a growing interest in plant growth – promoting rhizobacteria (PGPR) due to their efficacy as biological control and growth promoting agents in many crop (Thakuria *et al.*, 2004).

Paddy soils are a group of anthropogenic soils with a long history of rice cultivation that are currently producing one-quarter of the grain consumed in china, representing an extremely important farming system in terms of global food security (Gong, 1999). A recent study argued that paddy soils should have greater organic carbon sequestration potential than dry cropland soils (Lal, 2002).

Indian agriculture is moving rapidly towards commercialization but many farmers still give primacy to cereals in their cropping system on the consideration of food

security, low risk and easy market access. The rice-wheat cropping system (RWCS) has been, and remains the most preferred cropping in Punjab, due to its comparative economic advantages, assured marketing and stable productivity levels (World Bank, 2003 and Sidhu *et al.*, 2010). Rice cultivation is the principal activity and source of income for millions of households around the globe and several countries in Asia and Africa are highly dependent on rice as a source of foreign exchange earnings and government revenue (FAO, 2009). Globally, rice is the second most important crop in terms of area, but as food, rice is an important crop since it provides more calorie than any other cereal (Islam *et al.*, 2014).

Tamil Nadu one of the leading rice growing state in India, has been cultivating rice from time immemorial as this state is endowed with all favorable climatic conditions suitable for rice growing. The Paddy Breeding Station of Coimbatore which was established in 1913 has released CO1 to CO7 varieties up to 1929. The pure line CO4 is highly resistant to Blast and has been utilized as donor for the evolution of blast resistant strains. A total of 50 varieties and 3 rice hybrids were released from Paddy Breeding Station, Coimbatore. The climate of Tamil Nadu is basically tropical. Due to its proximity to the sea, the summer is less hot and winter is less cold. The maximum daily temperature rarely exceeds 43°C and the minimum daily temperature seldom falls below 18°C. The state is exposed to both South West and North East monsoons. Rice is a semi-aquatic plant and grows best under low land condition. In India, it grows in almost all types of soils - alluvial, red, laterite, black, saline and alkali, peaty and marshy soils and in acid soils. But, the soils having good water retention capacity with good amount of clay and organic matter are ideal for rice cultivation. Pongal is an ancient and undoubtedly the most popular and fervently celebrated festival of Tamils. It is the harvest festival of Tamil Nadu celebrated on the 1st day of Tamil month, *Thai*, that is 14th January of every year. “Pongal” is actually the name of a rice and lentil dish cooked commonly in Tamil Nadu, and specifically on the festive day of Pongal (Thiyagarajan and Kalaiyarasi, 2005 - 06).

The rising population of Asian countries means that it is necessary to increase food production. Fertilizer will be an essential part of this process. Asia is moving from agriculture, which depends on the natural fertility of the soil, to agriculture, which is fertilizer dependent. Most agricultural soils are deficient in N, which can conveniently be

supplied in the form of N fertilizer. Correct timing of nitrogen application is important to ensure that they coincide with the period of nitrogen uptake by the crop.

Soil organic matter (SOM) play a key role in the quality of soil because of its effect on the physical, chemical and biological processes of soil (Yan *et al.*, 2007). Use of organic fertilizers in paddy fields can reduce global warming by reducing the green house gas emission (Sampanpanish, 2012).

The major determinant of the success or failure of a crop production depends on the availability of plant nutrients either naturally/artificially in soil. The main source to replenish the plant nutrients in agricultural soils include organic, inorganic and bio-fertilizers (Masarirambi *et al.*, 2012). For a sustainable crop production, no single source of plant nutrients can meet the total nutrient requirement. The use of chemical fertilizer in agriculture is increasing and this reduces the fertility of soil by reducing the soil health. Soil health is needed for sustainable crop productivity (Korsaeth *et al.*, 2002).

The burning of organic materials (rice and wheat crop residues) results in depletion of soil fertility and poor physical properties of soil. The burning process produces pollution by way of heat and toxic gas production. The crop residue possesses a certain amount of organic matter that is essential for soil fertility and productivity (Shahzad *et al.*, 2010).

Soil organic matter is important to improve the water holding capacity of soil and to maintain better aeration for seed germination and root development of plants (Zia *et al.*, 1998). The use of organic manures such as farmyard manure, poultry manure, compost and green manure could enhance the plant growth and development. The use of herbicides such as butachlor, anilofos and pretichlor control the growth of weed in rice (Khare *et al.*, 2014).

India has to produce around 140 million tons of rice by 2020 to meet the food requirement of the growing population (Kavitha *et al.*, 2008). The use of fertilizers, both chemical fertilizers and manures to increase the soil fertility and crop productivity has often negatively affected the complex system of biogeochemical cycle (Steinshamn *et al.*, 2004). In the last five decades, the application of nitrogen, phosphorus and potassium (NPK) fertilizers has increased tremendously (Adesemoye and Kloepper, 2009).

Rice (*Oryza sativa*) is considered the most important food crop of the world, representing the staple diet for two-thirds of the human population. In response to current reduced availability and soaring prices of rice and other agricultural products, an urgent Emergency Rice Initiative was launched to assist African rice growing countries in exploring sustainable approaches for seed production and distribution as well as for mineral fertilizer procurement and distribution (Ramos and Austria, 2008).

The goals of that initiative are to : (1) promote an agronomic revolution in rice production and reduce existing yield gaps, (2) accelerate the delivery of new post – harvest technologies, (3) accelerate the introduction of higher yielding rice varieties, (4) strengthen and upgrade the rice breeding and research pipelines, (5) accelerate research on the thousands of rice varieties and disseminate the results, so that scientists can access the vast reservoir of useful information they contain, and educate a new generation of rice scientists and researchers for the public and private sectors (Yanni and Dazzo, 2010).

The main objective of the present investigation is

1. to study the growth of rice seedlings under different fertilizer application including leaf extract and
2. evaluate the changes taking place in the growth (shoot length, root length, fresh weight and dry weight) and biochemical (viz., chlorophyll, protein and carbohydrate) parameters.

CHAPTER - II

REVIEW OF LITERATURE

The available literature pertaining to the use of green manures and chemical fertilizers on growth and biochemical parameters relevant to the present investigation has been reviewed and presented in this chapter.

Meelu and Morris (1986) have studied the biomass production, N accumulation and effect of legume green manures on *Sesbania* crop yield and fertility in Philippines. The results revealed an increase in crop yield due to legume green manuring.

A field experiment with irrigated rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) rotations was conducted on a sandy loam soil in Punjab to evaluate the effect of incorporation of cowpea (*Vigna unguiculata* L.) or sesbania (*Sesbania aculeate* L.) green manure on crop productivity (Aulakh *et al.* , 2000). Nitrogen utilization by rice was greater when green manure was used than fertilizer nitrogen.

Vaishampayan *et al.* (2001) has investigated the surface of soil that acts as a site for aerobic phototrophic nitrogen (N) fixation by free- living cyanobacterial and their *Azolla-Anabaena* symbiont and reported that the cyanobacterial biofertilizer could have a strong potential in rice-growing countries to reduce the requirement of inorganic fertilizers.

A field study was conducted to investigate wheat growth and yield response to various green manure legumes (*Sesbania*, cluster bean, rice bean) and different P levels (0, 30, 60, 90 kg P₂O₅ ha⁻¹). Among the green manuring crops, *Sesbania* and cluster bean showed an increase over control by about 16% and 11% respectively (Sultani *et al.*, 2004).

In a field study, Mirza *et al.* (2005) have studied the effect of agroforestry (*Sesbania*) and farm yard manure on rice (*Oryza sativa*). Results revealed that both grain and straw yield of paddy were significantly improved by the application of *Sesbania* and farm yard manure. Green manuring with *Sesbania* improved the grain and straw yield by 15.4 % and 14.5 % respectively.

A field experiment was conducted in red soils to evaluate the effect of green manuring on soil properties and yield of wheat (*Triticum aestivum* L.) under different soil depths. The highest grain yield of 2638 kg ha⁻¹ was recorded under green manuring of sun hemp in deep soil (Dev and Lal, 2006).

Makeen *et al.* (2007) suggested that the main pigment of black gram is chlorophyll *a*. The study showed that incubation method is superior to other methods.

Yan *et al.* (2007) has analyzed the C and N mineralization and C and N content in soil, particulate organic matter (POM), light fraction organic matter (LFOM) and microbial biomass to assess the effect of long-term fertilization on labile organic fractions. Their results showed that the fertilizer N decreased or did not affect the C and N amounts in soil fractions, except N mineralization and soil total N.

An experiment was carried out to determine the effect of organic and inorganic fertilizers on growth and yield of broccoli. Four organic fertilizer doses (0, 40, 60 and 80 t ha⁻¹) and three inorganic fertilizer doses (0, 30 and 60 kg ha⁻¹) were used. Application of 60 kg inorganic fertilizer with 60 ton organic manure per hectare produced highest broccoli yield (40.05 t ha⁻¹). The results showed higher chlorophyll content when inorganic fertilizer was added along with organic manure (Ouda and Mahadeen, 2008).

A field experiment was conducted to study the effect of organic manures on plant growth, seed quality and yield of soyabean. It was observed that the application of recommended dose fertilizer (RDF) +FYM recorded higher seed quality parameters viz., germination (96.76%), seedling vigour index (3713) and field emergence (93.33%) (Maheshbabu *et al.*, 2008).

Jeyakumar *et al.* (2008) have studied varied responses of black gram (*Vigna mungo*) to certain chemical foliar spray and plant growth regulators. Results showed an increased plant height, number of branches and leaf area index.

Tejada *et al.* (2008) studied the effect of incorporating three green manures originating from residues of *Trifolium pretense* L. (TP), *Brassica napus* L. (BN) and mixture of TP+BN at the rate of 5384 and 8973 kg ha⁻¹ on soil biological properties, nutrition and yield parameters of maize (*Zea mays*).

Adesemoye and Kloepper (2009) has reported that, the use of chemical fertilizers and manures for enhancing soil fertility and crop productivity continuously, often resulted in unexpected harmful environment effects, including, leaching of nitrate, surface run off of phosphorus and nitrogen and eutrophication of aquatic ecosystems.

Akparobi (2009) reported an increase in the growth and yield parameters of *Amaranthus cruentus* by applying different levels of farm yard manure (0 t / ha, 15 t / ha and 35 t / ha) and also observed a significant differences ($p > 0.05$) among different levels of farm yard manure used.

Erulan *et al.* (2009) have studied the effect of seaweed liquid fertilizer (*Sargassum polycystum*) on seed germination, growth, yield, biochemical parameters and pigment characteristics of *Cajanus cajan*. The 50 % concentration of water and alcohol extracts of seaweed showed better results on seed germination, growth parameters, biochemical parameters and pigment constitution.

Liu *et al.* (2009) has studied the effect of alternative farming on soil quality parameters. Their results indicated that the development of effective fertilization practices, especially by manipulating the quantity and type of organic amendments, may improve long-term sustainability of paddy soil ecosystems.

Mohammed and Tarpley (2009) has studied the effect of high night time temperatures (HNT) and preventive exogenous effectors on growth, development, physiology and yield of rice plants. Their results indicated that exogenous application of salicylic acid reduced the negative effects of HNT.

The study to develop a biofertilizer based on filamentous nitrogen-fixing cyanobacteria selected from rice fields to generate a technological package compatible with its use for the rice crop in Chile has been done by Pereria *et al.* (2009).

Tolanur (2009) has conducted a study on the effect of different organic manures with different levels of inorganic fertilizer- N, 50 % N and 100 % N (recommended dose of N- RDN) on grain yield and uptake of major nutrients (N, P, K). The result showed highest grain yield and uptake of NPK in chick pea at 50% recommended dose level.

The isolation and characterization of plant growth promoting rhizobacteria (PGPR) from the rhizosphere soil of rice field for the enhancement of growth of rice has been reported by Ashrafuzzaman *et al.* (2009). Saijeen *et al.* (2009) evaluated the growth of *Gerbera* by organic fertilizer application.

The possibility of using *Azolla- Anabaena* in rice field, as a source of organic nitrogen has been studied by Bocchi and Malgioglio (2010).

The effect of NaCl on seed germination in some plant species belonging to the families *Brassicaceae* and *Solanaceae* has been studied by Bojovic *et al.* (2010). The results showed that the seeds of all the species germinated only in the lowest concentration.

Javadian *et al.* (2010) has studied cold-induced changes of carbohydrate and chlorophyll in wheat. Msuya and Stefano (2010) has recorded the seed germination capacity and vigour in maize (*Zea mays*) with regard to size and position of ear.

The study carried out by Yanni and Dazzo (2010) on the ability of biofertilizer inoculants containing *Rhizobium leguminosarum* to enhance the production of rice (*Oryza sativa* L.) has established a merit of deploying our biofertilization strategy using selected rhizobial strains to promote rice production, there by, reducing the need for additional chemical N-fertilizer inputs to maintain agricultural sustainability and acceptable production economy.

Waman Ajit Arun *et al.* (2011) has investigated the *invitro* seed germination in *Withania somnifera* L. cv 'Poshita' by the application of different chemicals. Their results revealed that seed germination differed significantly over a period of five weeks due to GA₃, KNO₃ and NaNO₃ treatments.

Jaime and Viola (2011) studied the effect of compost and inorganic fertilizer on baby corn performance. The results showed that a reduction in the application of inorganic fertilizer can be achieved through compost addition without compromising baby corn yields.

Jeon *et al.* (2011) have conducted field experiments to evaluate the effect of different seedling methods on biomass, soil properties and rice yield in rice based cropping system. Based on the results, broadcasting before rice harvesting and partial tillage seedling were found to be good practices for production of green manure in paddy soil. Chemical fertilizers can be replaced with hairy vetch as green manure in rice based cropping system.

The effect of organic manure and chemical fertilizers on vegetable crops and soil properties were studied in radish. The result showed highest radish yield in 100% RDF treatment. The highest N,P,K and S uptake were reported by Islam *et al.* (2011).

Maneemegalai and Nandakumar (2011) aimed to study the effect of biochemical activity on germinated seeds of *Vigna radiata*, *Vigna mungo* and *Pennisetum typhoides*. Their study confirmed a corresponding increase in the nutritive quality of the germinated seeds compared to dry seeds.

Murugan *et al.* (2011) suggested a study on the effect of biofertilizers *Rhizobium leguminosarum* in phaseoli (RHL) and *Pseudomonas fluorescense* (PSF) with neem cake (NEC) and farm yard manure (FYM) in different combinations. Their results indicated an improved soil nutrient and grain yield by integrated application of RHL and PSF with NEC that plays a significant role in improving soil fertility and pulse productivity.

Shah *et al.* (2011) attempted to grow green manure legume in the gap between wheat harvest and rice plantation for sustainable growth of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) cultivar. The effect of six leguminous green manure viz., mung bean, cowpea, soybean, sesbania, pigeon pea and guar (*Cyamopsis tetragonoloba*) was assessed on rice yield and soil organic fertility. The greatest paddy and straw yield were recorded in cow pea and sesbania grown soil. The N uptake in rice was also significantly increased by legumes.

Suge *et al.* (2011) have studied the effect of organic and inorganic sources of fertilizer on growth, yield and fruit quality of eggplant (*Solanum melongena* L.). Their results revealed that, among the different combinations of inorganic and organic manure treatment, eggplant responded well to the application of 100% FYM.

The results of experiment conducted by Xavier and Kavitha (2011) on the biochemical analysis of the plant extract of *Acacia concinna* (wild) have shown various colour changes when treated with different chemical reagents.

A field study of wheat-based cropping systems was conducted at Faisalabad using 7 treatments and Jantar (*Sesbania aculeate* L.) as a green manuring crop to increase the fertility status of soil. The results indicated that with jantar treatment, all the parameters increased when compared to control (Kamal, 2011).

Vijaya and Seethalaksmi (2011) have studied the response of eggplant (*Solanum melongena* L.) in integrated nutrient management amended soil. The effect of different organic manures and inorganic fertilizers on growth and yield of eggplant were observed.

Abbasniyazare *et al.* (2012) have investigated the effect of biofertilizers and chemical fertilizers on growth indices of *Spathiphyllum illusion*. The result showed an increase in number of leaves, dry and fresh weight of leaves and the size of spadix in “triple super phosphate+ Barvar 2” treatment.

Ainika *et al.* (2012) studied the effect of organic and inorganic fertilizer on the growth of *Amaranthus caudatus* L. The treatment included three levels of organic fertilizers and four levels of inorganic fertilizers. The results analyzed showed best edible growth of amaranth in 300 kg ha⁻¹ NPK and 5 t ha⁻¹ FYM.

Aynehband *et al.* (2012) studied the effect of green manure crops and nitrogen (N) levels on grain, straw and biomass production in wheat (*Triticum aestivum* L.). Results of their study indicated that, legume green manure crops could be better than non-legume green manure crops due to atmospheric N fixation and release of suitable nutrients especially N for subsequent crop production.

Dong *et al.* (2012) has examined the effect of different treatments viz., no fertilizer (CK), rice straw return (SR), chemical fertilizer (NPK), organic manure and green manure on soil pH and soil organic carbon in the paddy soil. Their results suggested that annual straw returning application could improve soil fertility.

A study was conducted to determine the effect of foliar spraying of bio-organic fertilizers and urea on root and vegetative growth of mung bean (*Vigna radiata* L.) in a green house condition by Khalizadeh *et al.* (2012). Results showed that foliar application of urea and organic manures substantially improved the plant height, leaf area, shoot and root length, dry weight, volume and number of roots of mung bean.

A judicious integration of inorganic fertilizers with organic residues, composts, biofertilizers and other plant tonics may help to improve crop yield and soil productivity in different cropping systems (Ali *et al.*, 2012). It was suggested that combined application of synthetic fertilizers and organic manures proved more efficient in improving paddy yield and economic returns of rice as compared to their separate use.

Egbe *et al.* (2012) has carried out a study in mount Cameroon region to evaluate the effect of green manure and fertilizer on the growth and yield of maize (*Zea mays* L.). Results showed that biomass of prunings was highest in *Calliandra* (5314 kg/ha) in the first pruning.

El-Sayed *et al.* (2012) has investigated the response of *Echinacea paradoxa* L. plant to nitrogen and potassium fertilizer and their combination. The results indicated that the highest levels of nitrogen (N) or potassium (K) or the combination (NK) significantly improved the growth characters.

A field experiment was conducted by Ng'etich *et al.* (2012) to evaluate the effect of farm yard manure on the growth of spider plant (*Cleome gynandra*). The result showed a significant increase in the growth parameter when compared to control. The chlorophyll content also increased in FYM treated plants when compared to control.

Shaheen *et al.* (2012) conducted experiments in *Vigna radiata* and *Vigna mungo* to determine the proximate composition such as moisture, ash, fibre, fat and protein content. The biochemical constituents were also analyzed. The research concluded that *V. radiata* had high percentage of moisture and protein content as compared to *V. mungo*.

Patil *et al.* (2012) had carried out studies using different combination of manures and recorded higher grain yield in Chick pea.S

Pariari and Khan (2013) studied the effect of cow dung manure on growth and seed yield of coriander (*Coriandrum sativum* L.). Their study revealed an increased plant height and number of primary branches and secondary branches per plant, whereas, the growth parameters seemed to show a low value with application of cow dung manure alone (100%).

Saritha *et al.* (2013) have studied the application of selected organic manures on the seed germination and seedling growth of cluster bean (*Cyamopsis tetragonoloba* L. Taub). It was observed that the treatment containing FYM could be an ideal and suitable potting mixture for better seedling and crop production in cluster bean.

The conjunctive use of organic and inorganic source of fertilizer significantly increased both the concentration and uptakes of N,P,K and S by rice at the time of harvest under rainfed condition (Pankaj *et al.*, 2013).

A study was carried out by Saravanan *et al.* (2013) to investigate the effect of organic manure and chemical fertilizers on the yield attributes and macronutrient levels of green gram. The results indicated that, the yield was maximum in FYM+ 10 per cent NPK. Maximum phosphorus and iron content were also recorded in the seed.

An investigation was carried out by Subhalakshmi *et al.* (2014) in split plot design with different treatments to assess the nutrient uptake at each stage of crop growth. The results revealed that among the organic sources used, the incorporation of subabul @ 5t/ha recorded highest nutrient uptake.

Subhalakshmi *et al.* (2014) have studied the effect of organic sources and fertilizer levels on the nutrient uptake by hybrid rice at different stages of crop growth. They found that the interaction effect between organic sources and fertilizer levels were significant.

CHAPTER - III

MATERIALS AND METHODS

A study was conducted to assess the effect of different fertilizer and leaf extract on growth and biochemical parameters of *Oryza sativa* L. var. IR 20.

COLLECTION OF VARIOUS MATERIALS

Red soil and clay soil were collected from sundatty village, kotagiri.

COLLECTION OF SEEDS

The paddy seeds (*Oryza sativa* L. var. IR 20) were procured from Department of Grains, Tamil Nadu Agricultural University, Coimbatore.

FARM YARD MANURE

Farm yard manure (FYM) is prepared basically using cow dung. It is highly useful and some of its properties are

1. Farm yard manure is rich in nutrients.
2. A small portion of nitrogen is directly available to the plants, while, a large portion is made available as and when the FYM decomposes.
3. Availability of potassium and phosphorus from farm yard manure is similar to that from inorganic source.
4. Application of farm yard manure improves soil fertility.

CHEMICAL FERTILIZER

Di- Ammonium Phosphate

Di- Ammonium Phosphate (DAP) is used as a chemical fertilizer. When applied to plant, it temporarily increases the soil pH resulting in increased growth of the plant.

ORGANIC FERTILIZER

Neem Cake

Neem cake is an organic fertilizer for farms, gardens and lawns. Neem cake is the main product of neem seed kernel and contains natural nutrients.

Neem cake as fertilizer

Neem cakes have adequate quantity of NPK in organic form for plant growth. Being totally a botanical product, it contains 100 % natural NPK content and other essential micronutrients.

Use of neem cake as a fertilizer

1. Very cost effective.
2. Better yield than conventional urea and fertilizers.
3. Rich source of NPK and other micronutrients.
4. In comparison to urea which is a nutrient collector, neem cake itself contains nutrients for the plant and maintain power of soil.
5. Though neem cake is a fertilizer, it also acts as a pesticide.

Collection of *Ocimum basilicum*

Ocimum basilicum leaves were collected from sundatty village, kotagiri.

Herbal extract

Ocimum basilicum

The other names are basil, garden basil and sweet basil. The plant belongs to the family Lamiaceae. Basil is a low growing (30-100cm) annual plant. It has slightly hairy stem and ovate, entire to slightly toothed leaves. The plant is generally used in treatment for problems concerning digestion and nervous system. Leaves are taken (fresh or dried) in case of fever, abdominal cramps, gastro-enteritis, nausea and poor digestion. Leaf extracts were taken afresh by grinding the leaves in a mixie and filtering with the help of a muslin cloth.

METHODS

The soil was cleaned by removing stones and other unwanted materials. The red soil and clay soil were mixed in the ratio of 1:1 and filled in pots having 5 kg capacity. A

study was conducted to assess the effect of farm yard manure, chemical fertilizer (di-ammonium phosphate), organic fertilizer (neem cake) and leaf extract (*Ocimum basilicum*) on the growth and biochemical parameters of rice seedlings. The treatments were given at every 2 weeks interval i.e., on 15th day, 30th day, and 45th day after sowing the seeds.

TREATMENTS

T ₀	-	Control
T ₁	-	FYM
T ₂	-	DAP
T ₃	-	Neem Cake
T ₄	-	Leaf extract (<i>Ocimum basilicum</i>)

I. GERMINATION PERCENTAGE

Paddy seeds were surface sterilized with 0.1% HgCl₂. Then, they were soaked in distilled water for 2 hours. Later, they were transferred to petriplates (200x20mm). After one week, the seeds were tested for germination. The germination percentage was calculated using the following formula

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

II. GROWTH PARAMETERS

Plant samples were uprooted carefully on 30th and 45th day and the following growth parameters were measured and recorded for all the treatments.

1. Root Length (cm)
2. Shoot Length (cm)
3. Fresh Weight (gm)
4. Dry Weight (gm)

Root Length (Shukla and Mishra, 1986)

The plants were taken from control pot and other treatment pots and washed to get rid off adhering soil particles. Then the length of the roots were measured with the help of a scale from root collar point to root tip and expressed in centimeter.

Shoot Length (Shukla and Mishra, 1986)

The shoot length of the plants were measured with the help of scale from the root collar point to shoot apex and expressed in centimeter.

Fresh Weight (Shukla and Mishra, 1986)

Fresh weight of the plants were measured with the help of an electronic digital balance and expressed in grams.

Dry weight (Shukla and Mishra, 1986)

The collected plant materials were kept in hot air oven at 55°C for 24 hours. Then, the dry weight of the plants were measured using an electronic digital balance and expressed in grams.

III.BIOCHEMICAL PARAMETERS

1. Chlorophyll - estimated on 30th and 45th day of seedling
2. Protein - estimated on 30th and 45th day of seedling
3. Total carbohydrate - estimated on 30th and 45th day of seedling

The above parameters were estimated by collecting leaf samples at that particular stage of growth.

Estimation of chlorophyll content

Chlorophyll 'a', 'b' and total chlorophyll were analysed following the method of Arnon (1949).

Materials required

Analytical grade acetone was diluted to 80 % acetone.

Procedure

1. One gram of freshly cut leaf samples were taken in a clean mortar.
2. The leaf bits were ground by adding 20ml of 80 % (w/v) acetone.
3. The mixture thus obtained were centrifuged at 5000 rpm for 5 minutes.
4. The supernatant was transferred to 100ml volumetric flask. This procedure was repeated until the residue became colorless.
5. The washings were collected and the volume was made up to 100 ml in the volumetric flask with acetone.
6. The absorbance of the solution was read in a spectrophotometer at 645 and 663 nm against the solvent blank (80% acetone).

Calculation

The amount of chlorophyll present in the extract was calculated and expressed as mg chlorophyll/gm tissue using the formula,

$$\begin{aligned} 1. \text{ mg chlorophyll 'a' / gm tissue} &= 12.7A_{663} - 2.69A_{645} \times \frac{V}{1000 \times W} \\ 2. \text{ mg chlorophyll 'b' / gm tissue} &= 22.9A_{645} - 4.68A_{663} \times \frac{V}{1000 \times W} \\ 3. \text{ mg total chlorophyll / gm tissue} &= 20.2A_{645} + 8.04A_{663} \times \frac{V}{1000 \times W} \end{aligned}$$

Where,

$$\begin{aligned} A &= \text{Absorbance at specific wave length.} \\ V &= \text{Final volume of chlorophyll extract in 80\% acetone.} \\ W &= \text{Fresh weight of the tissue.} \end{aligned}$$

ESTIMATION OF PROTEIN (Lowry *et al.*, 1951)

Principle

The blue color developed by phosphomolybdic phosphotungstic components in the Folin – ciocalteau reagent by the amino acids , tyrosine and tryptophan present in the protein, the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate was measured by Lowry's method.

Materials Required

1. Two percent sodium carbonate in 0.1 N sodium hydroxide (Reagent A).
2. 0.5 percent copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% potassium sodium tartarate (Reagent B).
3. Alkaline copper solution : Mix 50 ml of Reagent A and 1 ml of Reagent B prior to use (Reagent C).
4. Folin – ciocalteau reagent (Reagent D).
5. Protein standard (stock solution): Accurately 50 mg of bovine serum albumin (fraction v) was weighed and dissolved in distilled water and made up to 50 ml in a standard flask.
6. Working standard : 10 ml of stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 mg protein.

PROCEDURE

Estimation of protein from sample

Extraction was carried out with buffers. About 100 mg of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer, centrifuged and the supernatant was used for protein estimation.

Estimation of protein

1. About 0.2, 0.4, 0.6 ,0.8 and 1 ml of the working standard were pipetted out into a series of test tubes.
2. About 0.5 and 1ml of the sample were pipetted out in two other test tubes.
3. The volume was made upto 1 ml using distilled water in all the test tubes. A tube with one ml of water served as the blank.
4. About 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 min.
5. To this, 0.5 ml of reagent D was added, mixed well and incubated in dark for 30 mins. Blue color developed was read at 660nm.
6. A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in the sample was expressed in mg /gm.

$$= \frac{\text{mg of protein}}{\text{volume of test standard}} \times \text{concentration of the standard}$$

ESTIMATION OF CARBOHYDRATE (Hedge and Hofreiter, 1962)

Anthrone method

Principle

Concentrated sulphuric acid hydrolyses the glycosidic bond of carbohydrate to the given monosaccharide's, which were then dehydrated to furfural, reacted with anthrone (10-keto 9,10-dihydro anthracene) to give the blue colored complex which was measured colorimetrically at 630 nm.

Materials required:

- a. 2.5 N HCl
- b. Anthrone reagent was prepared by dissolving 200 mg anthrone in 100 ml of ice cold 95% H₂SO₄, prepared freshly before use.
- c. Stock standard solution: 100mg of glucose was dissolved in 100 ml of water.
- d. Working standard: Five ml of stock standard solution was diluted to 100 ml using distilled water (50 mg/ml).

Procedure

1. About 100 mg of the sample was taken in a boiling tube and was hydrolysed by keeping it in boiling water bath for three hours with 5 ml of 2.5 N HCl and cooled at room temperature.
2. Then, it was neutralized with solid sodium carbonate until the effervescence ceases.
3. The volume was made up to 100 ml and centrifuged.
4. The supernatant was collected and 0.5 ml and 1ml aliquots were taken for analysis.

5. The standard was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and a blank was maintained.
6. The volume was made up to 1 ml in all the tubes including the sample test tubes by adding distilled water.
7. Then, 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
8. Then, it was cooled rapidly and blue-green color developed was read at 630nm.
9. A standard graph was drawn by plotting concentration of the standard on the X-axis.
10. From the graph, the amount of carbohydrate present in the sample was calculated.

Calculation

Amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

CHAPTER – IV

RESULTS AND DISCUSSION

The paddy seeds sown in petriplates (Plate 1) showed 100% germination (Plate 2). The germinated seeds were allowed to grow in petriplate for one week. Later, the seedlings were transferred to paper cups (Plate 3 & 4). After one week, the seedlings from cups were transferred to pots containing soil (Plate 5). Different fertilizers such as farm yard manure (FYM), di-ammonium phosphate (DAP), neem cake (NC), and leaf extract (LE) were added one week after transplantation into pots. The growth of the rice seedlings in terms of root length and shoot length were measured on 30th day and 45th day of the seedlings. The same day seedlings were taken for biochemical analysis also.

The results of the experiment conducted in *Oryza sativa* L. var. IR 20 with farm yard manure, chemical fertilizer (di-ammonium phosphate), organic manure (neem cake) and leaf extract (*Ocimum basilicum*) on growth and biochemical parameters are given below.



PLATE 1
PADDY SEEDS SOWN IN PETRIPLATES



PLATE 2
GERMINATION OF PADDY SEEDS

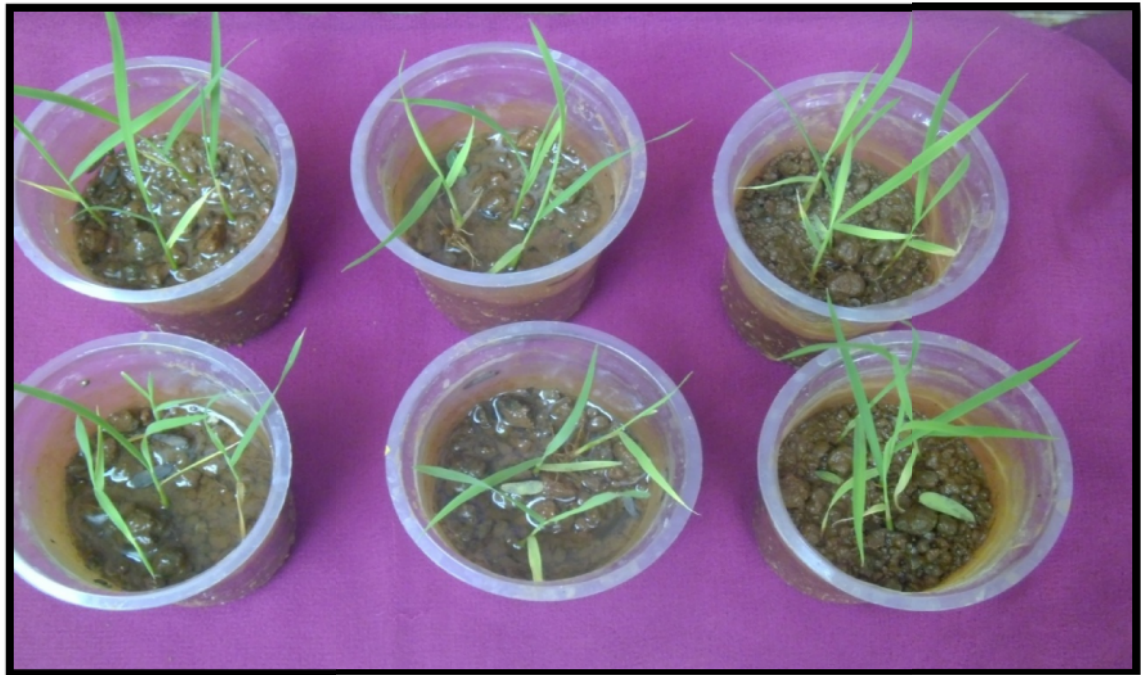


PLATE 3
PADDY SEEDLINGS TRANSFERRED TO CUPS



PLATE 4
PADDY SEEDLINGS TRANSFERRED TO CUPS

Growth parameters

Root length

The growth parameters of rice seedlings were analysed on 30th and 45th day and tabulated in Table 1 (Figure 1).

The maximum root length was observed in T₄ on 30th day and 45th day and the readings were 7.47 ± 1.45 and 8.83 ± 1.19 cm respectively. The minimum root length was observed in T₂ on 30th day and T₃ on 45th day and the values were 6.23 ± 0.49 and 7.53 ± 0.76 cm respectively.

Wang *et al.* (2008) have reported that the nitrogen supply in urea influenced the growth of maize plants especially in the roots .

Ne Meat Alla and El-Geddawy (2001) has reported that the use of foliar spray of macronutrients significantly increases the root length of suger beet.

Shoot length

The highest shoot length was obtained in T₃ (16.57 ± 2.43 cm) on 30th day and T₄ (18.83 ± 2.08 cm) on 45th day (Table 1; Figure 2). The minimum shoot length on 30th day was found in control plants and on 45th day, the minimum shoot length was found in plants that were supplied with DAP. The values were 14.33 ± 1.91 and 17.17 ± 0.15 cm respectively.

The results are in accordance with Shiralipour and Faber (1996) on broccoli (*Brassica Oleraceaves italica*) plant that had the tendency to increase the shoot length by the application of organic manure. This view has also been supported by Arisha *et al.* (2003) on the plant growth of broccoli.

Irshad *et al.* (2002) also reported that manure and urea fertilizer enhanced maize plant growth as compared to control. Shah *et al.* (2007) has supported the view with their results showing that integrated use of urea could produce maximum growth in maize plant.

TABLE 1

SHOOT LENGTH AND ROOT LENGTH OF RICE (*Oryza sativa* L.) SEEDLINGS

Treatment	Shoot Length (cm)		Root Length (cm)	
	30 th day	45 th day	30 th day	45 th day
T ₀	14.33 ± 1.91	18.03 ± 0.15	7.37 ± 0.74	8.37 ± 0.83
T ₁	15.70 ± 2.52	18.77 ± 0.83	7.03 ± 1.04	7.90 ± 1.29
T ₂	16.37 ± 2.05	17.17 ± 0.15	6.23 ± 0.49	7.53 ± 0.76
T ₃	16.57 ± 2.43	18.63 ± 0.35	6.63 ± 1.06	7.70 ± 1.31
T ₄	16.23 ± 1.20	18.83 ± 2.08	7.47 ± 1.45	8.83 ± 1.19
Sed	1.33666		0.86204	
CD(p<0.05)	2.78824		1.79819	
CD (p<0.01)	3.80342		2.45290	

Values are mean ± SD of triplicates

FIGURE 1

ROOT LENGTH OF RICE SEEDLINGS ON 30TH AND 45TH DAY

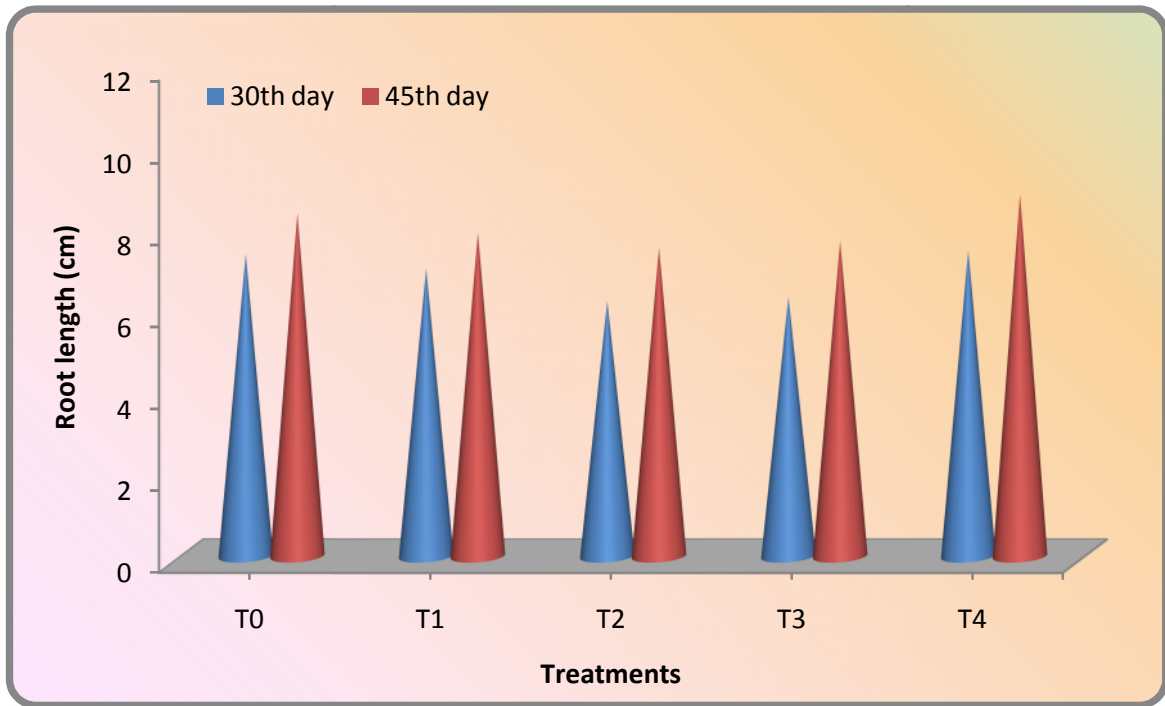


FIGURE-2

SHOOT LENGTH OF RICE SEEDLING ON 30TH AND 45TH DAY

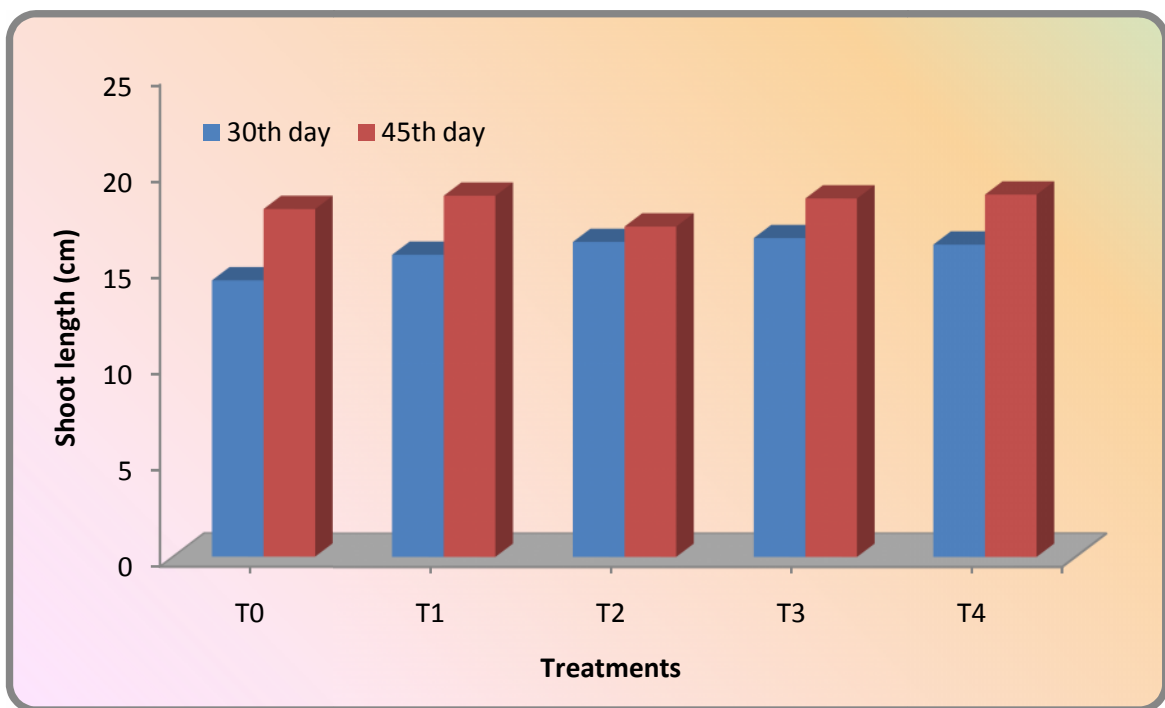


TABLE 2
FRESH WEIGHT AND DRY WEIGHT OF RICE (*Oryza sativa* L.) SEEDLINGS

Treatment	Fresh Weight (g)		Dry Weight (g)	
	30 th day	45 th day	30 th day	45 th day
T ₀	0.40 ± 0.02	0.50 ± 0.03	0.02 ± 0.01	0.04 ± 0.02
T ₁	0.30 ± 0.01	0.42 ± 0.02	0.02 ± 0.01	0.05 ± 0.01
T ₂	0.26 ± 0.02	0.31 ± 0.01	0.02 ± 0.02	0.03 ± 0.02
T ₃	0.32 ± 0.01	0.36 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
T ₄	0.48 ± 0.02	0.65 ± 0.04	0.05 ± 0.01	0.09 ± 0.01
SEd	0.01513		0.00989	
CD(p<0.05)	0.03156		0.02063	
CD (p<0.01)	0.04305		0.02814	

Values are mean ± SD of triplicates

FIGURE 3

FRESH WEIGHT OF RICE SEEDLINGS ON 30TH AND 45TH DAY

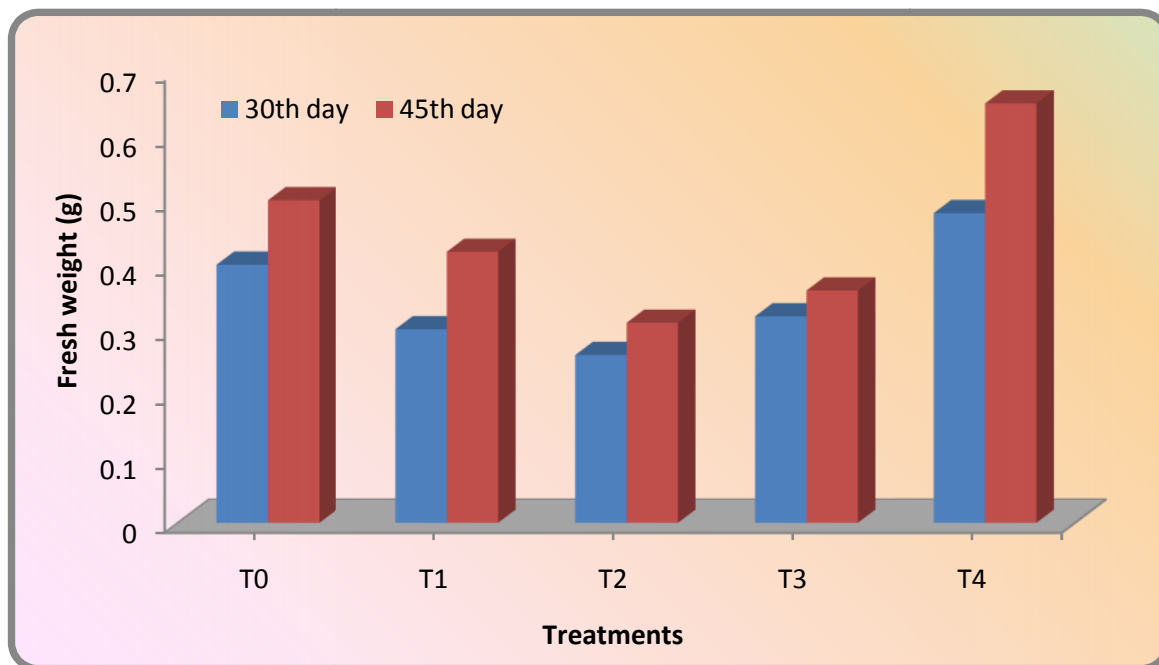
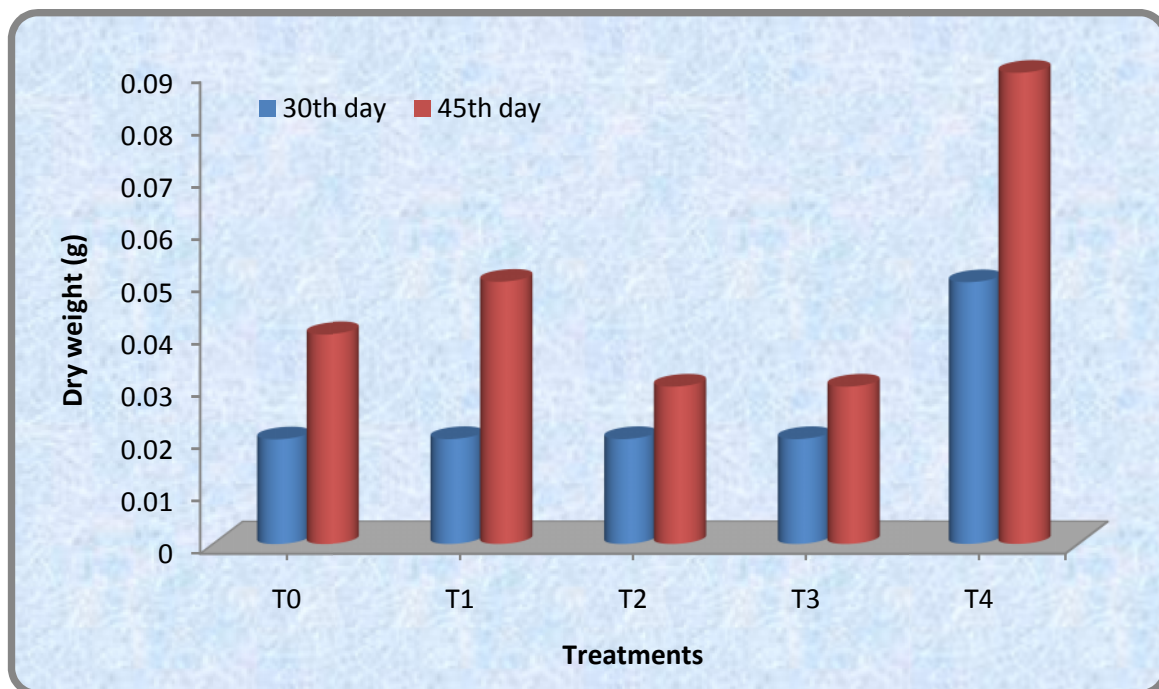


FIGURE 4

DRY WEIGHT OF RICE SEEDLINGS ON 30TH AND 45TH DAY



The results of shoot length are in agreement with the findings of Babalad (1999) in soybean, who have opined that there is a need of organic manure application along with inorganic fertilizer.

Fresh weight

The fresh weight was found to be maximum in T₄ on both 30th and 45th day (Table 2; Figure 3). The values were 0.48 ± 0.02 g and 0.65 ± 0.04 g respectively.

The minimum fresh weight was observed in T₂ on 30th and 45th day and the values were 0.26 ± 0.02 g and 0.31 ± 0.01 g respectively.

The results are in accordance with the result obtained in broccoli by application of 60 and 80 kg organic manure along with 60 kg inorganic fertilizer (Ouda and Mahadeen, 2008).

Dry weight

The dry weight was found to be maximum in T₄ on 30th and 45th day (Table 2; Figure 4) and the values were 0.05 ± 0.01 g and 0.09 ± 0.01 g.

The minimum dry weight of 0.02 ± 0.01 g was observed in all the treatments except T₄ on 30th day. A minimum dry weight of 0.03 ± 0.02 g and 0.03 ± 0.01 g were observed in T₂ and T₃ on 45th day respectively.

Wong *et al.* (1999) and Magnusson (2002) obtained an increase in weight by the use of organic manures in Chinese cabbage (*B. Chinesis*).

Biochemical parameters

The biochemical parameters such as chlorophyll *a*, chlorophyll *b*, total chlorophyll, protein and carbohydrate content of the rice seedlings were analysed on 30th and 45th day and the results are tabulated.

Chlorophyll content

The maximum chlorophyll 'a' was observed in T₄ on 30th day (Table 3; Figure 5) and the value was found to be 8.74 ± 0.01 mg/g. On 45th day, the chlorophyll content increased in T₄ and the value was 11.33 ± 0.04 mg/g.

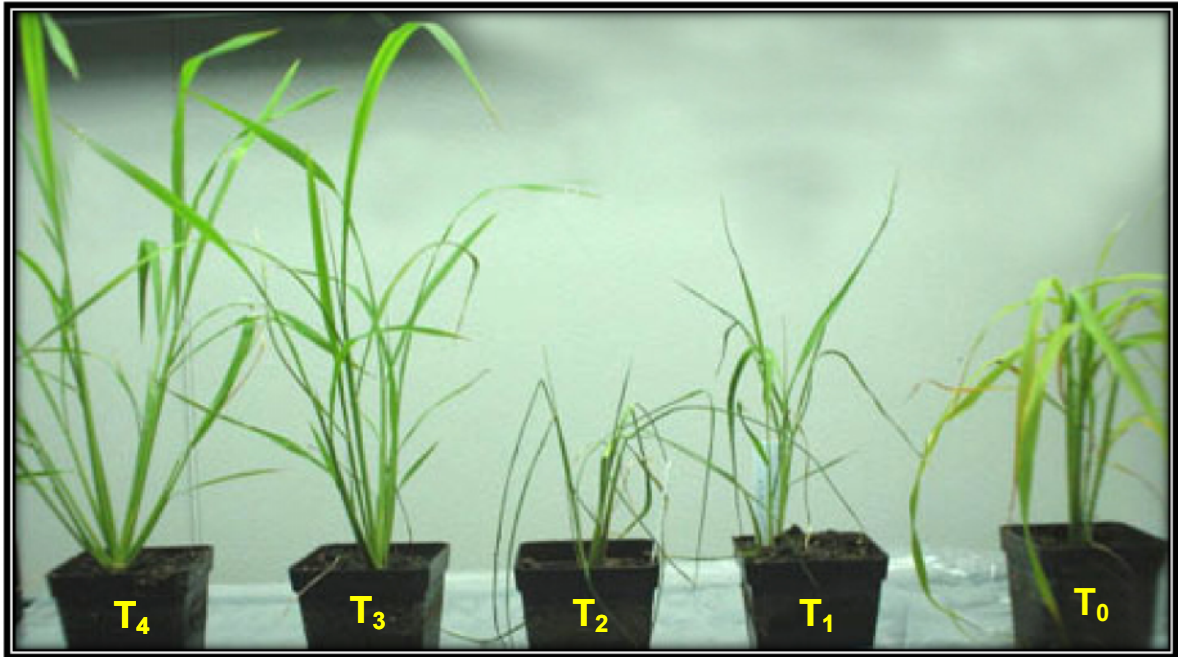


PLATE 5

GROWTH OF PADDY SEEDLINGS AFTER TRANSFER INTO PLANTS

TABLE 3
CHLOROPHYLL “a”, CHLOROPHYLL “b” AND “TOTAL” CHLOROPHYLL CONTENT OF RICE
(*Oryza sativa* L) SEEDLINGS (mg/g)

Treatment	Chlorophyll ‘a’ (mg/g)		Chlorophyll ‘b’ (mg/g)		Total Chlorophyll (mg/g)	
	30 th day	45 th day	30 th day	45 th day	30 th day	45 th day
T ₀	1.87 ± 0.02	4.63 ± 0.02	2.50 ± 0.05	4.78 ± 0.04	2.28 ± 0.01	4.24 ± 0.01
T ₁	8.27 ± 0.04	7.93 ± 0.01	4.27 ± 0.02	8.60 ± 0.050	3.90 ± 0.03	7.64 ± 0.02
T ₂	6.05 ± 0.02	8.71 ± 0.05	3.12 ± 0.01	8.90 ± 0.03	2.85 ± 0.03	7.94 ± 0.03
T ₃	5.54 ± 0.05	8.36 ± 0.03	3.06 ± 0.03	8.97 ± 0.01	2.82 ± 0.04	7.95 ± 0.05
T ₄	8.74 ± 0.01	11.33 ± 0.04	4.81 ± 0.03	9.18 ± 0.02	4.40 ± 0.05	8.22 ± 0.03
SEd	0.02625		0.02620		0.02625	
CD(p<0.05)	0.05475		0.05466		0.05475	
CD(p<0.01)	0.07468		0.07456		0.07468	

Values are mean ± SD of triplicates

FIGURE 5
CHLOROPHYLL 'a' CONTENT OF RICE SEEDLINGS
ON 30TH AND 45TH DAY

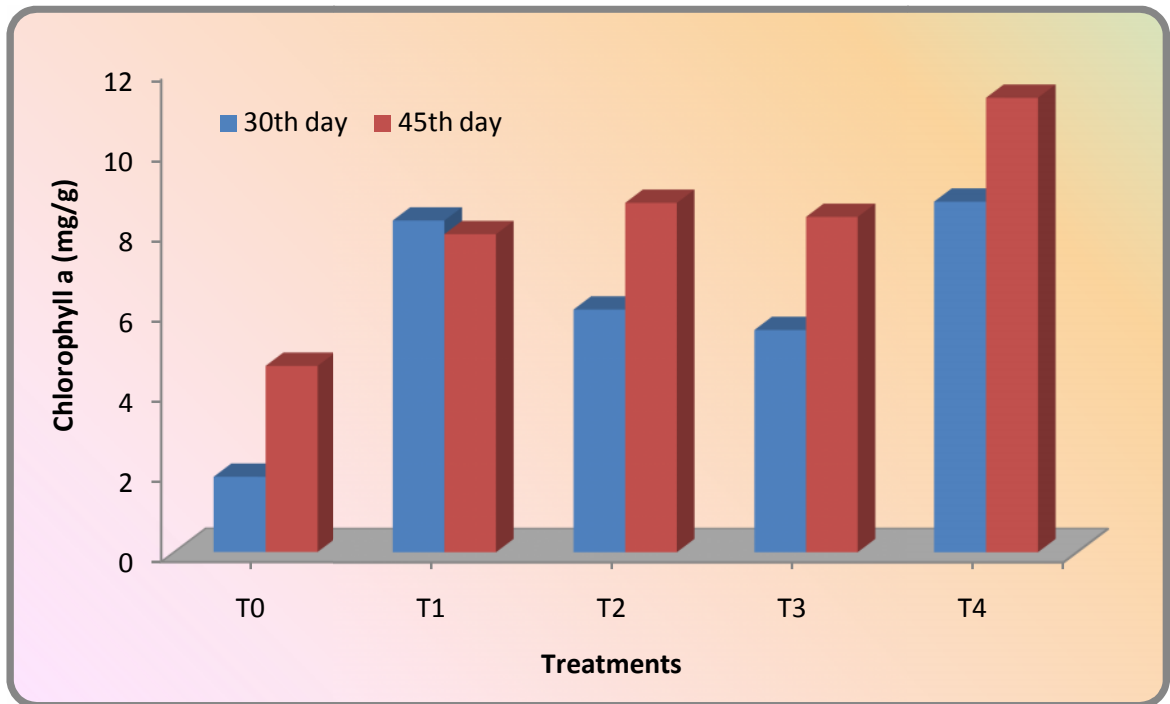


FIGURE 6
CHLOROPHYLL 'b' CONTENT OF RICE SEEDLINGS
ON 30TH AND 45TH DAY

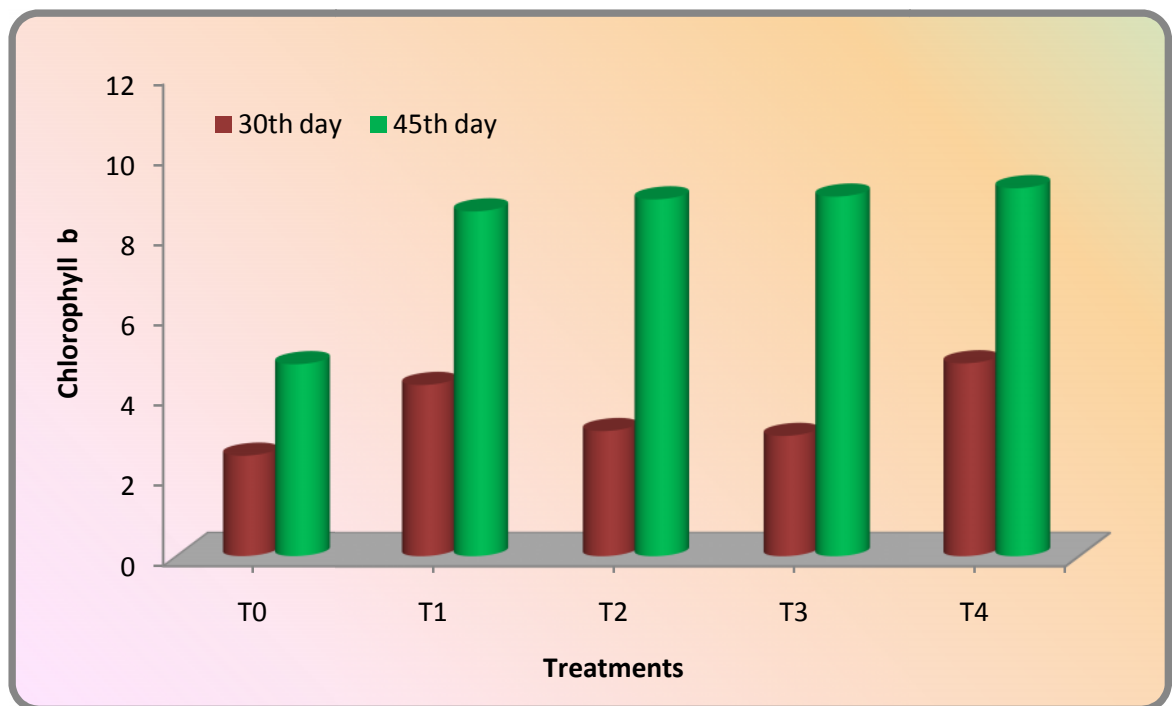
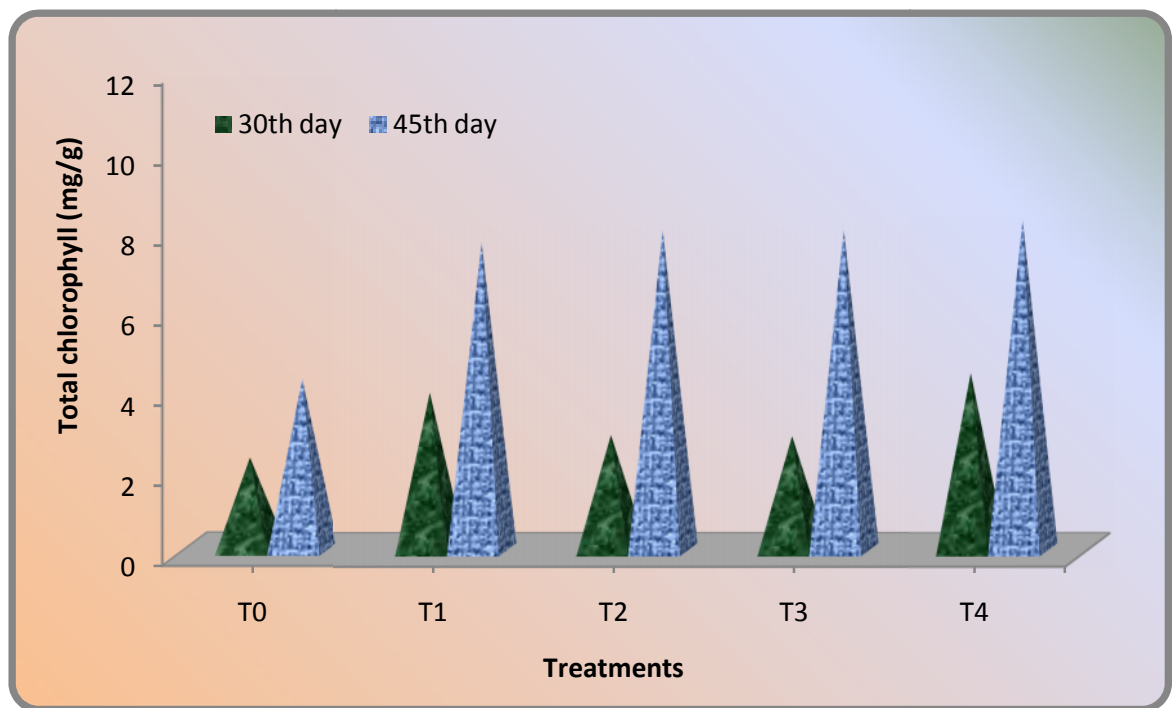


FIGURE 7
TOTAL CHLOROPHYLL CONTENT OF RICE SEEDLINGS
ON 30TH AND 45TH DAY



The chlorophyll 'b' content was more in T₄ on 30th day (4.81 ± 0.03 mg/g). On 45th day, the maximum chlorophyll 'b' was found in T₄ and the value was 9.18 ± 0.02 mg/g (Table 3; Figure 6).

The highest total chlorophyll content was obtained in T₄ on 30th day (Table 3; Figure 7) and the value was 4.40 ± 0.05 mg/g. On 45th day again, the maximum total chlorophyll content was found in T₄ and it was 8.22 ± 0.03 mg/g.

Least chlorophyll content was observed in control plants (T₀) on both 30th and 45th day. The amount of chlorophyll 'a' was found to be 1.87 ± 0.02 and 4.63 ± 0.02 mg/g. The least chlorophyll 'b' content on 30th and 45th day were found to be 2.50 ± 0.05 and 4.78 ± 0.04 mg/g.

The lowest total chlorophyll content of 2.28 ± 0.01 and 4.24 ± 0.01 mg/g were obtained on 30th and 45th day respectively in control plants.

The observations of the present work agrees with the previous findings obtained in other vegetable crops (Arisha and Beadisi, 1999 and Al-Tarawneh, 2005). Abbasniayzare *et al.* (2012) have shown that the use of biofertilizers (NK and Barvai) increases the chlorophyll content in plants.

Protein content

The protein content of rice seedlings were measured on 30th and 45th day (Table 4; Figure 8). The readings were taken by using 0.5ml of sample and 1ml of sample.

The highest protein content in 0.5 ml of sample on 30th day was observed in T₄ plant and the reading was 8.41 ± 0.04 mg/g, and in 1 ml sample, it was maximum in T₁ on 30th day and the reading was 9.46 ± 0.06 mg/g. On 45th day, the protein content increased in T₄ in both (0.5ml and 1ml) sample and the values were found to be 8.80 ± 0.05 and 7.40 ± 0.05 mg/g respectively.

The lower protein content was observed in control plant on 30th day in 0.5ml of sample and the reading was 1.05 ± 0.07 mg/g. In 1ml sample, minimum protein content was found in T₃ on 30th day and the reading was 4.60 ± 0.02 mg/g. On 45th day, the protein content decreased in T₂ in 0.5ml and it was 1.06 ± 0.03 mg/g.

TABLE 4
PROTEIN CONTENT OF RICE (*Oryza sativa* L.) SEEDLINGS (mg/g)

Treatment	30 th day		45 th day	
	0.5 ml	1 ml	0.5 ml	1 ml
T ₀	1.05 ± 0.07	5.04 ± 0.04	1.36 ± 0.01	1.06 ± 0.02
T ₁	3.25 ± 0.05	9.46 ± 0.06	7.59 ± 0.01	6.20 ± 0.04
T ₂	1.20 ± 0.05	7.61 ± 0.03	1.06 ± 0.03	7.20 ± 0.02
T ₃	6.02 ± 0.08	4.60 ± 0.02	2.31 ± 0.01	4.80 ± 0.01
T ₄	8.41 ± 0.04	7.04 ± 0.04	8.80 ± 0.05	7.40 ± 0.05
Sed	0.03350			
CD(p<0.05)	0.06771			
CD (p<0.01)	0.09060			

Values are mean ± SD of triplicates

FIGURE 8
PROTEIN CONTENT OF RICE SEEDLINGS

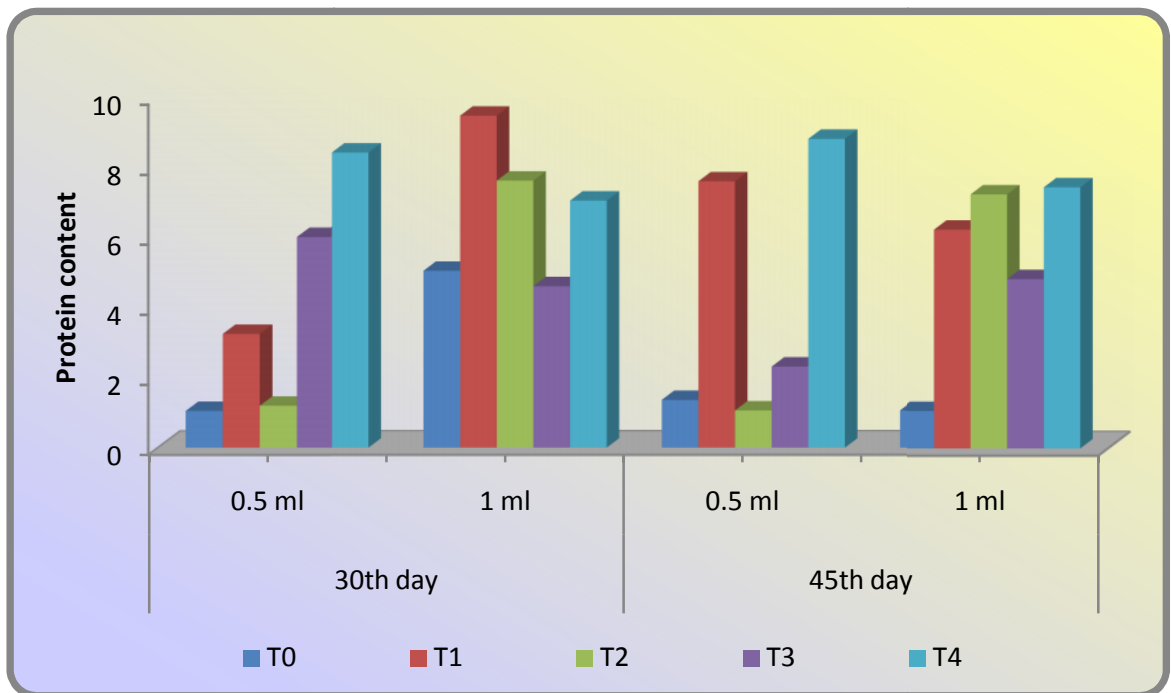
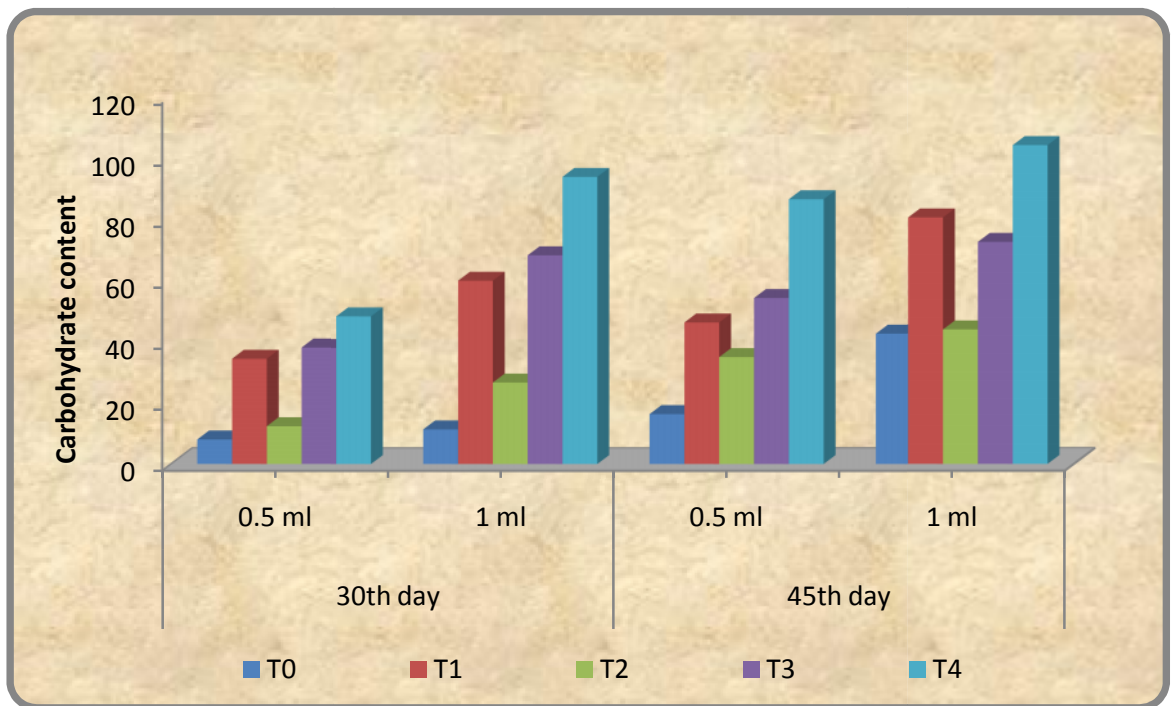


TABLE 5
CARBOHYDRATE CONTENT OF RICE (*Oryza sativa* L.) SEEDLINGS (mg/g)

Treatment	30 th day		45 th day	
	0.5 ml	1 ml	0.5 ml	1 ml
T ₀	8.00 ± 1.00	11.33 ± 1.53	16.33 ± 1.53	42.67 ± 2.08
T ₁	34.33 ± 1.53	60.00 ± 1.00	46.33 ± 1.53	80.67 ± 2.08
T ₂	12.33 ± 2.52	26.67 ± 5.86	35.00 ± 3.61	44.00 ± 2.00
T ₃	38.00 ± 1.00	68.33 ± 1.53	54.33 ± 2.52	72.67 ± 3.06
T ₄	48.33 ± 1.53	94.00 ± 2.00	86.67 ± 3.06	104.33 ± 3.51
SEd	2.03852			
CD(p<0.05)	4.12009			
CD(p<0.01)	5.51311			

Values are mean ± SD of triplicates

FIGURE 9
CARBOHYDRATE CONTENT OF RICE SEEDLINGS



The minimum protein content of 1.06 ± 0.02 mg/g was obtained in control plants on 45th day in 1ml sample.

Experiment in chick pea by Mohammadi *et al.* (2010) reveals that application of green manure increases the protein content of the seed.

In *Amaranthus dubius*, Manoharan *et al.* (2011) showed an increase in the amount of carbohydrate and protein content in plant treated with cyanospray compared to other treatments.

Carbohydrate content

The carbohydrate content on 30th as well as 45th day was higher in T₄ plants in both 0.5ml and 1ml sample (Table 5; Figure 9) and the (30th day) values were found to be 48.33 ± 1.53 and 94.00 ± 2.00 on 30th day and 86.67 ± 3.06 and 104.33 ± 3.51 mg/g on 45th day respectively.

Minimum carbohydrate content was shown by control plants on 30th and 45th day. The readings were observed to be 8.00 ± 1.00 and 11.33 ± 1.53 mg/g on 30th day and 16.33 ± 1.53 and 42.67 ± 2.08 mg/g on 45th day.

Rajula and Padmadevi (2000) has recorded an increase in biochemical parameters like chlorophyll, protein and carbohydrate in *Helianthus annuus* L. by the use of cyanopith and cyanospray.

CHAPTER – V

SUMMARY AND CONCLUSION

Manure enrich the soil. The word “Manure” most often refers to any kind of fertilizer. The benefit of using green manure by organic farmers is that, it adds organic matter to the soil by releasing important plant nutrients, including nitrogen and helps to increase crop growth. Thus, the use of manures that supply nutrients to the soil is needed for plant growth.

An experiment was conducted to study the influence of farm yard (FYM), chemical fertilizer (DAP), organic manure (neem cake) and leaf extract (*Ocimum basilicum*) on the growth and biochemical parameters of paddy (*Oryza sativa* L. var. IR 20).

The paddy seeds soaked in water were transferred to petriplates containing cotton and filter paper. The germination was 100% in the plates. After transfer into pots, the growth of the seedlings were observed.

The growth and biochemical parameters were evaluated on 30th and 45th day. The root length and shoot length were significantly higher in T₃ and T₄ on 30th day and 45th day respectively. The fresh and dry weight of the seedlings showed a significant increase in T₄ plants on both 30th and 45th day.

The biochemical parameters were also measured on 30th and 45th day. The values indicated that the chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll contents were higher in T₄ on 30th day and 45th day. Similarly, the protein content was maximum on 30th and 45th day in 0.5 ml extract of T₄ plants. In 1 ml of sample, on 30th day, the protein content was maximum in T₁ and on 45th day, it was maximum in T₄. The carbohydrate content of plants that were supplied with leaf extract showed a significant increase in both 0.5 ml and 1 ml sample on both 30th day and 45th day.

To reduce carbon dioxide, methane and nitrous oxide, farmers should be encouraged to discard organic waste instead of burning and also they should be trained to use organic fertilizers instead of chemical fertilizers. The application of organic

fertilizer in agriculture, particularly, in paddy field farming, would prevent pollution and conserve the environment.

The use of nitrogen, the main nutrient element in crop growth, tends to be inefficient. A great deal of applied nitrogen is lost by leaching, volatilization and other natural processes. This is not only wasteful, but burdens the natural environment with excessive nitrogen. The problem is particularly marked in paddy fields, since nitrogen losses are high under flooded conditions.

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