Effect of Biofertilizer and Phosphorus on the growth, Biomass production, Nodulation and Enzymic Antioxidants in Soyabean (Glycine max)

By

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A thesis submitted to Avinashilingam University for Women, Coimbatore – 641 043.

In partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BIOCHEMISTRY

April 2008

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Certified as bonafide research work

Signature of the Head of the Department

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

1.0 INTRODUCTION

Soyabean (*Glycine max*) is one of the most important oil and protein crops of the world. The supplies of oils and protein from animal sources are becoming scarce and expensive in developing nations. As a source of oils and proteins, soyabean can play a major role in elevating nutritional standards of foods in developing nations, where human beings are facing protein deficiencies (Khan *et al.,* 2004). The growth of soyabean production was stimulated by the increase of demand of soyabean meal used in industrial farming of pork, meat, eggs, milk and beef (Ortega *et al.,* 2004).

Soyabean is one of the grain legumes of India which not only helps in maintaining soil fertility but is also a rich source of protein and fats. Soyabean meets its most of nitrogen requirement through biological nitrogen fixation in association with appropriate *Bradyrhizobium japonium* (Jain and Trivedi, 2005). The symbiosis established among *Rhizobium* and legume plants requires a complex interchange of molecular signals between both the symbionts. In this way, compounds like flavonoids produced by the plants induce the synthesis and excretion of lipochitinooligosaccharides or nod factors as signals that activate the nodule organogenesis on roots of leguminous plants (Garcia *et al*., 2001).

Growers typically rely on tillage for weed control in organic food grade soyabean production systems. Disadvantages with crop systems that rely on tillage include increased erosion risk, loss of soil structure, and decrease in soil organic carbon levels and increases in machinery and fuel costs (Thelen *et al*., 2003). Biofertilizers are commonly called as microbial inoculants, which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form, by the crop plants through their biological processes. Biofertilzers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the Rhizophere (Ram Rao *et al.,* 2007).

Biofertilizers increases the physicochemical properties of soil structure, texture, water holding capacity, cation exchange capacity and pH by providing natural nutrients and sufficient organic matter. They can also be used as a possible tool to reclaim saline or alkaline soil because of their ameliorating effect on the physicochemical properties of the soil (Majumdar *et al*, 2007).

Biofertilizers not only supply the major nutrients but also micronutrients like Zn, Fe, Cu, etc (Raj, 2002). *Rhizobium* interaction is a well known symbiotic association occurring in nature and responsible for biological nitrogen fixation (Bikrol, 2005). *Rhizobium japonicum* significantly increases the nodulation and nodule dry weight. *Rhizobium* promotes the nodule formation on the roots of leguminous plants and is essential for the synthesis of certain amino acids and oils. It is also called as "master nutrients" for oil seed production (Gupta and Abraham, 2003). The presence of a specific inducer at right concentration would induce the nod gene transcription and therefore increases nodulation, which facilitates nitrogen fixation and increase the crop yield (Joshi, 2000).

Next to nitrogen, phosphorus is an important nutrient for plants. The soil contains high organic matter including rich organic forms of phosphorus, which comes mainly by way of decaying vegetation. Several fungi species belonging to the genera *Penicillium* and *Aspergillus* are also involved in solubilization of phosphates in soil. The above acids decrease the pH of soil and dissolve bound forms of phosphate. Phosphorus is applied to ensure good symbiotic performance and overall plant growth. Enhancing phosphorus availability to crop through phosphate solubilizing bacteria (PSB) holds a great promise in enhancing growth and yield of lentil. Applications of FYM not only provide nutrition to crop on decomposition but also increase the nutrient and water holding capacity of soil.

Chemical fertilizers do not produce basic soil health but weaken soil structure. Plants growing in highly chemicalised soil do not have a natural resistance to disease. Phosphorus deficiencies are limiting crop production in many agricultural soils worldwide where conventional fertilizers are inaccessible. Only 1 to 5% of total soil phosphorus is in a soluble, plant available form (Archand and Schneider, 2006).

Phosphorus deficiency is considered to be one of the main biophysical constraints to food production. The phosphorus released from directly applied ground phosphorus rock is often too low to provide sufficient phosphorus for crop uptake (Vassilev *et al*, 2001). The effect of phosphorus precipitation is significant in acidic soils where twice the amount of added phosphorus per unit surface area is fixed compared to neutral or calcareous soils (Whitelaw, 2000).

Hence a study has been undertaken to study the effect of biofertilizer and phosphorus on the growth, biomass production and nodulation as well as the level of enzymic antioxidants in soybean.

2.0 REVIEW OF LITERATURE

The review of literature pertaining to the present study "Effect of biofertilizers and phosphorus on the growth, biomass production, nodulation and enzymic antioxidants in soyabean (*Glycine max*)" is discussed under the following headings

2.1 Soybean (*Glycine max)*

2.2 Biofertilizers

2.2.1 *Rhizobium*

2.2.2 *Phosphobacteria*

2.2.3 *Rhizobium* and *Phosphobacteria*

2.3 Chemical Fertilizers

2.3.1 Phosphorus

2.1 Soyabean (*Glycine max*)

Soyabean plays a unique role in the world agriculture both as a food and oil seed crop. Its yield potential (40-45q/ha), protein (40-42%) and oil content (210 %) have proved its significance in agriculture. Yield is a complex character and controlled by heritable and non-heritable components (Dev Vart *et al.*, 2005). Soyabean is one of the important grain legumes of India. This not only helps in maintaining soil fertility, but is also a rich source of protein and fats. Soyabean meets its most of nitrogen requirement through biological nitrogen fixation in association with appropriate *Bradyrhizobium japonium* (Jain and Trivedi, 2005). Soyabean is leading exports with a fast growing production from 2 million tons in the beginning of the 70's to 53 million t in 2003. Brazil is the second largest soyabean producer, behind the United States that had a crop of 74 million t in 2003 (Ortega *et al,* 2004).

At present, export of soymeal fetches foreign earnings equivalent to Rs.15000 million. Soyabean oil produced to the tune of about 0.8 million t is consumed in the country mostly for edible use. There exists a wide gap between the national productively (1t/ ha) and the production potentials of varieties bred in India (3 to 4 t / ha). The yield levels achieved in front-line demonstrations, under real farm conditions have been around 2t/ ha i.e. almost double of the national average yield (Tiwari *et al*, 2001).

Phosphorus is required for better root growth, nodulation and nitrogen fixation and supplied as inorganic fertilizers. Soyabean is photosensitive crop and performance varies with latitude, longitudes elevation and agroclimatic conditions of the region (Agarwal *et al.,* 2005).

Soyabean seeds are put to a myriad of uses both nutritional and industrial, and form a very important part of our diet and that of other animals due to its high levels of protein (40%) and fat (20%) (Liu *et al*., 2005). Although soyabeans contain less oil than sunflower about 18% soy oil compared with 26% oil for sunflower, soyabeans can be produced without or nearly zero nitrogen. This makes soyabean advantageous for the production of biodiesel. A nitrogen fertilizer is one of the most energy costly inputs in crop production (Pimentel and Patzek, 2005).

Soyabeans are widely accepted as a healthy food and some of their pharmacological effect could be attributed to the presence of these valuable constituents (Blockhina *et al.,* 2003). Soyabean oil obtained from the extraction of oil from its seeds is fairly rich in glycosides of the unsaturated fatty acids particularly linoleic and linolenic with few oleic fatty acids, which do not oxidize readily because they contain natural antioxidants (Ashaye and Olusoji, 2006).

Soyabean oil readily absorbs oxygen on exposure to air and could form in some cases tough elastic but resistant film used as solvents for pigments in paints and varnish industries (Karabulut *et al.,* 2002). Soy foods have received considerable attention for their potential role in reducing the formation and progression of certain types of cancers and some chronic diseases, such as cardiovascular disease, Alzheimer's disease, and osteoporosis (Zhaol *et al.,* 2002). It contains natural calcium, magnesium and phosphorus suitable for the growth of children. Due to high protein content soyabean is also known as "poor man's meat" (Balamurugan, 2005).

2.2 Biofertilizers

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere, when applied through seed or soil (Kumutha *et al.,* 2000).

The biofertilizers enriched with bacteria and fungi have proven to be great importance in improving the yield and quality of various agricultural crops. Biofertilizers are not efficient under acidic condition, the efficiency of rock phosphate and super phosphate can be increased by organic manures in acid soil (Majumdar *et al.,* 2007).

Biofertlizers are environment friendly low cost agricultural in put playing a significant role in improving nutrient availability to the crop plants. In wide term it includes a diverse category of bioinoculants such as nitrogen fixers (symbiotic and asymbiotic), phosphate solubilizers and plant growth promoting *Rhizobacteria*. Performance of biofertilizers is highly unpredictable due to their biological nature and susceptibility of biotic and abiotic stress. However there is a consistent trend of increased effectiveness on account of the use of biofertilizers supplemented with chemical fertilizers (Tilak and Singh, 1996).

Biofertilizers play a vital role in maintaining long-term soil fertility and sustainability. Soil is a highly complex system with heterogeneous nature and therefore biofertilizers generally adjust the soil ecosystem and benefits the crops (Sivakumar *et al.,* 2002*).*

Biofertilizers are products consisting of selected and live microorganisms, which help to improve plant growth and productivity mainly through supply of plant nutrients. Biofertilizers are also known as microbial inoculants or bioinoculants. Biofertilizers have come to stay in Indian agriculture, since last three decades. In view of the cost effectiveness, contribution to crop productivity, soil sustainability and ecofriendly characters, biofertilizers form an integral part of integrated plant nutrient supply system (Hedge, 2002).

Enhanced plant growth and yield were reported in various crops due to the combined inoculation of various biofertilizers (Chendrayan *et al.,* 2003) Biofertilizers significantly increase the yield of galegaorientalis jam by 68-200 %. Biofertilizers not only promote the growth and development of plants, but also reduce the damage by insects and pests (Arkhipchenko *et al.,* 2005). In sesbania cannabia, nodulation was profuse and effective. Nitrogen fixed was nearly 1.22kg ha^{-1} at 100 DAS on application of biofertilizer, also there was considerable beneficial influence from growing sesbania on soil carbon and nitrogen status (Rao and Gill, 2000).

Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen in the available form for plants either by living freely in the soil or being associated symbiotically with plants (Subba Rao, 1997). Biofertilizers are inputs containing microorganisms, which are capable of mobilizing nutritive elements from non-usable form to usable from through biological processes (Shaheen *et al*., 2007).

2.2.1 *Rhizobium*

The bacteria in root nodules of legumes are called **"***Rhizobium".* They are important agents in the fixation of atmospheric nitrogen. Chemical fixation of nitrogen came to known only several years after our understanding of the implication of biological nitrogen fixation**.**

The nature of the compounds, which induce the activation of nod genes in *Rhizobium,* depends on the species itself. It is stated that the most potent inducers for nod genes of *Bradyrhizobium japonicum* are the genistein, daidzein and isoliquintigenin. The presence of a specific inducer at right concentration could induce the nod gene transcription and therefore increase nodulation, facilitate nitrogen fixation and increase crop yields. It has been shown that some cultivars seem to supply limited quantities of flavonoids. So the inclusion of these inducers on the culture medium could be an important alternative in the production of high quality biofertilizers (Garcia *et al*., 2001).

The preincubation of *Bradyrhizobium japonicum* with genistein increases the amount of nodules and the nitrogen fixation level. Concentration of the isoflavonoid is increased by the early development of the root and decreases after the fixation process has begun.

Soil nitrogen is a consumptive element in plant nutrition and it must be replenished via chemically fixed nitrogen fertilizer or by biologically fixed nitrogen. Atmosphere contains 80% molecular nitrogen and around 6400 kg of nitrogen are available above every hectare of land and water. Legume plants fix and utilize this nitrogen by working symbiotically with *Rhizobium* in nodules on their roots. The host plants provide a home for the bacteria and energy to fix atmospheric nitrogen, inturn the plant receives fixed nitrogen as protein. *Rhizobia* are characterized by their unique ability to invade root hairs of leguminous plants and induce effective nitrogen fixing nodules on the roots. Though *Rhizobia are* common habitants of soils, they often fail to produce effective nodulation, which may be due to very low population. *Rhizobium* legume association is a unique plant bacterial association that commonly results in fixation or reduction of dinitrogen within the root nodules, which are induced by rhizobia on the compatible host legumes. Effective symbiosis can only be achieved when the nodules are formed by efficient and effective rhizobia (Kumutha *et al.,* 2000).

Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that can actively colonize plant roots and increase plant growth. These PGPR can prevent the deleterious effects of phytopathogenic organisms and stressors from the environment. The *Bacillus* species strains enhance soyabean nodulation and growth under low temperature stress (Han and Lee, 2005).

2.2.*2 Phosphobacteria*

The soil contains high organic matter including rich organic forms of phosphorus that comes mainly by way of decaying vegetation. Rock phosphate is one of the basic materials for phosphate fertilizer production. In India 100 million t of rock phosphate deposits are available. Super phosphate is a common form of phosphatic fertilizer used in India. Phophobacteria was first prepared in Russia and other east European countries for increasing the yield of crops by 5 to 10% over corresponding control. In India IARI, used phosphobacteria for wheat, maize, rice, pigeon pea and berseem. Microorganisms bring about insoluble soil phosphate in to soluble forms by secreting several organic acids like formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. These acids decrease the pH of soil and dissolve the bound forms of phosphate.

Several fungi species belonging to the genera *Penicilium* and *Aspergillus* are also involved in solubilization of phosphates in soil. As particular inhabitants in rhizosphere some microorganisms play a critical role in mediating the availability of soil phosphorus to the host plants. Such functional microorganisms may directly act to phosphate solubilization and mineralization. An extensive range of microorganisms that are able to solubilize varies for different forms of soil bound phosphorus have been reported (Whitelaw, 2000)

Using phosphate-solubilizing microorganisms as root specific biofertilizers has been proposed as a method to enhance the efficiency of applied phosphorus fertilizers and / or phosphorus containing organic compounds leading to enhancement of the land productivity for screening of phosphate solubilizing microorganisms (Gyaneshwar *et al.,* 2002).

Some hydroxy acids chelate with calcium and iron and effectively solubilize and utilize the phosphates. Phosphorus is one of the essential and vital nutrients for plant growth. Phosphorus nutrition is absolutely essential for the activities of food synthesis, cell reproduction, growth of shoot and root, flower formation, fruits and seed setting.

2.2.*3 Rhizobium and Phosphobacteria*

Soyabean meets its most of nitrogen requirement through biological nitrogen fixation in association with appropriate *Bradyrhizobium japonicum*. Phosphorus is required for better root growth, nodulation and nitrogen fixation and applied as inorganic fertilizers. The use efficiency of phosphorus fertilizers is generally low (20% to 30%) and therefore enhancing of applied fertilizer. Phosphate solubilizing bacteria hold promise in the present scenario of escalating prices of phosphatic fertilizers in the country. A positive response of PSB inoculation on seed yield of chickpea and inoculation could save 13.10 kg phosphorus ha^{-1}(Jain and Trivedi, 2005).

Phosphorus is applied to ensure good symbiotic performance and overall plant growth. Enhancing phosphorus availability to crop through phosphate solubilizing bacteria (PSB) holds a great promise in enhancing growth and yield of lentil. Application of FYM not only provides nutrition to crop on decomposition but also increases the nutrient and water holding capacity of soil.

2.3 Chemical Fertilizers

Chemical fertilizers are synthetically produced plant nutrients or inorganic materials. Several chemical fertilizers have high acid content like sulfuric acid, and hydrochloric acid, which inturn lead to high soil acidity. This would in turn result in the destruction of nitrogen fixing bacteria. (Yang and Liu, 2001).

It is practically impossible to use chemical fertilizers with out damaging the environment. Many chemical fertilizers are mineral, salt or more synthetically derived from coal or petroleum products. Some chemical fertilizers are called slow release or synthetic organic chemical fertilizers that need water for their nutrients to be released. This means that nitrogen, phosphorus, and potassium are leached in to water, contaminating them and negatively affecting aquatic life in the lakes.

The health effects associated with the phosphate fertilizer industry include poisoning of domestic animals caused by fluorine in smoke. Industrial fluoride in livestock is a disorder known by veterinarians in all industrialized countries (Ana *et al*., 2005).

2.3.1 Phosphorus

Phosphorus deficiencies are limiting crop production in many agricultural soils worldwide, where conventional fertilizers are inaccessible to total soil. Phosphorus only 1 to 5 % is in a soluble plant available form. Phosphorus deficiency is considered to be one of the main biophysical constraints to food production (Archand and Schneider, 2006).

In regions where soils are high in total phosphorus including some temperate soils, deficiencies may also occurs if soluble forms of P are not replenished following plant uptake of phosphorus from soil solution. For example, organically managed soils have been found to become deficient in plant available phosphorus over long term without external inputs of phosphorus (Oehl *et al.,* 2002).

The use of conventional phosphorus fertilizers is highly limited in developing regions due to cost and is prohibited for use by organic farmers. As a result, locally available sources of phosphate rock are increasingly recognized as potential phosphate fertilizers (Goenadi *et al.,* 2000). The phosphorus released from directly applied ground phosphate rock is often too low to provide sufficient phosphorus for crop uptake (Vassilev *et al.,* 2001). Low technology alternatives to the energy intensive and costly methods of conventional phosphate fertilizer production have been proposed including enhancing plant and micro biological mechanisms that promote phosphate rock solubilization (Trolove *et al.,* 2003).

The soil phosphorus cycle is a dynamic process involving the transformation of phosphorus by geochemical and biological processes. Plant available phosphorus occurs in the soil solution as orthophosphate anions, predominantly $H_2po_4^{-}$ and Hpo_4^{2-}. Solid inorganic and organic forms of phosphorus are found is labile and poorly soluble forms in the soil and as a result, can replenish plant available phosphorus with varying degrees of effectiveness. Plant available phosphorus or solution phosphorus is in equilibrium with a relatively labile fraction of phosphorus that is adsorbed to aluminum or ferric hydrous oxides, clays, calcium carbonates and organic matter that is associated with the solid phase of the soil (Pierzynski *et al.*, 1994).

As a result, solution phosphorus is easily replenished in response to plant uptake through desorption of phosphorus from the labile solid fraction. However only a small fraction of phosphorus in the solid phase remains in a labile form, as it can become strongly adsorbed to the soil or participate in precipitation reactions. Phosphorus may become strongly fixed and eventually precipitate as variscite and strongite in acid soils or with ca^{2+} in alkaline soils (Whitelaw, 2000).

The effects of phosphorus precipitation are significant in acidic soils where twice the amount of added phosphorus per unit surface area is fixed compared to neutral or calcareous soils. The organic phosphorus pool generally constitutes 30 to 80% of the total soil phosphorus and represents a labile phosphorus fraction that may supply phosphorus to plants through mineralization by the microbial biomass (Oberson *et al.,* 1996).

The microbial biomass is a small fraction of the total soil organic phosphorus, containing anywhere between 3 to 24% depending on cultivation However it is significant in its role as recycler of phosphorus and as a relatively labile phosphorus source (Kwabiah *et al.,* 2003).

3.0 EXPERIMENTAL PROCEDURE

The methodology of the study on "Effect of biofertilizer and phosphorus on the growth, biomass production, nodulation and enzymic antioxidants in Soyabean (*Glycine max*)" is presented under the following headings

3.1 Soil preparation
3. 2 Treatment of seeds
3.3 Layout of treatment pots
3.4 Sowing and watering
3.5 Harvest methodology
3.6 Biometric parameters
3.7 Biochemical analysis
3.8 Statistical analysis

3.1 Soil preparation

All the pots used for the study were filled with 8 kg of soil and treated with phosphorus in the form of super phosphate at the concentrations of 50kg and 100kg P/ha. The soil was tested for its nitrogen, phosphorus and potassium. The soil was enriched by the basal application of farmyard manure (FYM) at the rate of 10 t / ha.

3. 2 Treatment of seeds

Seeds of soybean were collected from Tamil Nadu Agricultural University, Coimbatore. Seeds were soaked in cold water; shade dried and treated with *Rhizobium and Phosphobacteria* inocula with rice gruel as an adensive agent. Plate 1 and 2 Picturises the untreated and treated seeds of soyabean

PLATE 1

SEEDS OF UNTREATED SOYABEAN

PLATE 2

SEEDS OF BIOFERTILIZERS TREATED SOYABEAN

PLATE 3

GROWTH OF SOYABEAN ON 30 DAS

PLATE 4

GROWTH OF SOYABEAN ON 45 DAS

PLATE 5

GROWTH OF SOYABEAN ON 60 DAS

3.3 Layout of treatment

The study was carried out as pot culture with four replications for each treatment.

The treatments were as follows

T_1 - Control

T_2 - *Rhizobium*

T_3 - *Phosphobacteria*

T_4 - *Rhizobium + Phosphobacteria*

T_5 - 50 kg of Phosphorus

T_6 - 100 kg of Phosphorus

3.4 Sowing and watering

About fifteen (Soyabean) seeds were sown in each pot and allowed to germinate. After germination of the seeds, waterlogging condition was maintained.

3.5 Harvest methodology

At the end of the 30^{th}, 45^{th}, and 60^{th} day, the plants were uprooted from the pots with out any damage to the root system. The plants were washed gently with water and blotted with filter paper to absorb the water droplets. Then the plants were subjected to biometric and biochemical analysis.

3.6 Biometric parameters

3.6 .1 Fresh weight

The fresh plants after removing the soil particles were weighed immediately and expressed as g/ plant.

3.6.2 Dry weight

The uprooted plants after recording the fresh biomass were oven dried at 70ºc for 36 hours. Weighed and expressed as g / plant.

3.6.3 Root and shoot length

The length of the root and the shoot were measured from the collar region to the tip of the root and shoot respectively and expressed in cm/ plant.

3.6.4 Number of nodules

Soil particles were removed from the uprooted plants and the total number of root nodules per plant were counted and recorded.

3.7 Biochemical analysis

The biochemical constituents analyzed with soyabean plants were total chlorophyll, total carbohydrate, protein, total nitrogen, phosphorus, potassium and the ammonia assimilating enzymes namely glutamate dehydrogenase, glutamate synthase and the oxidative enzymes like catalase and peroxidase.

The details of the parameters studied, the various parts of the plant used, the method of analysis, and the relevant references are given in Table I.

3.8 Soil analysis

The procedures of the estimation of soil nitrogen, phosphorus, and potassium are described in appendices Table I.

3.9 Statistical Analysis

The results of the experiment were analyzed by subjecting to statistical analysis by the arithmetic mean and two-way analysis of the variance.

TABLE I

ANALYSIS OF BIOCHEMICAL PARAMETERS AND ENZYME ASSAY

PARAMETERS	PARTS OF PLANT USED	METHOD OF ANALYSIS	REFERENCE	APPENDIX NO
BIOCHEMICAL ANALYSIS				
Total Chlorophyll	Fresh Leaves	Spectro Photometry	Yoshida *et al.*, 1971	I
Total Carbohydrate	Plant Dry Sample	Spectro Photometry	Hedge and Hotrieter, 1962	II
Protein	Fresh Root	Spectro Photometry	Lowry *et al.*, 1951	III
Total Nitrogen	Plant Dry Sample & Soil	Microkjehldel Method	Humphries, 1956	IV
Phosphorus	Plant Dry Sample & Soil	Spectro Photometry	Jackson,1973	V
Potassium	Plant Dry Sample & Soil	Flame Photometry	Jackson, 1975	VI
ENZYME ANALYSIS				
Glutamate Synthase	Fresh Root	Spectro Photometry	Van de caseele *et al.,*1975	VII
Glutamate Dehydrogenase	Fresh Root	Spectro Photometry	Doherty, 1970	VIII
Catalase	Fresh Leaves	Titrimetry	Thenmozhi and Sadasivam, 1989	XI
Peroxidase	Fresh Leaves	Spectro Photometry	Reddy *et al.,* 1995	X

4.0 RESULTS AND DISCUSSION

A study was conducted to find out the "Effect of biofertilizers and phosphorus on the growth, biomass production, nodulation and enzymic antioxidants in soyabean (*Glycine max*)".

The study was carried out in pot cultures in completely randomized block design with four replications for each treatment. Farmyard manure was added at the rate of 10 t ha^{-1} to enrich the soil nutrition. 8 kg of soil was filled per pot. Phosphorus in the form of superphosphate at the concentrations of 50 kg ha^{-1} and 100 kg ha^{-1} was added to various pots. Soyabean seeds were treated with 2g each of *Rhizobium* and *Phosphobacteria* separately and in combination. 3ml of rice gruel was added to the inoculum and made in to a sticky paste to which soyabean seeds were added, thoroughly mixed and dried in shade for 30 minutes and about 15 soyabean seeds were sown in each pot and allowed to germinate.

The treatments were as follows,

T_1 – Control
T_2 – *Rhizobium*
T_3 – *Phosphobacteria*
T_4 – *Rhizobium* and *Phosphobacteria*
T_5 – 50 kg of Phosphorus
T_6 – 100 kg of Phosphorus

At the end of the 30^{th}, 45^{th} and 60^{th} day after sowing (DAS), plants were uprooted from the pots with out any damage to the root system. The plants were washed gently with water and blotted with filter paper to absorb the water droplets. Then the plants were subjected to biometric and biochemical analysis.

The results of the study were discussed as follows:

4.1 BIOMETRIC PARAMETERS

4.1.1 Fresh weight

4.1.2 Dry weight

4.1.3 Shoot length

4.1.4 Root length

4.1.5 Number of nodules

4.2 BIOCHEMICAL PARAMETERS

4.2.1 Total chlorophyll

4.2.2 Total carbohydrate

4.2.3 Total protein

4.2.4 Total Nitrogen, Phosphorus and Potassium

4.3 AMMONIA ASSIMILATING ENZYMES

4.3.1 Glutamate dehydrogenase

4.3.2 Glutamate synthase

4.4 OXIDATIVE ENZYMES

4.4.1 Catalase

4.4.2 Peroxidase

4.1 BIOMETRIC PARAMETERS

The growth attributes of soyabean such as shoot and root length, fresh and dry weights were recorded on the 30th, 45th and 60th day after sowing. Plate 3, 4 and 5 depict the growth of the plant on the 30th, 45th and 60th day of growth.

4.1.1 Fresh weight and Dry weight

The fresh and dry weights of soyabean for various stages of growth were depicted in Table II.

Table II clearly indicates that the fresh weight of the treatment T_2 *(Rhizobium)* and T_6 (Phosphorus) was enhanced significantly ($P<0.05$) on the 30th day of growth, which was followed by T_4 and T_3 respectively.

TABLE II

THE FRESH AND DRY WEIGHTS OF SOYABEAN AS INFLUENCED BY THE BIOFERTILIZER AND PHOSPHORUS

Treatments	Fresh weight (g/plant)			Dry weight (g/plant)		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T_1	0.34	0.44	0.97	0.02	0.08	0.15
T_2	0.50	0.58	1.47	0.08	0.14	0.27
T_3	0.44	0.46	1.04	0.07	0.16	0.20
T_4	0.46	0.49	1.21	0.08	0.17	0.20
T_5	0.34	0.68	0.98	0.05	0.15	0.22
T_6	0.50	0.64	1.14	0.06	0.18	0.20
CD (0.05)	**0.0586**			**0.0433**		

The treatment, T_5 had shown a higher fresh weight on the 45th day, followed by T_6 and T_2. Again the treatment, T_2 recorded a maximum fresh weight on the 60th day after sowing. This was followed by T_4 and T_6 respectively. The treatments T_2 and T_4 have recorded the maximum dry weight on the 30th DAS, which was followed by T_3 whereas on the 45th day after sowing, T_6 recorded the highest dry weight followed by T_4 and T_3 respectively. The treatment T_2 had shown the maximum dry weight on the 60th day when compared to all the other treatments.

The fresh and dry weights of soyabean were also depicted in figure 1 and 2.

FIGURE 1

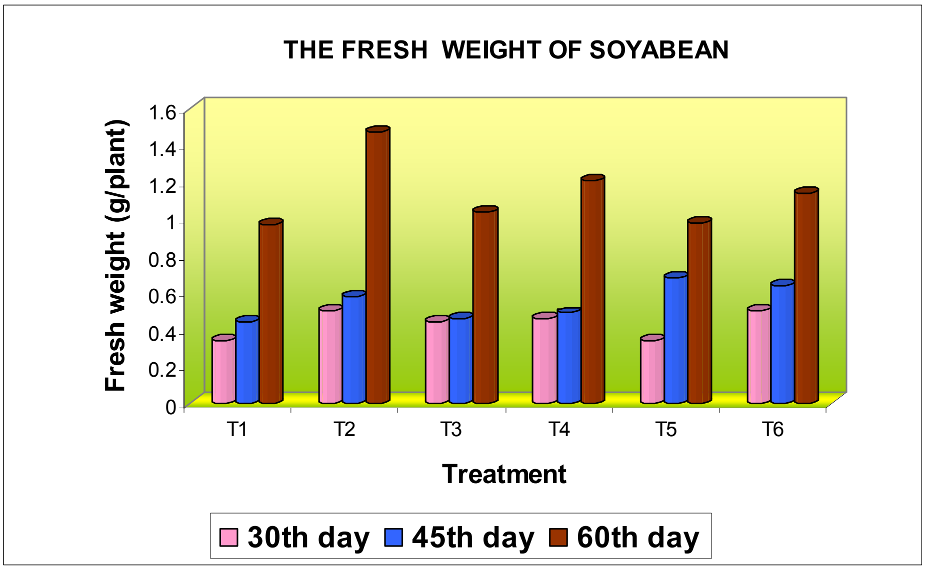
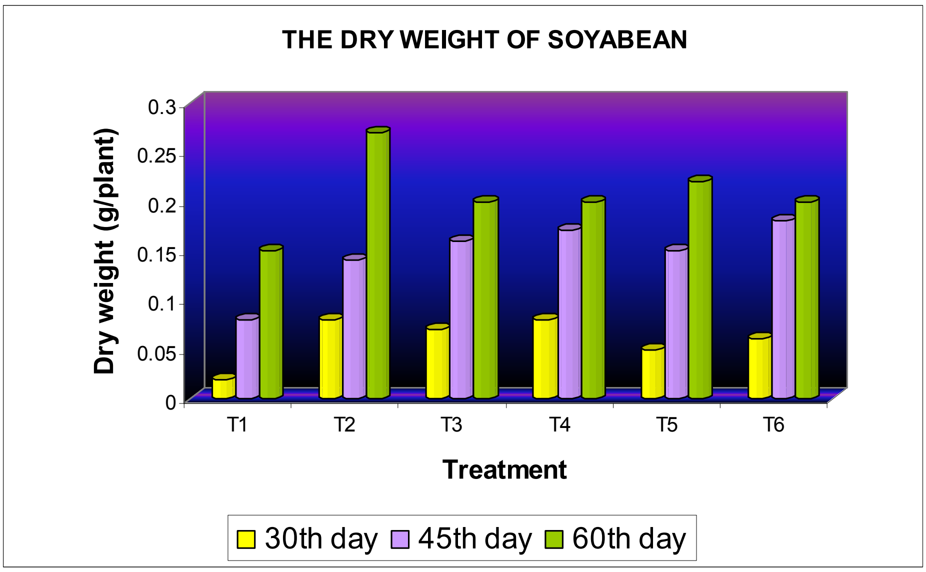


FIGURE 2



Total dry matter production, its distribution and partitioning are the integral part of the growth and development over the entire growing period and are directly related to the seed yield. Positive relationship exists between soyabean seed yield and total dry matter production (Kumar *et al.,* 2006).

4.1.3 Shoot length

Table III and Figure 3 and 4 indicate the shoot and root length of soyabean on the 30th, 45th and 60th day after sowing.

TABLE III

THE SHOOT AND ROOT LENGTH OF SOYABEAN

Treatments	Shoot length (cm)			Root length (cm)		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T_1	22.45	32.82	69.75	3.60	7.70	12.40
T_2	27.15	31.97	71.82	7.50	9.50	10.30
T_3	25.10	29.65	90.82	5.90	8.50	15.90
T_4	36.62	45.65	111.75	6.40	9.80	19.60
T_5	33.32	37.82	58.82	4.70	8.20	14.00
T_6	43.70	41.22	66.02	5.40	7.80	17.30
CD (0.05)	**0.802**			**0.671**		

From the above data it was evident that the shoot length increased with the growth period for all the treatments and the increase was found to be significant ($P<0.05$) when compared to the control. Among the treatments T_6 and T_4 were recorded the maximum shoot length on the 30th, as well as on the 45th day where as on the 60th day T_4 was found to be superior among all the other treatments. Similar results were reported in maize and wheat plants where the inoculation with *Azospirillum* along with synthetic fertilizers mostly increased the length of shoot and root (Rousta *et al.,* 1988). The combined applications of biofertilizers (Rhizobium and Phosphobacteria) have enhanced the shoot length very much compared to all the other treatments.

FIGURE 3

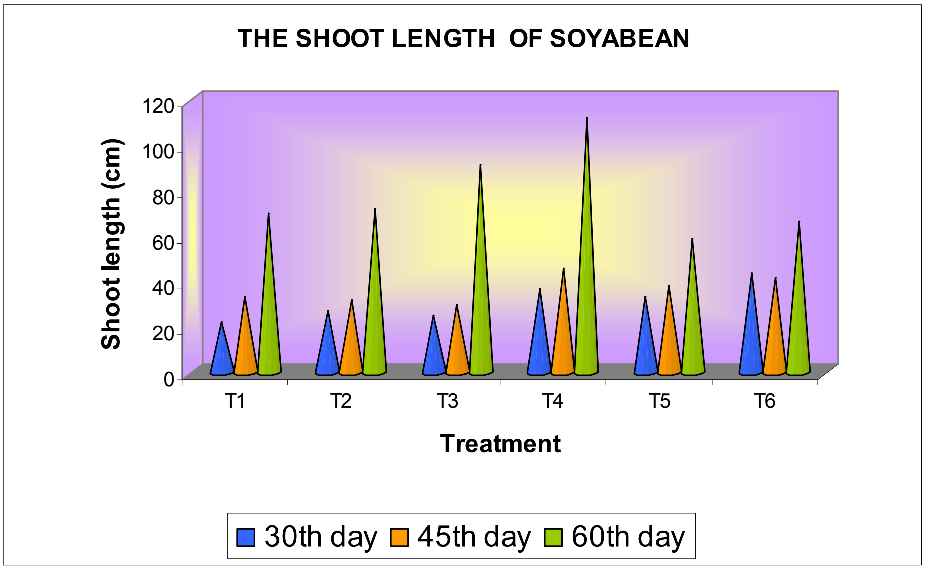
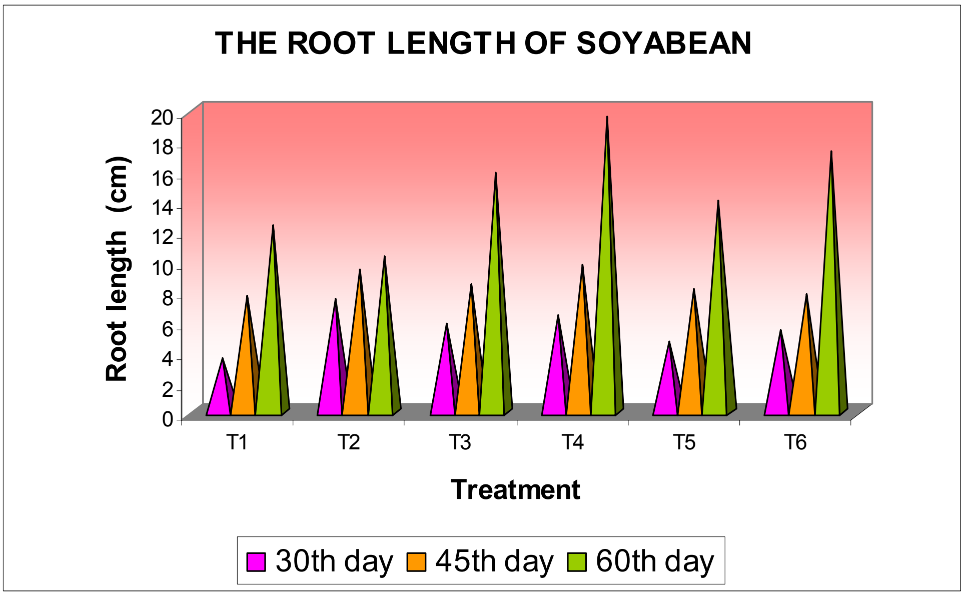


FIGURE 4



4.1.4 Root Length

The root length of all the treatments were increased significantly when compared to the control as shown in Table III and figure 4. Among the treatments, T_2 and T_4 had registered the maximum root length on the 30^{th} as well as on the 45^{th} day of growth. Where as the trend was changed to T_4 and T_6 on the 60^{th} day of growth. A study by Molla *et al.,* (2001) also reported that the root length was increased by the application of *Rhizobium* in soyabean.

4.1.5 Number of Nodules

The nodules of soyabean increased significantly when compared to the control as shown in Table IV and Figure 5.

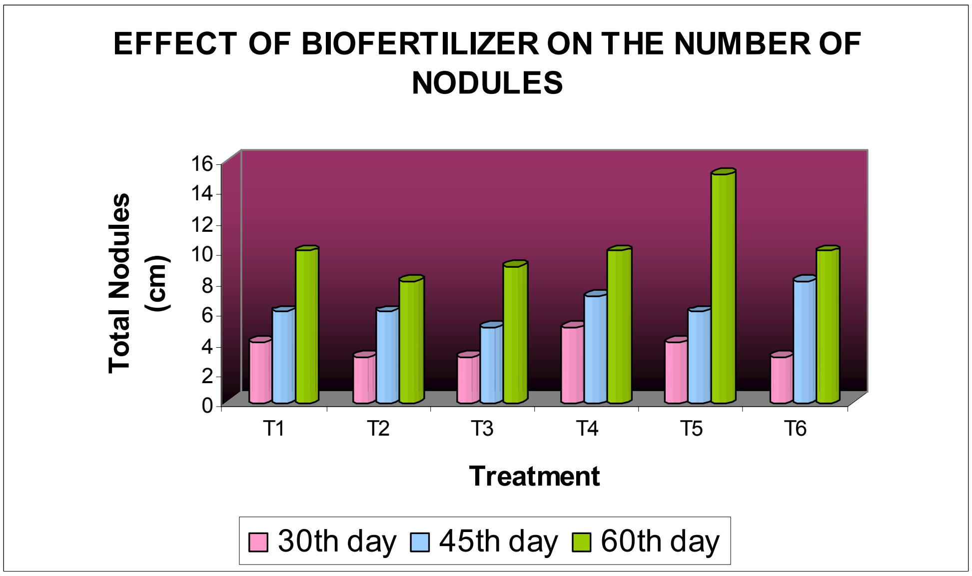
TABLE IV

EFFECT OF BIOFERTILIZER ON THE NUMBER OF NODULES

Treatments	Total nodules (cm)		
	Days after sowing		
	30	45	60
T_1	4	6	10
T_2	3	6	8
T_3	3	5	9
T_4	5	7	10
T_5	4	6	15
T_6	3	8	10
CD (0.05)	**0.777**		

Among the treatments, T_4 had registered the maximum number of nodules on the 30^{th}day of growth. T_6 and T_4 have produced the large number of nodules on the 45^{th} day whereas T_5 was found to be superior on the 60^{th} day of growth. The rest of the treatments were on par with each other with identical or similar number of nodules.

FIGURE 5



A decreased specific nitrogenase activity in nodules of phosphorus deficient soyabean plants was associated with decreased energy status of host plant cells of nodules. These later observations imply specific involvement of phosphorus in symbiotic nitrogen fixation.

However, conversion of inorganic phosphorus in to different forms of organic phosphorus is not known, especially concerning the formation and functioning of symbiotic nodules Tsvetkova and Georgiev (2003).

4.2 Biochemical Parameters

4.2.1 Total chlorophyll

The levels of chlorophyll and total carbohydrate content of soyabean as influenced by the application of biofertilizer, and phosphorus were depicted in Table V and figure 6 and 7.

TABLE V

EFFECT OF BIOFERTILIZER AND PHOSPHORUS ON THE CHLOROPHYLL AND TOTAL CARBOHYDRATE CONTENTS OF SOYABEAN

Treatments	Total chlorophyll (mg/plant)			Total carbohydrate (mg/plant)		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T_1	0.07	0.13	0.23	8.96	5.43	6.61
T_2	0.10	0.18	0.37	8.08	4.35	6.29
T_3	0.08	0.20	0.31	7.76	4.35	5.45
T_4	0.07	0.22	0.34	6.92	2.26	4.58
T_5	0.09	0.30	0.32	6.05	1.47	4.53
T_6	0.08	0.29	0.34	6.00	7.49	3.96
CD (0.05)	0.026			0.073		

FIGURE 6

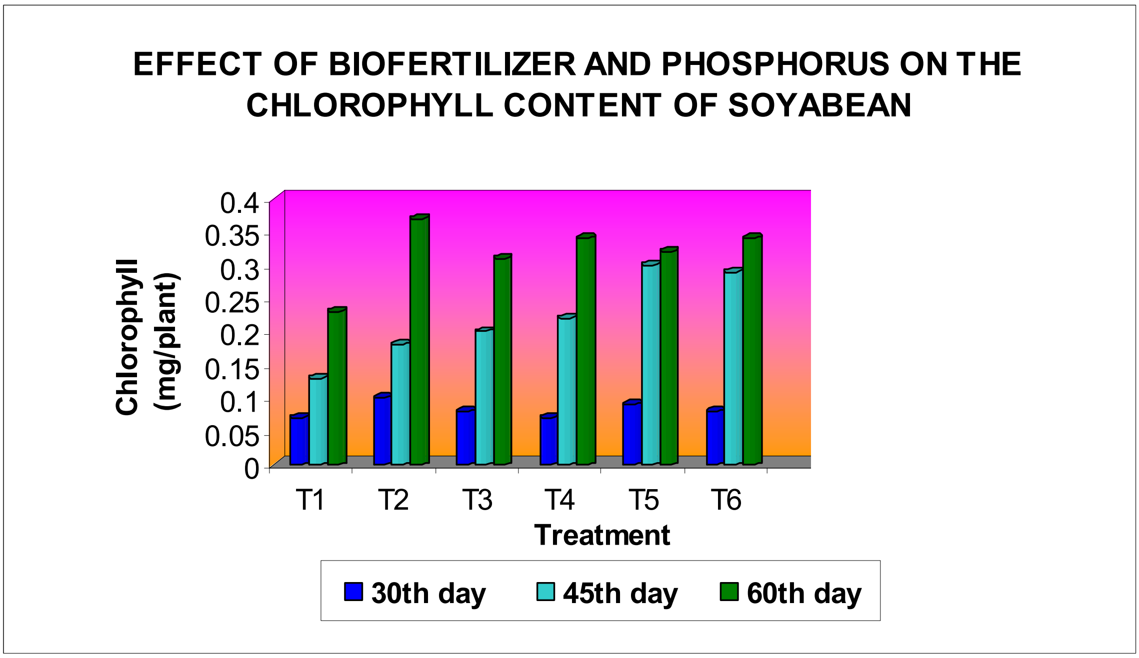
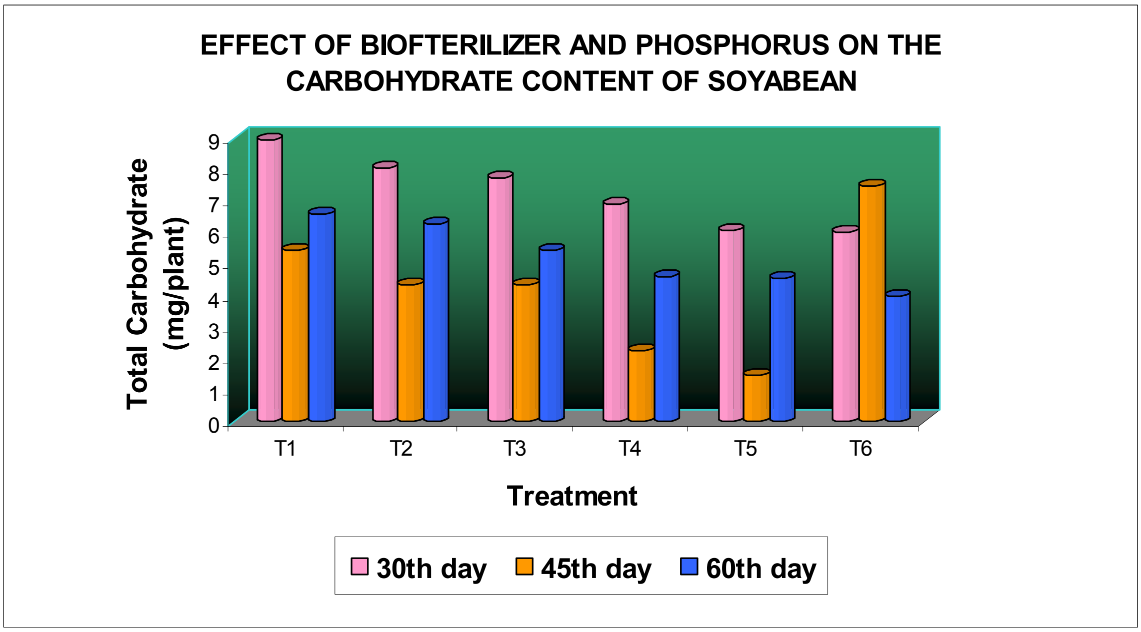


FIGURE 7



There was a statistically ($P<0.05$) significant increase in the chlorophyll content of all the treatments when compared to the control as well as between the days of growth. Among the treatments T_2 exhibited the highest chlorophyll content on the 30^{th} as well as on the 60^{th} day of growth where as T_5 and T_6 have shown the highest chlorophyll content on the 45^{th} day of growth. The rest of the treatments have registered a comparable chlorophyll values throughout the growth period.

Thus it was clear that the individual application of *Rhizobium* or phosphorus can induce the synthesis of chlorophyll. The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency. Thus it is can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin, 2000).

The study of Padma (1998) also indicated that the inoculation of *Azospirillum* in tobacco had increased the chlorophyll content of the plant. The higher chlorophyll content will enhance the rate of photosynthesis and hence the plant growth.

4.2.2 Total carbohydrate

It was clear from Table V that the total carbohydrate content of all the treatments was found to be insignificant when compared to the control. Also the carbohydrate content of the plant was decreased with the growth period. This might be due to the redistribution of sugar from the vegetative part to the reproductive part of the plant (Chandrasekhar *et al.,* 2005).

4.2.3 Total protein

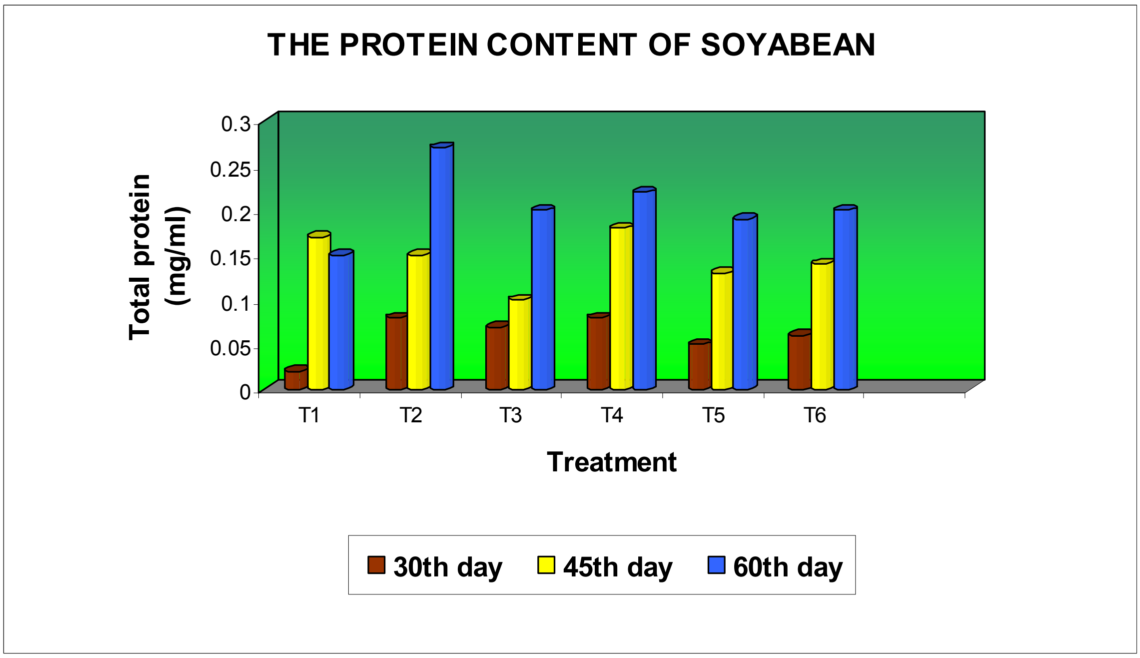
It was clear from Table VI and figure 8 that the total protein content of all the treatments were found to be increased tremendously when compared to the control except on the 45th day and the increase was found to be statistically significant ($P<0.05$). T_2 and T_4 were recorded the highest protein content on the 30th and 60th day followed by T_3 and T_6 respectively.

TABLE VI

THE PROTEIN CONTENT OF SOYABEAN

Treatments	**Total protein (mg/ml)**		
	Days after sowing		
	30	**45**	**60**
T_1	0.02	0.17	0.15
T_2	0.08	0.15	0.27
T_3	0.07	0.10	0.20
T_4	0.08	0.18	0.22
T_5	0.05	0.13	0.19
T_6	0.06	0.14	0.20
CD (0.05)	**0.0266**		

FIGURE 8



A soyabean protein diet may protect against bone loss indirectly by mechanisms independent of its estrogenic effects on bone similar to the estrogen enhancing effects on calcium uptake invitro (Arjmandi *et al,* 1993).

4.2.4 Total Nitrogen, Phosphorus and Potassium

The Nitrogen, Phosphorus and Potassium contents of *Glycine max* on the 30^{th}, 45^{th} and 60^{th} day of growth were illustrated in Table VII and figure 9, 10 and 11 respectively.

Total nitrogen

The nitrogen content of soyabean for the 30^{th}, 45^{th} and 60^{th} day was illustrated in Table VII. There was a statistically ($P<0.05$) significant increase in nitrogen content of all the treatments when compared to the control except T_4 on the 30^{th} and 45^{th} day and T_5 on the 60^{th} day of the growth. The treatment T_6 recorded the highest nitrogen content on the 30th day of growth, followed by T_3 and on the 45th day, the treatment T_3 registered the maximum nitrogen content, whereas on the 60th day after sowing T_4 had shown the highest nitrogen content.

TABLE VII

EFFECT OF BIOFERTILIZER AND PHOSPHORUS ON THE NITROGEN, PHOSPHORUS AND POTASSIUM CONTENTS OF SOYABEAN

Treatments	Total Nitrogen (%)			Phosphorus (%)			Potassium (%)		
	30	45	60	30	45	60	30	45	60
T_1	1.06	1.70	1.74	0.01	0.01	0.03	0.82	0.92	2.09
T_2	1.22	1.28	1.91	0.06	0.06	0.09	1.63	1.72	3.19
T_3	1.71	2.28	1.91	0.09	0.09	0.09	1.64	1.68	3.19
T_4	0.95	1.51	2.56	0.09	0.09	0.10	1.82	1.62	3.27
T_5	1.28	1.44	1.77	0.06	0.07	0.11	1.60	1.88	3.17
T_6	1.81	1.65	2.20	0.07	0.07	0.12	1.59	1.60	3.18
CD (0.05)	**0.0616**			**0.0048**			**0.0106**		

FIGURE 9

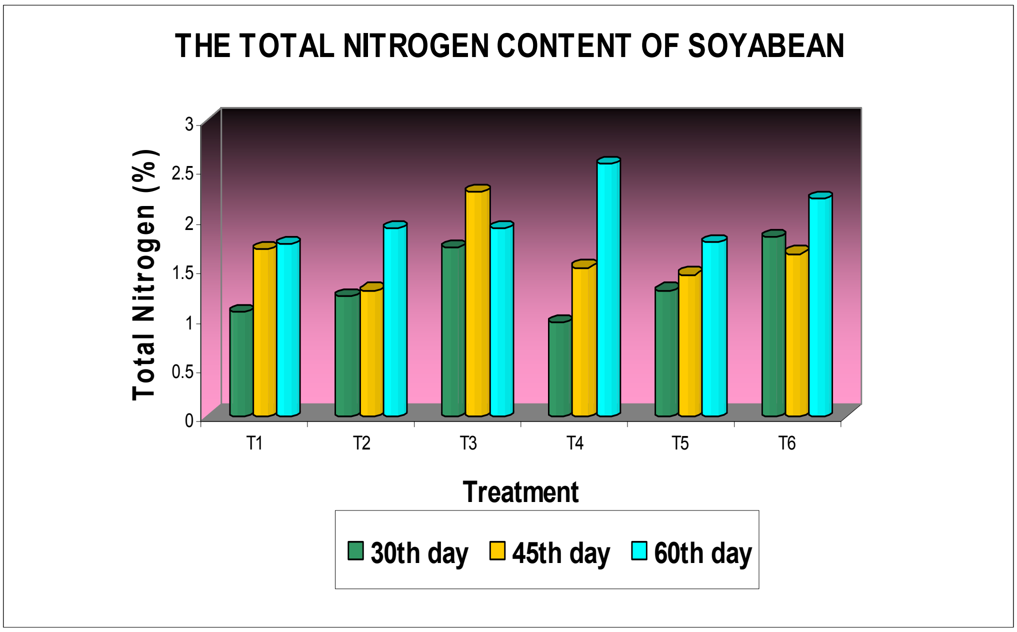


FIGURE 10

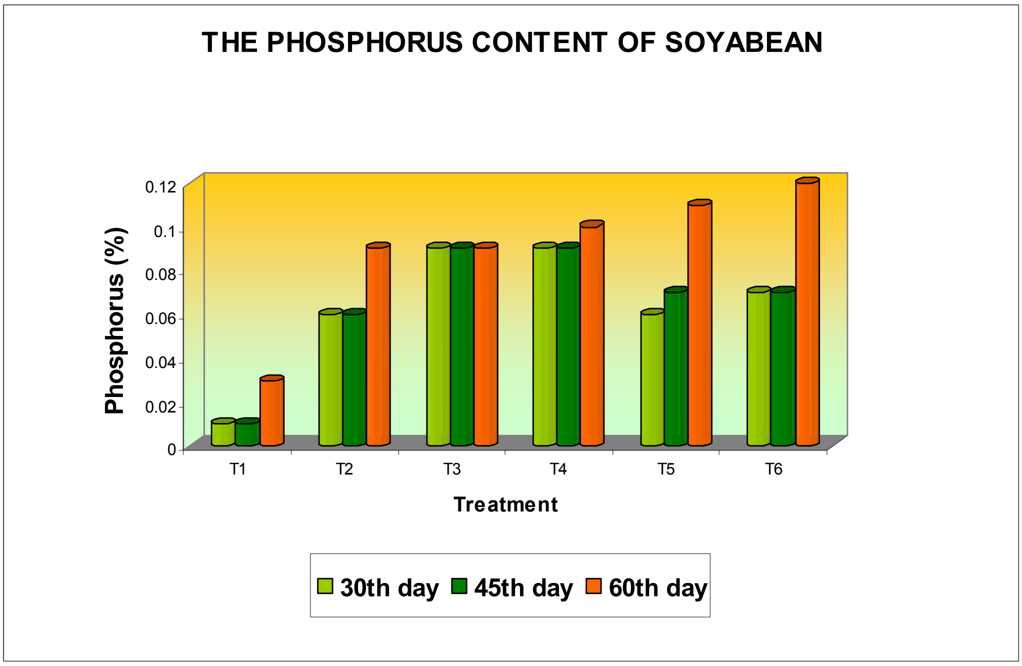
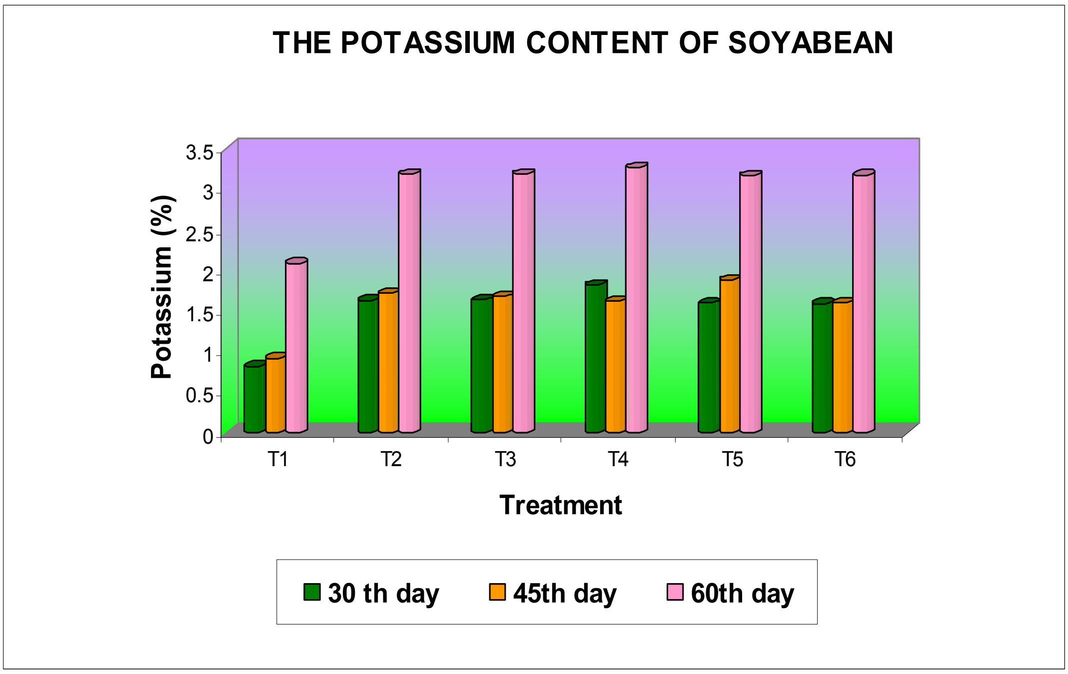


FIGURE 11



Nitrogen is required in large quantities for plants to grow, since it is the basic constituent of protein and nucleic acids. Nitrogen is provided in the form of synthetic chemical fertilizer (urea). Such chemical fertilizers pose a health hazard and microbial population problem in soil besides being quite expensive and making the cost of production high. In such a situation the biofertilizers play a major role (Chandrasekhar *et al*., 2005).

Phosphorus

As depicted in Table VII there was a statistically significant increase in the phosphorus content of *Glycine max* for all the treatments when compared to the control. Also the phosphorus levels of the treatments were found to be three times higher when compared to the control on the 60th day of growth. The uptake of phosphorus increased with the application of *Rhizobium* and *Phosphobacteria* in wheat was also reported by Mahendran and Chandramani (1998).

Phosphorus is an essential macronutrient for plant growth and function. The requirements of host plants for optimal growth and symbiotic dinitrogen fixation processes for phosphorus have been assessed by determination of nodule development and functioning. The increase of whole plant growth and plant nitrogen concentration in response to increased soil phosphorus supply have been noted for several leguminous species including soyabean (Sa and Israel, 1991)

Potassium

From Table VII it was clear that the potassium content of all the treatments were found to be twice the control on the 30th day of growth. Soyabean seeds are relatively high in potassium in comparison to corn, wheat, grain, and sorghum. This means removal of potassium from soyabean is greater than for those crops on a per bushel basis when the grain is removed. Yield increases from potassium are comparable to those with phosphorus under very low soil levels (Kilgore and Fjell, 1997).

4.3 Ammonia Assimilating Enzymes

The enzyme involved in glutamate/glutamine production from ammonia such as glutamate dehydrogenase (GDH), glutamate synthase (GOGAT) and glutamate synthetase (GS) are known as the ammonia assimilating enzymes.

The glutamate synthase and glutamate dehydrogenase content of *Glycine max* for the 30^{th}, 45^{th} and 60^{th} day of growth were illustrated in Table VIII and figure 12 and 13.

4.3.1 Glutamate dehydrogenase

Table VIII represent the glutamate dehydrogenase activity of *Glycine max.* The GDH activity was significantly ($P<0.05$) increased with the growth period as well as for all the treatments when compared to the control. Among The treatments T_4 exhibited the maximum glutamate dehydrogenase activity on the 30^{th} as well as on the 45^{th} day of growth. Where as T_2 and T_3 had shown the highest glutamate dehydrogenase activity on the 60^{th}day of growth. A study by Mirzakrimbaig *et al.* (2001) had shown that the biofertilizer inoculation had increased the ammonia assimilation rate. Wani (1997) also suggested that the *Rhizobium* inoculation generally increased the nitrogen assimilation there by increase the ammonia assimilating enzymes.

TABLE VIII

EFFECT OF BIOFERTILIZER AND PHOSPHORUS ON THE ACTIVITIES OF GLUTAMATE DEHYDROGENASE AND GLUTAMATE SYNTHASE

Treatments	Glutamate synthase #			Glutamate dehydrogenase #		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T_1	2.39	3.23	3.45	0.16	0.86	0.95
T_2	4.04	4.85	5.39	1.20	1.54	2.40
T_3	4.75	4.83	4.87	0.79	1.32	2.30
T_4	6.49	6.68	6.27	1.26	1.78	2.03
T_5	6.65	8.64	9.45	0.70	1.52	2.12
T_6	7.65	8.92	9.02	0.75	1.62	2.13
CD (0.05)	**0.181**			**0.0473**		

Nanomole of NADH per min per mg Protein

FIGURE 12

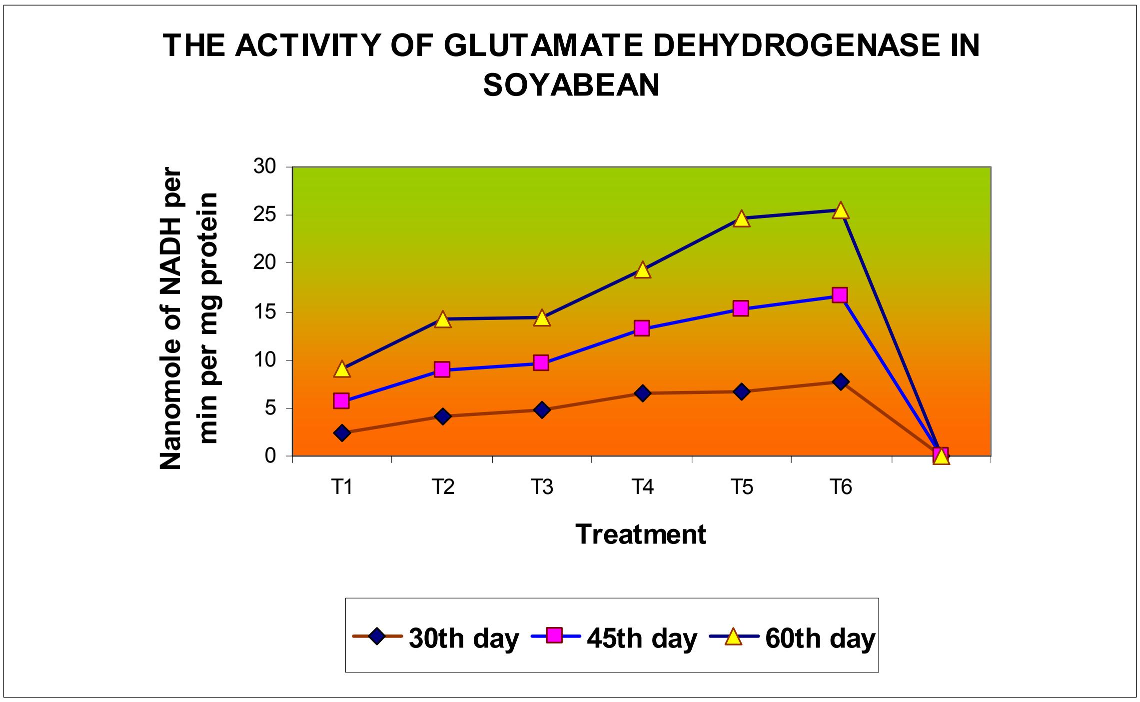
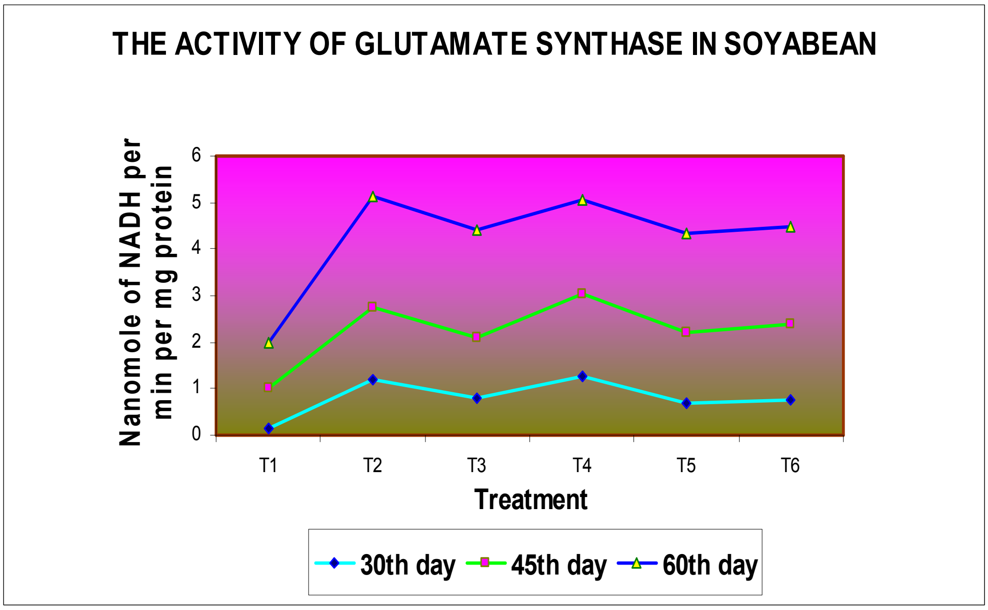


FIGURE 13



4.3.2 Glutamate Synthase

From the Table VIII it was clear that there was a significantly enhanced glutamate synthase activity for the all treatments when compared to the control as well as between the number of days of growth. Among the treatments, T_6 exhibited the highest glutamate synthase activity on the 30th as well as on the 45th day of growth. Whereas T_5 had shown the highest glutamate synthase activity on the 60th day of growth, which was followed by T_6. Gunasekharan and Sivakumar (1996) reported that the inoculation of *Rhizobium* significantly increased the ammonia assimilatory enzyme activity in rice. The enhanced activities of glutamate synthase and glutamate dehydrogenase by the application of phosphorus and biofertilizers indicated that they have enhanced the nitrogen fixation by the *Glycine max.*

4.4 Oxidative Enzymes

Catalase and peroxidase are the oxidative enzymes. Catalase has been shown to associate with disease resistance, there by indicating direct role in host metabolism during growth and development. Peroxidase shows a greatest response to the pollutants and the increase in enzyme activity may be an indication of a stress situation in plant. Hydrogen peroxidase produced directly in plants is metabolized by the catalase and peroxidase of plants (Zaddy and Perevolotsky (1996).

4.4.1 Catalase

Table IX gives the Catalase and Peroxidase activities of *Glycine max* on the 30th, 45th and 60th day of growth and figure 14 and 15.

TABLE IX

EFFECT OF BIOFERTILIZER AND PHOSPHORUS ON THE ACTIVITIES OF CATALASE AND PEROXIDASE

Treatments	Catalase #			Peroxidase (min /g)		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T_1	0.126	0.198	0.298	0.16	0.18	**0.95**
T_2	0.146	0.298	0.318	1.14	1.24	**1.40**
T_3	0.264	0.356	0.554	0.79	0.86	**1.03**
T_4	0.233	0.466	0.528	1.26	1.30	**1.13**
T_5	0.113	0.219	0.475	0.72	0.80	**1.03**
T_6	0.118	0.365	0.528	0.75	0.89	**1.12**
CD (0.05)	0.346			0.047		

ml of 0.01 N $kMnO_4$/ min/ml enzyme

There was a statistically significant increase in the catalase activity of all the treatments when compared to the control through out the growth period. Among the treatments, T_3 exhibited the maximum catalase activity on the 60th day. Whereas T_3 had shown the highest value of catalase activity on the 30th day as well as on the 60th day of growth. This was followed by T_4 and T_6 on the 60^{th} day with identical catalase activity. Thus the inoculation of *Phosphobacteria* either alone or in combination with *Rhizobium* had improved the activity of catalase.

FIGURE 14

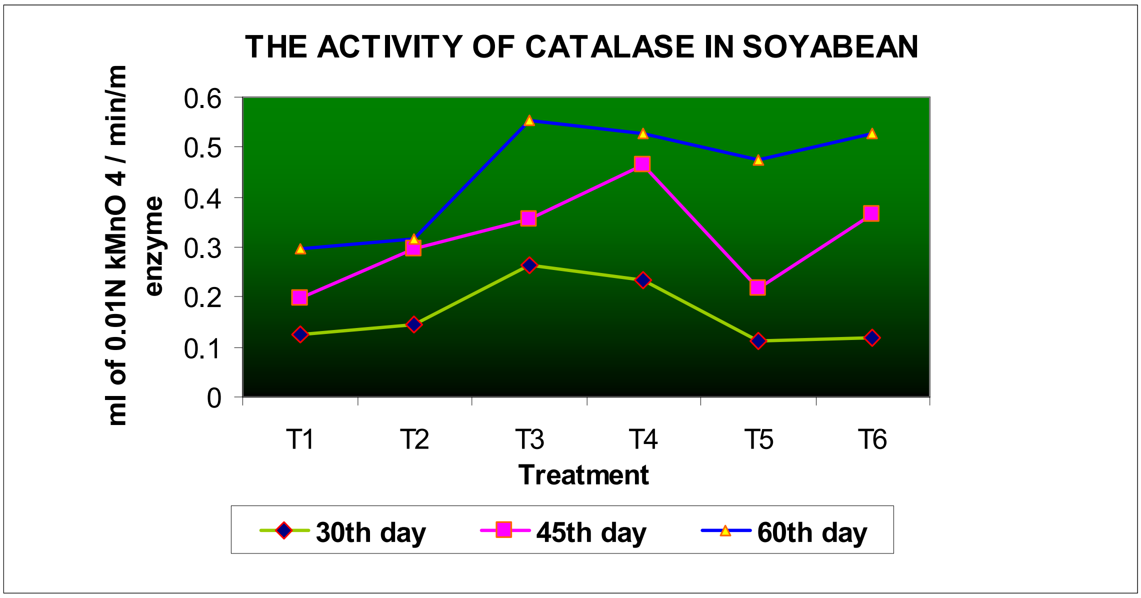
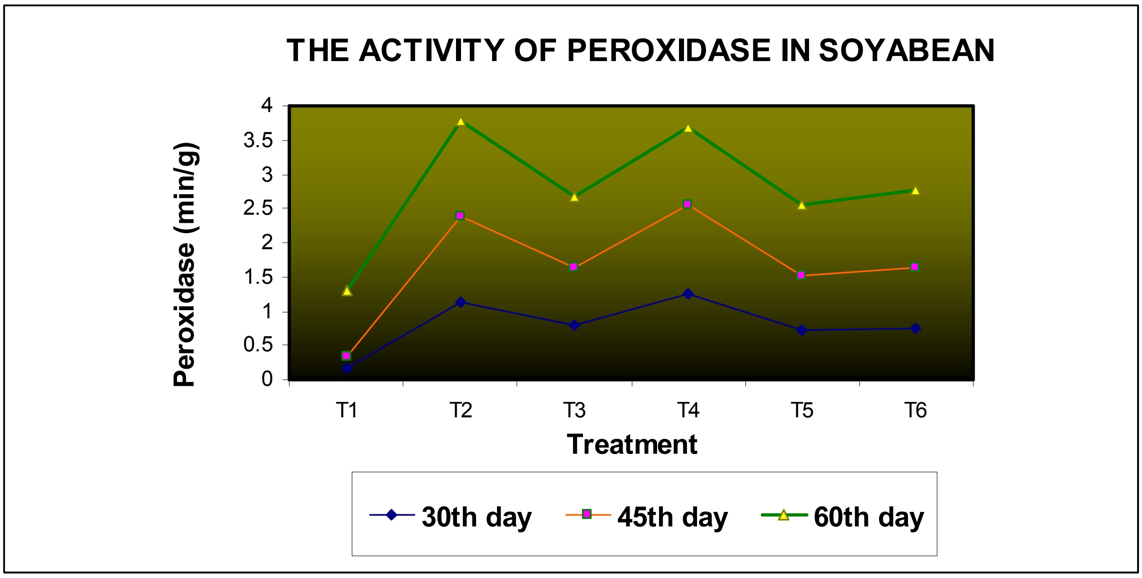


FIGURE 15



4.4.2 Peroxidase

As represented in Table IX and figure 15, the peroxidase activity was increased with the increase in growth period and with the biofertilizer and mineral application. The treatments, T_2 and T_4 were shown a higher peroxidase activity throughout the growth period when compared to other treatments. It was clear that the individual as well as the combined inoculation of *Rhizobium* and *Phosphobacteri*a had influenced the activity of peroxidase very much compared to the other treatments. Devi and Reddy (2004) also reported that the application of biofertilizer increased the activity of oxidative enzymes.

3.8 Soil Analysis

The nitrogen, phosphorus and potassium contents of the soil at the initial stage and post harvesting stages were described in Table X.

TABLE X

SOIL ANALYSIS BEFORE AND AFTER HARVESTING THE PLANTS

Treatments	Nitrogen (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)
Initial stage	218	14	30
Post harvest stage			
T_1	240	140	45
T_2	320	180	63
T_3	259	158	72
T_4	255	149	98
T_5	275	198	103
T_6	290	230	112

It was clear from the Table X, that at the post harvesting stage the nitrogen, phosphorus and potassium contents were found to be improved very much when compared to the initial stage of the soil by the application of biofertilizers and phosphorus. *Rhizobium* and high concentration of phosphorus (100 Kg/ha) were found to enrich the soil nitrogen, phosphorus and potassium very much when compared to other treatments.

5.0 SUMMARY AND CONCLUSION

The present study entitled "Effect of biofertilizer, and phosphorus on the growth, biomass production, nodulation and enzymic antioxidants in soyabean *(Glycine max*)" was conducted with an objective to study the effect *of Rhizobium and phosphobacteria*, individually and in combination, phosphorus in the from of superphosphate, on the biometric and biochemical parameters of soyabean. The study was carried out in pot cultures in completely randomized block design with four replications for each treatment.8 kg of soil was filled in all the pots along with farmyard manure at the rate of $10t/ha^{-1}$ and superphosphate at the concentrations of 50 kg and 100 kg ha^{-1} to various pots.

Before the seeds were sown, soyabean seeds were treated with *Rhizobium,* and *Phosphobacteria*, both individually and in combination.

The treatments were as follows.

T_1 – Control

T_2 – *Rhizobium*

T_3 – *Phosphobacteria*

T_4 – *Rhizobium and Phosphobacteria*

T_5 – 50 kg of Phosphorus

T_6 – 100 kg of Phosphorus

At the end of the 30^{th}, 45^{th} and 60^{th} day after sowing, the plants were uprooted and subjected to various biometric and biochemical analysis.

The results of the study were summarized as follows

1. The fresh and dry weights of soyabean were found to be maximum in the treatment T_2 on the 60^{th} day of growth. This was followed by the treatment T_4 for the fresh weight and lower dose of phosphorus T_5 for the dry weight.

2. Among the treatments, T_6 and T_4 recorded the maximum shoot length on the 30^{th} as well as on the 45^{th} day, whereas T_4 and T_3 had shown the enhanced shoot length on the 60^{th} day. Thus the application of *Rhizobium* individually and in combination with *Phosphobacteria* stimulated the growth of soyabean. When compared to the biofertilizers, phosphorus application had shown less influence on the shoot length of soyabean.

3. Root length of soyabean was found to be increased in treatments T_2 and T_4 on the 30^{th} and 45^{th} day of the growth. T_4 and T_6 have recorded the maximum root length on the 60^{th} day of growth. Thus the phosphorus absorption influences the growth of root.

4. The lower dose 50 kg of phosphorus (T_5) was found to be sufficient to induce the number of nodules. The rest of the treatments were on par with each other with similar number of the nodules.

5. The treatment T_2 exhibited the highest chlorophyll content on the 30^{th} day as well as on the 60^{th} day, followed by T_4 and T_6 respectively. The biofertilizer application had improved the chlorophyll content very much when compared to the chemical fertilizers.

6. The total carbohydrate content of all the treatments was found to be insignificant when compared to the control.

7. The treatments T_2 and T_4 recorded the maximum protein content on the 30^{th} and 60^{th} day followed by T_3 and T_6 respectively.

8. The nitrogen content was found to be higher in T_6 on the 30^{th} day after sowing. Among the treatments, T_3 recorded the maximum nitrogen content on the 45^{th} day whereas T_4 had shown greater nitrogen content on the 60^{th} day of growth.

9. The total phosphorus level of all the treatments were found to be three times higher when compared to the control on the 60^{th} day of growth. Phosphorus content of the soyabean was also increased with the increased dose of phosphorus.

10. The combined application of *Rhizobium* and *Phosphobacteria* (T_4) recorded the maximum potassium content among the various treatments on the 30^{th} and 60^{th} day of growth, whereas T_5 had the highest potassium content on the 45^{th} day.

11. Among the treatments, T_2 and T_3 exhibited a maximum glutamate dehydrogenase activity on the 60^{th} day of growth, where as T_4 recorded a maximum glutamate dehydrogenase activity on the 30^{th} day as well as on the 45^{th} day of growth.

12. The activity of glutamate synthase was found to be higher in T_6 on the 30^{th} as well as on the 45^{th} day of growth. Where as T_5 had shown the highest glutamate synthase activity on the 60^{th} day of growth, which was followed by T_6.

13. Among the treatments, T_3 exhibited the maximum catalase activity on the 60^{th} day. Whereas T_3 had shown the highest value of catalase activity on the 30^{th} day as well as on the 60^{th} day of growth. This was followed by T_4 and T_6 on the 60^{th} day with identical catalase activity. The treatments, T_2 and T_4 were shown a higher peroxidase activity throughout the growth period when compared to other treatments. Thus the biofertilizers were found to be superior in enhancing then activities of the oxidative enzymes compared to the chemical fertilizer.

14. It was clear from the Table X that at the post harvesting stage the nitrogen, phosphorus and potassium contents were found to be improved very much when compared to the initial stage of the soil.

From the results summarized above we can conclude that the treatment T_2 with *Rhizobium* individually as well as the combined application of biofertilizer (T_4) exhibited an improved plant growth, biomass production, chlorophyll, and total carbohydrate content, phosphorus , protein , potassium and total nitrogen. The activities of ammonia assimilating enzymes were also enhanced by the application of *Rhizobium* individually as well as the higher dose of phosphorus. Combined application of *Rhizobium* and *Phosphobacteria* individually and in combination influenced the activities of oxidative enzymes and increased the total phosphorus content.

The combined application of the biofertilizers also enhanced the plant growth and potassium content. Thus the individual as well as the combined application of biofertilizers seem to invigorate the growth, biomass production and biochemical constituents than the chemical fertilizers.

Research prospects

- The *Glycine max* seeds can be treated with fungicide and its influence on the nitrogen fixation can be studied.
- The effect of other chemical fertilizers apart from phosphatic fertilizers can also be studied.

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