Influence of AM Fungi and *Rhizobium* Inoculation on Quality and Productivity in Field Pea (*Pisum sativum* L.)

THESIS

Submitted to the

LOVELY PROFESSIONAL UNIVERSITY,
PHAGWARA, PUNJAB, INDIA

In partial fulfillment of the requirements for the award of degree of

MASTER OF SCIENCE
IN
(AGRONOMY)

BY

Rupinder Singh Dhillon
Registration Number: 11307586

Under the supervision of
Dr. Balkrishna Sopan Bhople

Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, India June, 2015

CERTIFICATE

This is to certify that the thesis entitled **"Influence of AM Fungi and *Rhizobium* Inoculation on Quality and Productivity in Field Pea (*Pisum sativum L.*)"** submitted by **Rupinder Singh Dhillon** to the Lovely Professional University, Phagwara in partial fulfilment of the requirements for the degree of **Master of Agriculture/Horticulture** in the discipline of Agronomy has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

Chairperson Advisory Committee	External Examiner
Member	Member
Member	

Head of the Department

Dean, School of Agriculture

Certification

This is to certify that the thesis entitled "**Influence of AM Fungi and *Rhizobium* Inoculation on Quality and Productivity in Field Pea (*Pisum sativum L.*)"** submitted in partial fulfilment of the requirements for the degree of Master of Science with major in Agronomy of the Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, is a record of bonafide research carried out by Rupinder Singh Dhillon, Registration No.11307586 under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

(Signature of the Major Advisor)	**(Signature of co-supervisor)**
Dr. Balkrishna Sopan Bhople	Dr. Arun Kumar
Designation: Assistant Professor	Designation: Assistant Professor
ID. No. 16709	ID No. 18703

Declaration

I hereby declare that this thesis is a presentation of my own work and has been generated by me as the result of my own research work and efforts. This thesis is submitted by me in partial fulfillment of the requirement for the award of degree M.Sc. in Agronomy from Lovely Professional University, Phagwara, Punjab comprises only my original work and due acknowledgement has been made in the text to all other material used.

This thesis work was done under the guidance of my advisor.

_____

(**Signature of the student**)

Rupinder Singh Dhillon
Registration number: 11307586

Acknowledgement

With unending humility, at the very outset, I would like to thank "The Almighty" who blessed with the limitless internal strength and favorable circumstances, to face and pass through all odds successfully at this juncture.

With an overwhelming sense of legitimate pride and genuine obligation, I seize this rare opportunity to express my deep sense of gratitude, indebtedness and personal regards to my esteemed teacher, Dr. Balkrishna Sopan Bhople, Assistant Professor, Dept. of Agronomy, School of Agriculture Lovely Professional University Jalandhar, Punjab (India), for his expert, invaluable, criticism during the entire course of my investigations.

I am thankful to esteemed Dr. Madhu Sharma (HOD, Horticulture), Dr. Arun Kumar Dr. Chandra Mohan, Dr. Amit Kesarwani and other faculty members of School of Agriculture and Food Technology, for their proper guidance during the entire course of present studies.

I expressed my heartfelt thanks to my friends Gurjinder singh, Manpreet singh, Raju, Navninder, Bhawandeep and Satpal for their co-operation, encouragement, moral and timely help when demanded.

I avail the blessings, affection and moral encouragement of my gracious parents, constant moral encouragement inspired me to search ahead.

Table Contents

List of Tables

List of Figures

Abbreviation

%	Percentage
^{o}C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungi
B:C	Benefit Cost Ratio
CEC	Cation Exchange Capacity
DAS	Days After Sowing
DMA	Dry Matter Accumulation
EC	Electrical Conductivity
K	Potassium
LPU	Lovely Professional University
Meq	Milliequivalent
N	Nitrogen
NFB	Nitrogen Fixing Bacteria
NPK	Nitrogen, Phosphorus and Potassium
P	Phosphorus
PAU	Punjab Agriculture University
PGR	Plant Growth Regulator
pH	Potential of Hydrogen
RBD	Randomized Block Design
RDF	Recommended Dose of Fertilizers
RH	Relative Humidity
RHZ	*Rhizobium*
VAM	Vascular Mycorrhizal Fungi

Abstract

The present study aim to investigate the influence of AM fungi and *Rhizobium* inoculation on quality and productivity in field pea (*Pisum sativum)* . The field experiment was done in a Randomized block design with three replications, comparing seven treatments involving of *Rhizobium* culture, AM fungi, inorganic NPK, recommended fertilizer doze and absolute control .To study the various growth parameters, yield and quality parameter and to analysis the outcome from the experiment. The study revealed that treatment T7 (RHZ +AMF+ N50%+ P50% + K100%) lead to significantly increase in quality and yield parameters as compare with other treatments .where treatment T5 (AMF+ N100%+ P50% +K100%) lead to significant increase in growth parameters. Moreover AM fungi and *Rhizobium* inoculated treatments indicated an increase in yield of about 46 % as compare with absolute control. AM fungi and *Rhizobium* as amendment play an vital role in enhancing crop quality and productivity.

Keywords: AM Fungi, *Rhizobium*, Field pea (*Pisum sativum).*

Chapter I

1. INTRODUCTION

Pea *(Pisum sativum* L.) is an important vegetable crop grown throughout the world. In India, it is mainly grown as winter vegetable in the plains of North India and as summer vegetable in the hills. It is generally used as fresh vegetable and in the form of canned, processed or dehydrated .The origin of *Pisum sativum* is not well known. The Mediterranean region, western and central Asia and Ethiopia have been indicated as centers of origin. Recently the Food and Agriculture Organization (FAO) designated Ethiopia and western Asia as centers of diversity.

Pea occupies an area of 0.408 million hectares with the production of 3.74 million tones in India (Anonymous,2013).Where in Punjab it was grown on 1.7 thousand hectares during year 2012-2013 and with the production of 2.23 thousand tones. The average yield was 1309 kg per hectare (524 kg/acre). It is mainly grown in punjab in Hoshiarpur district under rainfed conditions. The crop can successfully be grown in other parts of the State under limited irrigation conditions with low inputs. Field pea is an annual plant, with a life cycle of one year. It is a cool-season crop grown in many parts of the world. The planting can take place from winter to early summer, depending on location. The Optimum temperature for good growth is between 10 C to 18 C. Peas can be grown on all types of soils but it prefers well-drained sandy loamy soils. The soils should rich an organic matter as it enhances better growth by supplying nutrients at a slower rate. It does not thrive in highly acidic or alkaline soils or saline soils. It grows best at a pH of 6.5.

Dry pea is a pulse crop and a member of the family Leguminacea. This crop like many other legumes is capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with Rhizobium. The crop thus, improves soil, economizes crop production reducing the requirement of added synthetic nitrogenous fertilizers. When properly inoculated with an appropriate Rhizobium inoculant, pea can derive up to 80% or more of its nitrogen through nitrogen fixation.

The rest of the N must be provided from the soil or from fertilizer applications (Ali-Khan and Zimmer, 1989; Bowren *et al*., 1986). Seed inoculation is the most widely used methods of inoculant application. Many researchers (Rahman *et al.*, 1994; Solaiman and Rabbani, 2005) have reported the beneficial effects of inoculation of grain legumes.

Nodule formation and subsequent nitrogen fixation are very sensitive to external nitrogen sources, including fertilizer and available soil nitrogen. Nitrogen fertilizer applications generally inhibit biological N-fixation by *R. leguminosarum*. The inhibitory effect of N fertilizer on nodule formation results from the fertilizer's contribution to the soil N pool. Bowren *et al*. (1986) concluded that nodule formation became inhibited as soil N levels approached 40 kg ha^{-1} and were progressively inhibited as levels exceeded this amount. This suggests that N fertilizer applications will reduce biological N-fixation by *R. leguminosarum*, except where the amount of N applied as fertilizer plus that contained in the soil is <40 kg ha^{-1}. Small N fertilizer applications have stimulated nodule formation on pea roots in some low N environments and other pulse crops (Kauskik *et al*., 1995). It can take 3-4 weeks after planting before nodules are fully functioning. Early plant growth may be poor in soils with low nitrogen levels and plants may appear yellow prior to the beginning of effective nitrogen fixation due to a nitrogen deficiency. This early deficiency can be overcome by adding low levels of starter nitrogen at seeding. Although, high levels of starter nitrogen may appear to help the crop overcome a nitrogen deficiency during early crop growth stages, final seed yields may not increase. Bowren *et al*. (1986) suggested that applications of 9-18 kg N ha^{-1} enhanced pea seedling growth prior to nodule development in low N soils. The soil of the region where the study was conducted has low nitrogen and organic matter content and their availabilities are low due to the various soil and other environmental factors. Therefore, it was aimed to determine the effects of nitrogen fertilizer and *Rhizobium* inoculation on the yield and growth parameters, as well as crude protein rate of seeds in field pea.

On the other hand where as the AM Fungi belongs to endomycorrhiza group which penetrate the root cell wall. These fungi enter in root cells and form hyphal masses within the cells. This group is most common and most widespread. The roots of the most common agronomic crops including cotton, wheat, potatoes, beans, alfalfa, sugarcane and dryland rice have AM association. Cultures of VAM are also being used on a limited scale. VAM helps in P nutrition by not only increasing P availability but also increasing its mobility. Through AM, plants are able to take P from soil zone not visited by the root system. As AM fungi cannot be grown on synthetic medium, its large scale application is limited to perennial crops and transplanted crops where the requirement of the inoculums is reduced by about 20 times due to less area covered in the nursery. When seed of pulses are inoculated with AM, enhance uptake of P, Zn, S, water as well as resistance to root disease and improve hardiness to transplant stock (Yawalkar *et al*., 2006).

About 93-99 per cent of the total phosphorus is insoluble and hence directly not available to plants. Only about a quarter of water soluble phosphate is taken up by plants in the season of the application and the remaining is converted into insoluble (unavailable) forms (Verma 2003). Inoculation of AM in the rhizosphere of crop and soil increases the availability of P from insoluble sources of phosphate, desorption of fixed phosphates and also increases the efficiency of phosphatic fertilizers (Gaur 2001). The inoculated AM secrete acidic substances and solubilize unavailable soil phosphorus and make available to plants. The culture can hence prove broad spectrum bio-fertilizer which may Supplement phosphorus upto 30 kg P2O5/ha and increase yield of crops (Legumes, vegetables etc.) by 10-30 per cent (Tilak and Annapurna 2013).

Hence, keeping in view the above facts an experiment “Influence of AM Fungi and Rhizobium Inoculation on quality and productivity on Field pea” was conducted at agricultural farm of L.P.U Jalandhar with the following objectives:

Aim and Objectives

Aim: The aim of the experimentation is assessing quality and productivity through inoculation with *Rhizobium* and AM fungi in field pea with following specific objectives.

Objectives

1. To study the effect of AM fungi and *Rhizobium* inoculation on quality and productivity of field pea

2. To work out the economics of the treatments.

Chapter II

2. REVIEW OF LITERATURE

A review of literature pertinent to present study is presented in this chapter; an attempt has been made to cite the relevant literature on **Influence of AM fungi and *Rhizobium* inoculation on quality and productivity of Field pea (*Pisum sativum L.*)**. The available literature strongly conveys that little work has been done on response of pea on its quality and productivity by bio-fertilizers. The similar work on other legume crops is also being reviewed in this chapter.

2.1. Effect of AM fungi and *Rhizobium*

2.1.1 Growth Attributes

Dadhich *et al.* (2001) reported that seed inoculation with PSB (0 and 500 g ha^{-1}) significantly increased the dry matter accumulation in clusterbean over control. Devi and Reddy (2001) found that seed inoculation of groundnut with VAM and *Rhizobium* increased the growth and number of nodules per plant over control. Yadav (2001) observed that inoculation of cowpea seed with PSB significantly enhanced the plant height, dry matter and weight of nodules per plant as compared to untreated control.

Vijayakumar and Lakshminarasimhan (2004) reported that seed inoculation of groundnut with *Glomus fasciculatum* (VAM) and *Rhizobium* gave significantly higher root and shoot dry weight, number of nodules per plant, nodules dry weight per plant and pod yield /ha over control.

Bahadur *et al.* (2006) conducted an experiment to investigate the effect of organic amendments and bio-fertilizers on growth, yield and quality attributes of garden pea cv. Azad P-3. The treatments comprised of individual application of FYM, in combination with bio-fertilizers *viz.* Azatobacter, PSM, *Rhizobium* and VAM. The results showed that seed inoculation with *Rhizobium* inoculation increased maximum nodulation and root proliferation and pod yield than conventional fertilizer application.

Singh *et al.* (2006) observed significantly higher number of nodules and their fresh weight with the treatment *Rhizobium* + VAM + PSB + 75% RDF of NPK.

Negi *et al.* (2006) conducted an experiment to access the effect of biofertilizers (*Rhizobium*) at 250 g/10 kg seed, nutrient sources (FYM at 20 t/ha and NPK at 25:25:25 kg/ha) and lime (at 4 t/ha) on growth and yield of garden pea. The Results showed that composite inoculation of bio-fertilizers significantly increased the growth and yield of pea.

Stancheva *et al.* (2006) studied the effects of combined inoculation of pea plants with VAM and *Rhizobium* on nodule formation and nitrogen fixation activity. The results showed that dual inoculation of pea plants increased plant biomass, nodules parameters, N_2-fixation activity at varying levels compared to single inoculation with *Rhizobium* and depended on VAM fungi species.

Biswas and Patra (2007) reported that application of 40 kg P_2O_5 ha^{-1} with soil inoculation of VAM and seed inoculation with PSB recorded higher plant height, dry matter accumulation, leaf area index, crop growth rate, net assimilation rate and nodulation in summer green gram than that of control/lower dose of P.

Singh *et al.* (2009).Inoculation of *Rhizobium*, VAM and N levels in green gram resulted of significant improvement in nodule dry weight, rhizobial counts, root colonization, and spore density over the control.

Djebali *et al.* (2010) studied the effect of inoculation by VAM on plant growth and development and the cultivar used. A significant increase in shoot fresh weight, number of nodules and root dry weight was observed in pea.

Ramana *et al.* (2010) studied the effect of bio-fertilizer VAM and PSB along with application of fertilizers on growth, yield attributes and yield of frenchbean. The results revealed that the application of 75 per cent RDF + VAM @ 2 kg ha^{-1} + PSB @ 2.5 kg ha^{-1} significantly increased the plant height (cm), number of branches per plant, leaf area (cm^2) and dry weight (g) .

Tabassum Yaseen *et al.* (2011) studied that VAM fungal inoculation had a

significant effect on productivity of cowpea attributed to growth, plant height, number of nodules, *mycorrhizal* dependency and number of flower per plant.

Pramanik and Bera (2012) observed that the growth parameters like plant height significantly improved by inoculation with biofertilizers (*Rhizobium*, PSB and VAM) in chickpea (*Cicer arietinum* L.).

2.1.2 Yield and Yield Attributes

Tarafdar and Rao (2001) carried out a field experiment and noted significant increase in seed yield of clusterbean with inoculation of VAM (*Glomus mosseas*) to soil.

Tomar *et al.* (2001) observed that inoculation of *Rhizobium* + VAM + PSB recorded maximum increase in nodulation, nodule dry weight, grain yield, N and P content in plant and grain of black gram (*Vigna munga* L.).

Singh *et al.*(2004) observed the effect of integrated nutrient management on nutrient uptake and yield with the application of FYM (15 t/ha) + *Azospirillum* + VAM + PSB + amount of NPK. The results obtained through INM treatments on pea was an increment in dry matter yield, fruit yield and nitrogen as well as phosphorus and potassium uptake.

Kristek *et al.* (2005) reported that seed inoculation with mycorrhizal species *Glomus mossae* increased dry matter yield 59.40 g/m^2, grain yield 346.20 g/m^2 in field pea as compared to non mycorrhizal species.

Singh *et al.* (2006) observed that inoculation of *Rhizobium* + VAM + PSB along with 75% NPK recorded significantly higher yield attributes and finally seed yield over each and all treatments in pea.

Dadhich *et al.* (2006) observed effect of VAM and PSB on the yield of soybean, nutrient uptake by plants and phosphorus balance in soil under field conditions. They found that co-inoculation of VAM along with PSB significantly improved nodulation, seed yield, mineral uptake and available P in soils.

Sajitha *et al.* (2007) reported that inoculation of *Rhizobium* and VAM along with vermicompost and vermiwash yielded better than uninoculated and controlled treatments in garden bean.

Mahanta and Rai (2008) studied effect of different sources of phosphorus (SSP and RP) and biofertilizers (PSB + VAM) on productivity, nutrient uptake, P balance in the soil, phosphorus use efficiency and economics of soybean wheat system. They found that half the dose of P could be saved through inoculation with both P-solubilizing and mobilizing micro-organism to obtain higher productivity and profitability.

Singh *et al*. (2009) observed the increase in grain yield and straw yield of green gram with inoculation of *Rhizobium*, VAM and N levels over the control under temperate conditions.

Bhardwaj, S.K. *et al.* (2010) observed that application of 100% NPK and dual inoculation of *Rhizobium* + VAM was found superior in yield (74.16, 74.60 q ha -1) and pod length (13, 13.1 cm) production in French bean..

El-Shaikh *et al.* (2010) recorded that application of 22.5 kg of phosphorus and inoculation with VAM in pea cultivar in early perfection increase total green pod yield along with highest number of pods/ plant.

Sarawgi *et al.* (2012) recorded that application of 30 kg P_2O_5 ha^{-1} through rock phosphate (RP) + PSB + *Rhizobium* inoculation (RI) + VAM in soybean (*Glycine max*) registered significantly higher seed yield, net returns and return / rupee invested in P compared to application of 60 kg P_2O_5 ha^{-1} through rock phosphate without bio-fertilizers.

Dania *et al.* (2013) observed 48 per cent higher grain yield in mycorrhizal pigeon pea compared to non-mycorrhizal pigeon pea for both the intercrop and the sole pigeon pea. Thus the VAM significantly improved the growth, shoot biomass, grain yield and nutrient uptake of maize and pigeon pea.

2.1.3 Nutrient Content and Quality

Tomar *et al.* (2001) observed that inoculation of *Rhizobium* + VAM + PSB recorded maximum increase in N and P content in plant and grain of black gram (*Vigna munga* L.). Dual inoculation of *Rhizboium* with VAM or PSB was generally significant in the effect and better than that of VAM + PSB. Hence, P accumulation in plant and grain was more with VAM + PSB. Among single inoculation, PSB however registered significant increase in P concentration in plant and grain over VAM and *Rhizobium.*

Robie and Humiany (2004) resulted the interactions between single, dual and triple inoculants of nitrogen fixing bacteria (NFB), Phosphate Solubilizing Bacteria (PSB) and VAM fungus on the growth and nutrition of cowpea plants in calcareous soil amended with rock phosphate, compost and mineral N fertilizers. They found that nitrogenous activity of cowpea plant were significantly improved by using bio-preparations especially mycorrhizal inoculation in the presence of 25% dose of mineral nitrogen fertilizer. The use of bio-preparations of VAM, NFB and PSB as biofertilizer could reduce at least 50-75% economic cost compared to mineral nitrogen fertilizers use.

Bahadur *et al.* (2006) conducted an experiment on plant growth, yield and quality attributes in garden pea with the application of FYM (organic amendments) or digested sludge (DS) or in combination with biofertilizers *viz.*, *Azotobacter*, phosphate solubilzing micro-organism (PSM), *Rhizobium* and VAM. They found that organic amendments and biofertilizers inculants *viz.*, Azotobactor, PSM, *Rhizobium* and VAM significantly influenced the carbohydrates and vitamin content in garden pea cv. Azad P-3.

Stancheva *et al.* (2006) reported that co-inoculation of pea plant with VAM and *Rhizobium leguminoserum* cv. Viciae strain D 293 increased significantly total P content in plant tissue and percentage of root colonization.

Sajitha *et al.* (2007) reported that *Rhizobium* and VAM alongwith flower

waste vermicompost and spraying of vermiwash registered higher protein content. The results also indicated that the garden bean being responded very well to inoculation of *Rhizobium*, VAM and vermicompost and its wash for providing all necessary nutrients in available form to crop.

Mali and Shah (2010) observed the effect of phosphorus and potassium in combination with VAM fungi on uptake of NPK in soybean. They found increase in uptake of nutrient content in mycorrhizal soil.

Choudhary *et al.* (2011) observed that application of 25 kg N + 50 kg P_2O_5 + 40 kg K_2O along with *Rhizobium* + VAM + PSB recorded significant higher available N 164.1 kg/ha and available P 26.6 kg/ha in groundnut.

Sarawgi *et al.* (2012) observed that application of 30 kg P_2O_5 ha^{-1} through rock phosphate + PSB + *Rhizobium* inoculation + VAM registered significantly higher seed yield, net returns and return / rupee invested in P compared to application of 60 kg P_2O_5 ha^{-1} through rock phosphate without biofertilizers. The P supplied through rock phosphate and inoculated with PSB, RI and VAM increased the N and P content in soil. PSB and VAM application over respective levels of P enhance the availability of different fraction of inorganic P in soybean crop.

Dania *et al.* (2013) resulted that VAM inoculation significantly improved their growth, shoot biomass, grain yield and nutrient uptake of maize and pigeon pea.

Chapter III

3. METHODOLOGY

The present study was carried out at the Experimental Farm of the Department of Agriculture, Lovely Professional University, Jalandhar, Punjab (India) during 2014-15 with the broad aim of assessing the influence of AM Fungi and *Rhizobium* Inoculation on quality and productivity of Field Pea (*Pisum sativum* L.). The experimental site is characterized as "Central Plain Zone (PB-3)" of Punjab. The rainfall in the region varies from 500-800 mm and about 80 per cent of which is received in a short period 3 months (mid June to mid September). Major constraints of the region are declining water table and soil sodicity and salinity. It comprises parts of eight districts of Punjab viz. Amritsar, Tarn taran, Kapurthala, Jalandhar, Ludhiana, Fatehgarh Sahib, Sangrur and Patiala. The soils predominantly belong to Central Alluvial Plain or sandy loam. The major crops grown in the region are mainly wheat, rice, maize, groundnut, cotton, gram, barley, pear and guava. The experimental site is located at 31º 15' N latitude and 750 41' E longitudes at an elevation of 245 m above mean sea level. The climate of the experimental area is characterized as hot and dry summer and wet and humid monsoons, distinctly experiences all the four seasons. The soil of experimental field was Sandy loam. The experimental soil was subjected to various estimations before the commencement of experiment. The figure 3.1 showed the general view of the experimental site along with field pea crop.

Figure 3.1 General view of the Experimental Field

Table 3.1 Initial Status of the Experimental Soil 0-15 cm

Sr. No.	Parameter	Value
1.	Textural class	Sandy Loam
	Mechanical separates (%)	
	Sand	30
	Silt	64
	Clay	6
2.	Chemical properties	
	Soil reaction	Alkaline (pH 7.2)
	Organic carbon (%)	0.561
	Available macronutrients (kg ha^{-}	
	N	150.528
	P	14.1
	K	133
3	Cation Exchange Capacity	0.347
4	Electrical Conductivity	0.614 dS/m

Table 3.2 Initial Status of the Experimental Soil 15-30 cm

Sr. No.	Parameter	Value
1.	Textural class	Sandy Loam
	Mechanical separates (%)	
	Sand	25
	Silt	50
	Clay	25
2.	Chemical properties	
	Soil reaction	Alkaline (pH 7.5)
	Organic carbon (%)	0.40
	Available macronutrients (kg ha^{-1})	
	N	188.16
	P	13.6
	K	131
3	Cation Exchange Capacity	0.478
4	Electrical Conductivity(EC)	0.916 ds/m

3.1 Experimental Details

A total of 7 treatments were evaluated in a Randomized Block Design (RBD) with three replications. The relevant information is given in Table 3.3.

3.1.1 Design and Layout

The experiment laid out in RBD (randomized complete block design) and consisted of seven treatments with three replications and each replication received seven treatments randomly. Thereby it was 21 total experimental plots and plot size was 2mx2.5m (Fig 3.2). The field preparation was done by applying the primary and secondary tillage, using mould board plough and harrow respectively which were mounted on a tractor. It was followed by planking of the field using. Once the field was leveled uniformly, the layout was carried out manually.

Table 3.3 Details of Treatments evaluated in Field Pea during Rabi* (2014-15)

T	Treatment detail	Treatment Code
T1	No NPK0% + No Bio-fertilizers: Uninoculated i.e. plot with no inoculated seeds (Control) and no chemical fertilizer use	Control: No NPK0% + No Bio-fertilizers
T2	NPK100% + No Bio-fertilizers: Application of Full recommended dose of NPK with no Bio-fertilizer	NPK100% + No Bio- fertilizers
T3	*Rhizobium* Inoculation + 50% kg N ha^{-1} + 100% kg P_2O_5 ha^{-1} + 100% kg K_2O ha^{-1}	*RHZ* + N50%+ P 100% + K100%
T4	*Rhizobium* Inoculation + 75% kg N ha^{-1} + 100% kg P_2O_5 ha^{-1} + 100% kg K_2O ha^{-1}	*RHZ* + N75%+ P 100% + K100%
T5	AMF Inoculation + 100% kg N ha-1 + 50% kg P_2O_5 ha^{-1} + 100% kg K_2O ha^{-1}	AMF+ N100%+ P50% + K100%
T6	AMF application + 100%kg N ha^{-1} + 75% kg P_2O_5 ha^{-1} + 100% kg K_2O ha^{-1}	AMF+ N100%+ P75% + K100%
T7	AM Fungi Application+ *Rhizobium* Inoculation + 50% kg N ha^{-1} + 50% kg P_2O_5 ha^{-1} + 100% kg K_2O ha^{-1}	*RHZ* +AMF+ N50%+ P50% + K100%

Note: Rabi season: The season that started from October/November and ended in March/April

AMF=Arbuscular Mycorrhizal Fungi, *RHZ= Rhizobium*

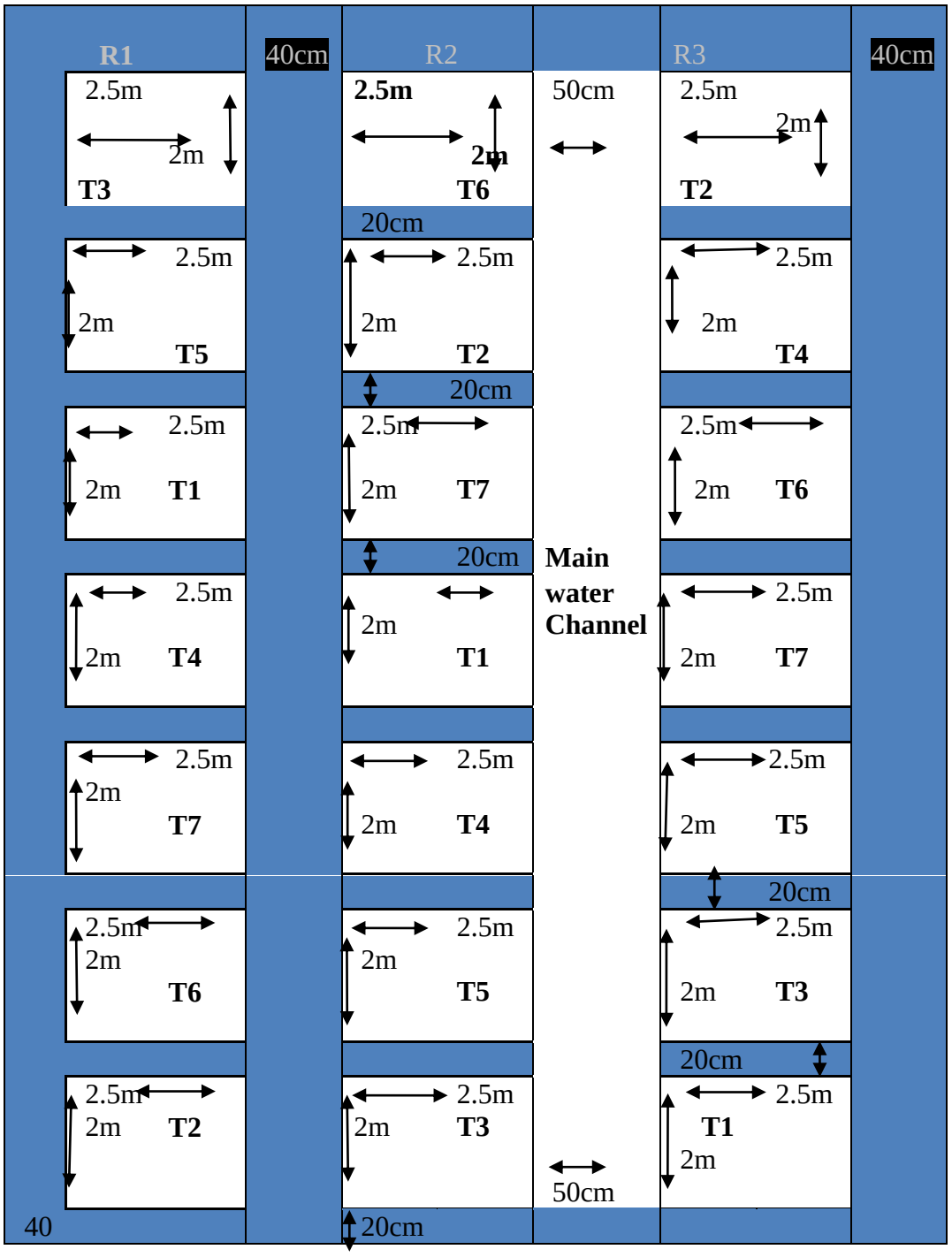


Fig: 3.2 Field Layout with Treatments Randomly in each Replication

3.2 Experimental Details

T: Treatment **R**: Replication

Treatments	7
Replications	3
Total number of plots	21
Design	RBD
plot size	$5m^2$
Variety	punjab-89

3.2 Variety and Bio-Fertilizer Description

3.2.1 Seed

Seed used in this research was obtained from Punjab Agriculture University, Punjab 89, early season variety developed through selection cross between Pusa2 x Morrasis 55. Developed by Punjab Agriculture University, Ludhiana .

3.2.2 Rhizobium

The *Rhizobium* culture, used in this research was *Rhizobium leguminosarum L.* as the culture suited to different pea legumes variety and is obtained from agriculture extension department of Punjab Agriculture University.

3.2.3 Arbuscular Mycorrhizal Fungi

The AM fungi used in this research was Arbuscular Mycorrhizal *Glomus musseae.* as the culture suited to different pea legumes variety and is obtained from agriculture extension department of Punjab Agriculture University.

3.3 Field Preparation and Subsequent Operations

The first plowing was done and this was followed by harrowing and leveling the soil to provide a good seedbed before sowing. All crop residues and weeds were removed as necessary to control weeds during the growing period. The urea, SSP and AMF were basally applied in plots according to the treatment assigned in each plot. Before sowing, one part of seeds of field pea were treated with *Rhizobium*

culture based on treatments definition and then left to dry under shade for about 30 minutes. In each plot 30 cm was maintained as planting distance between two successive rows. The other normal agricultural practices required including n, irrigation water canal cleaning were done. Details in below table (3.4)

Table 3.4 the Schedule of Main Agronomic Operations in Field Pea

S/N	Operation	Date
1	Ploughing and planking of field	22nd November, 2014
2	Before sowing irrigation	23rd November, 2014
3	Lay out of the field	24th November, 2014
4	Basal application of AMF	25th November, 2014
5	Seed Inoculation of *Rhizobium*	25th November, 2014
6	Sowing	25th November, 2014
7	First Weeding	9th January , 2015
8	Second Weeding	3rd February, 2015
9	Irrigation	10th February, 2015
10	Natural Irrigation (8 Rainfalls)	9/01, 3/02, 15/02, 1/03, 2/03, 08/03/, 15-16/03/2015
11	Harvesting	29th March, 2015

3.4 Soil Studies

Table 3.5 Analytical methods employed for soil Analysis

Sr./ No.	Parameter	Method employed
1	**Textural class**	International pipette method (Piper, 1950)
	Mechanical separates	
2	**Cation Exchange Capacity**	Acetate Method (Chapman, 1965)
3	**Chemical Properties**	
	Soil reaction	1: 2.5 soil: Water Suspension (pH-Meter by Jackson 1967)
	Organic carbon	Rapid Titration Method (Walkley and Black 1934)
4	**Available nutrients**	
	N	Alkaline Permanganate Method (Subbiah and Asija, 1956)
	P	0.5 M $NaHCO_3$, pH=8.5 (Olsen, 1954)
	K	1N Neutral Ammonium Acetate (Black 1965)
5	**Electrical Conductivity**	Water Suspension (EC Meter) (Hanlon et al., 1993)

3.4.1. Procedures of Soil Analysis

3.4.1.1. Triangle Method for soil textural class

Soil textural class was determined by using U.S. texture triangle method (Soil Survey Staff, 1998).

3.4.1.1.1. Particles Distribution (%): International pipette method (Piper, 1950)

For determination of soil texture, 50g of soil was dried in air and sieved through 2 mm sieve and put in 500 ml bottle of water. 100 ml was added to above dispersion solution in 50g soil in 500 ml plastic bottle. A set of sample bottles were shaken at regular intervals for half an hour on shaking machine for preparing homogeneous solution. The above sample solution was transferred to 1000 ml glass measuring cylinder and solution of 1000 ml was made by dilution with water. As per International approved system, the sample solution was shaken for 30 sec. Depending on the solution temperature and sedimentation chart, first pipetting was done with 50 ml pipette at 10 cm depth. In first pipetting, 50 ml solution sucken and transferred in 60 ml china dish. This sample solution contains a mixture of clay and silt particles. Depending on the solution temperature and sedimentation chart, second pipetting was done with 50 ml pipette at 10 cm depth.

In second pipetting 50 ml solution shaken and transferred in 60 ml china dish. This solution contained clay particles in soil sample. Transfer remaining soil solution in 1 lit. Measuring cylinders by using 0.02 mm sieved and washed the material through the sieve using jet of water. Sand particles on sieve were collected in china dish. Pipetted solution was transferred in 3 dishes and kept overnight in an oven at temperature of 105^{o}C. Dishes were cooled in desiccators and weight was taken quickly. The weight of fine was determined by deducting the weight of clay, silt and coarse sand particle from 100.

3.4.1.2. Soil Reaction or Determination of soil pH (Water suspension by Jackson, 1967)

12.5 g of dried soil were weighed and placed in a 150 ml beaker; 25 ml of distilled water was added and stirred at four times with a period of half an hour. This time was required for the soil and water to attain equilibrium. After half an

hour again the soil suspension was stirred and pH was measured with the help of pH-meter.

3.4.1.3. Electrical Conductivity (Water suspension by Hanlon *et al*., 1993)

To find out the electrical conductivity of soil, 25 g of dried soil were taken and put in a 100 ml beaker and add 50 ml of distilled water. It was stirred intermittently for 4-5 times and then left it overnight for getting a clear supernatant solution. EC measurement of this supernatant was recorded with the help of EC-Meter.

3.4.1.4. Organic Carbon (Rapid titration by Walkley and Black 1934)

To determine organic carbon of soil, 2 g of dried soil were weighed and put in a 250 ml conical flask. To the quantity, 10 ml of 1 N K_2CrO_7 solution was added and mixed accordingly. Then 20 ml of concentrated H_2SO_4 was added, swirling the flask during addition. The flask was left to cool the content and to make the reaction complete. 2 g of NaF powder or 10 ml of orthophosphoric acid was added (both NaF and orthophosphoric acid are the flocculating reagents) and 100 ml of distilled water was put and shaken vigorously afterwards.10 drops of diphenylamine indicator was added, which gave a violate color to the suspension.

The end point in this titration was the change of the color from violet to bright green. The volume of the ferrous ammonium sulphate solution used was recorded and results were calculated.

3.4.1.5. Cations Exchange Capacity (Acetate method by Chapman, 1965)

Quantity of 4 g of dried soil for medium or fine textured soil or 6 g of coarse textured soil were transferred into a 50 ml of centrifuge tube. 33 ml of 1 N CH_3COONa solution of a pH value of 8.2 was added to the soil. Tubes were shook for 5 minutes in a reciprocating shaker. The tube was centrifuged for 10 minutes at about 2000 rpm. The clear supernatant liquid was decanted as completely as possible and discarded. Sample was treated in the same manner with 33 ml portions of sodium acetate solution a total of four times, the supernatant was

discarded each time. The sample was suspended with 33 ml of 95% ethanol, shaken for 5 minutes in a reciprocating shaker, centrifuge until the supernatant was clear, decanted and discard the supernatant. The sample was washed with 33 ml portions of ethanol a total of three times. The EC of supernatant liquid from third washing should be less than 40 micro-mhos/cm. The adsorbed Na was replaced from the sample by shaking with three 33 ml portions of neutral normal ammonium acetate for 5 minutes in reciprocating shaker, and it was centrifuged until the supernatant was clear. The third ammonium acetate extracts was collected in a 100 ml volumetric flask, made to volume, mixed and the sodium concentration in the extract on a flame photometer was determined. A series of standard of Na was prepared, setting 10 ppm Na to 100 reading of the galvanometer. The Na concentration of the sample was read, after necessary dilution, from the standard curve.

3.4.1.6. Available Nitrogen (Alkaline Permanganate Method by Subbiah and Asija, 1956)

To determine the available nitrogen in the soil, 5 g of dried soil were taken and transferred into the distillation flask of micro-Kjeldhal distillation assembly. 52 ml of 0.32% $KMnO_4$ solution was added into the distillation unit. In 150 ml conical flask, 10 ml of N/50 H_2SO_4 was pipitted out and mixed with two drops of meyhyl-red indicator. This conical flask and the delivery tube of the distillation unit were placed in such a way that the delivery tube is well placed in the content of the conical flask. 25 ml of 2.5% NaOH solution was added into the distillation flask containing soil and $KMnO_4$ through the set provided in the distillation tube and immediately that inlet was closed with the help of the stop-cock.

The distillation was started and subsequently about 30 ml the distillate was collected. The content of the conical flask with N/50 NaOH used was titrated for determining the end point (pink to yellow).

3.4.1.7. Available Phosphorus (0.5 M $NaHCO_3$, pH=8.5 by Olsen et al. 1954)

1 g of soil was weighed and added in 150 ml conical flask. A pinch a Darco-G 60 and 20 ml of 0.5 $NaHCO_3$ was added to the solution. The flask was shaken for half an hour on an electrical shaker and then filtration of the suspension through Whatman No.1 filter paper was done. Similarly, a blank solution was prepared. 5 ml of the extract in a 25 ml volumetric flask was pippited out and 0.5 ml 5N H_2SO_4 was added and shaken for a while till CO_2 evolution disappeared. Subsequently, 4 ml of ascorbic acid solution (solution B) was added to it and the volume with distilled water and mix the content of the flask was made. The intensity of the blue color developed within a calorimeter at a wavelength of 760 mu was measured, using a red filter. The reading given by the calorimeter in the standard curve was located and the results were calculated.

3.4.1.8. Available Potassium (1 N Neutral ammonium acetate by Black, 1965)

5g of soil was weighed and added in 150 ml conical flask. 52 ml of neutral ammonium acetate solution was added and shaken for five minutes. Filtration of the suspension in Whatman No.1 filter paper was done. 5 ml of this extract was pipetted out into 25 ml volumetric flask and its volume with distilled water was completed. This solution was fed to the atomizer of the flame photometer (in which 100 of Galvanometer has been set by feeding 10 ppm solution of K to the atomizer) and the reading was recorded and indicated by the galvanometer needle. Then with help of the standard curve (which can be prepared as given) the amount of available potassium was calculated in the soil under test.

3.5 Growth Parameters

3.5.1 Plant Height (cm)

Five plants were randomly selected from each plot, tagged permanently and used for measurement of plant height. Height of each tagged plant was measured at harvest from ground to the tip of the plant by meter scale and average of five plants was computed as mean plant height.

3.5.2 Dry Matter Accumulation (g)

The dry matter accumulation was recorded at 90 day of growth. The randomly selected plants were removed from each plot. Above plant samples were dried in an oven at 60^{o}C for 72 hours and their weights were recorded.

3.6 Yield Parameter

3.6.1 Straw Yield (kg)

The dry seed pods are harvested from each treatment and the left over straw are weighed out and expressed as straw mean yield per treatment.

3.6.2 Grain Yield (kg)

The dry seed pods harvested from each treatment were weighed out and expressed as seed mean yield per treatment.

3.6.3 Number of Pods per Plant

The randomly selected plants were used for counting number of pods per plant and their average worked out.

3.6.4 Pod Length (cm)

The average of five randomly selected pods was calculated as a pod length from each plot.

3.6.5 Number of Seeds per Pod

At the time of picking, five pods were randomly selected from each plot and total seeds were counted to record the average number of seeds per pod.

3.7 Nutrient Content and Quality Parameters

3.7.1 Crude Protein Content

Above parameter was estimated in chickpea seeds through the estimation of total nitrogen (Jackson, 1973), in various samples. The value thus, obtained was multiplied by factor 6.25 to obtain crude protein content to obtain crude protein content.

3.7.2 Plant Analysis

Plant samples (leaves and pods) collected at final picking from all the field plots, were air dried and then, dried in an oven at 60^{o}C for 72 hours. The dried samples were now ground in a Willey Mill fitted with stainless steel parts, and passed through 1 mm sieve and stored in paper bags for analysis. The analytical procedures employed for the estimation of N, P and K is given in Table 3.6

Table 3.6 Analytical Methods Employed for Plant Analysis

Sr. No.	Parameter	Method employed	Reference
1.	Nitrogen	Micro-kjeldahl method	Jackson (1973)
2.	Phosphorus	Vanado-molybdo-phosphoric acid yellow colour method	Jackson (1973)
3.	Potassium	Wet Digestion method	Black (1965)

3.8 Statistical Analysis

All data were statistically analyzed using SPSS 17.0 software. Significance difference of data at $p<0.05$ was put to comparison of treatment means by DMRT (Duncan's Multiple Range Test) for separation of mean. Microsoft excel 2010 was used to present data graphically.

Chapter IV

4. RESULTS AND DISCUSSION

The experimental results pertaining to the current study entitled "**Influence of AM Fungi and *Rhizobium* Inoculation on Quality and Productivity in Field Pea***"* have been presented in this chapter under following headings:

4.1 Effect of different Treatments on Growth Parameters

4.2 Effect of different Treatments on Yield Parameters

4.3 Effect of different Treatments on Quality Parameters

4.1 Effect of Different Treatments on Growth Parameters

4.1.1 Plant Height

The data presented in table 4.1 revealed that at 30 DAS, highest magnitude of increase in plant height was registered under treatment T5 "AMF+ N100%+ P50% +K100%" followed by T7 "RHZ +AMF+ N50%+ P50% + K100%".However, a significant average height is recorded between 17 and 18 cm. hence there is less difference in height at early stage of growth and all the treatment shows less difference. The lowest plant height was observed under absolute control treatment T1.

At 60 DAS, highest plant height was recorded under T5"AMF+ N100%+ P50% +K100%" followed by T7"RHZ +AMF+ N50%+ P50% + K100%" and T4"RHZ + N75%+ P 100% + K100%"with the value of 30.5 cm,28.03cm and 26.2 cm. Above treatments gave statistically similar plant height (table 4.1). However, treatment T5"AMF+ N100%+ P50% +K100%"and T7"RHZ +AMF+ N50%+ P50% + K100%"are statistic significant as compare to other treatments. The lowest plant height was observed under absolute control treatment T1.

At 90 DAS, highest plant height was noted under T5 "AMF+ N100%+ P50% +K100%" followed by T2 "NPK100% + No Bio fertilizers" and T7"RHZ +AMF+ N50%+ P50% + K100%" (Fig 4.1). Above treatment gave statistically similar plant height and significant as compare to other treatment. The minimum plant

height was noted under absolute control 24.3 cm. Similar results has been reported by Pramanik and Bera (2012) observed that the growth parameters like plant height significantly improved by inoculation with biofertilizers (*Rhizobium*, PSB and VAM) in chickpea (*Cicer arietinum* L.).Where as the AM fungi associated with legumes are responsible for adequate P nutrition. Increased P assimilation influence positively nitrogenase activity that in turn promotes root and plant growth. The conducive effect of dual inoculation of roots with AM fungi and *Rhizobium* on growth, nutrient uptake and N2 fixation in soybean (Bethlenfalvay et al.,1990.

Table 4.1 Effect of Treatments on Different Plant Growth Parameters

Treatments	Plant height 30DAS (cm)	Plant height 60DAS (cm)	Plant height 90DAS (cm)	Dry matter per plant(g)
T 1. Control(NPK)0%+No Biofertilizers	17.6±0.11	24.33±1.59	24.33±1.76	13.17a±0.36
T2. NPK100% + No Bio fertilizers	17.46±0.29	25.7±0.57	43.2±0.83	14.07a±1.48
T 3. RHZ + N50%+ P 100% +K100%	17.53±1.47	25.43±0.46	37.33±1.04	13.28a±0.35
T4.RHZ + N75%+ P 100% + K100%	17.66±0.86	26.9±0.75	41.6±0.72	14.52a±1.18
T5. AMF+ N100%+ P50% + K100%	18.66±0.70	30.56±0.56	44.66±1.22	14.82a±0.82
T6. AMF+ N100%+ P75% + K100%	17.33±0.06	24.96±0.88	40.4±2.10	14.74a±1.16
T7.RHZ +AMF+ N50%+ P50% +K100%	18.06±0.48	28.33±1.53	42.53±1.18	14.51a±0.88

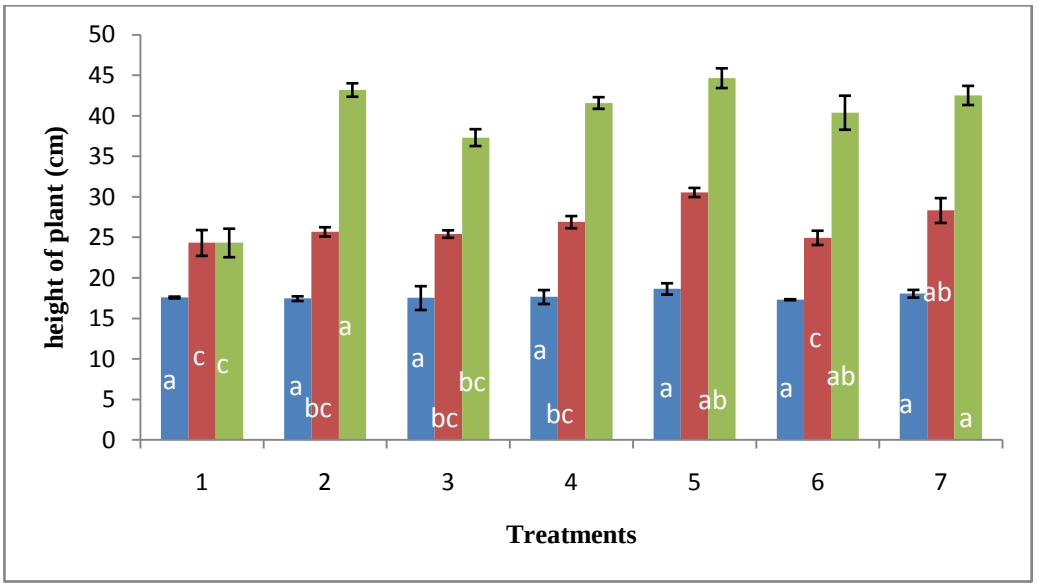


Fig 4.1 Effect of Treatments on plant height at 30, 60, 90 (DAS)

4.1.2 Dry Matter Accumulation per Plant (g)

It is apparent from Fig. 4.2 that highest and similar magnitude of increase in dry matter accumulation was recorded under T5“AMF+ N100%+ P50% +K100%” and followed by T4“RHZ + N75%+ P 100% + K100%”. All the treatments observed statistically no difference .hence treatments are non-significant at par with one another. However, a significant increase of 11.14% and 9.3% above parameter was found under T5“AMF+ N100%+ P50% +K100%” and T4“RHZ + N75%+ P 100% + K100%” in comparison with Control T1 “NPK 0% + No Bio fertilizers”. The lowest dry matter accumulation was found under absolute control T1 “NPK 0% + No Bio fertilizers”. Similar results had been reported by Stancheva *et al.* (2006) studied the effects of combined inoculation of pea plants with VAM and *Rhizobium* on nodule formation and nitrogen fixation activity. The results showed that dual inoculation of pea plants increased plant biomass, nodules parameters, N_2 -fixation activity.

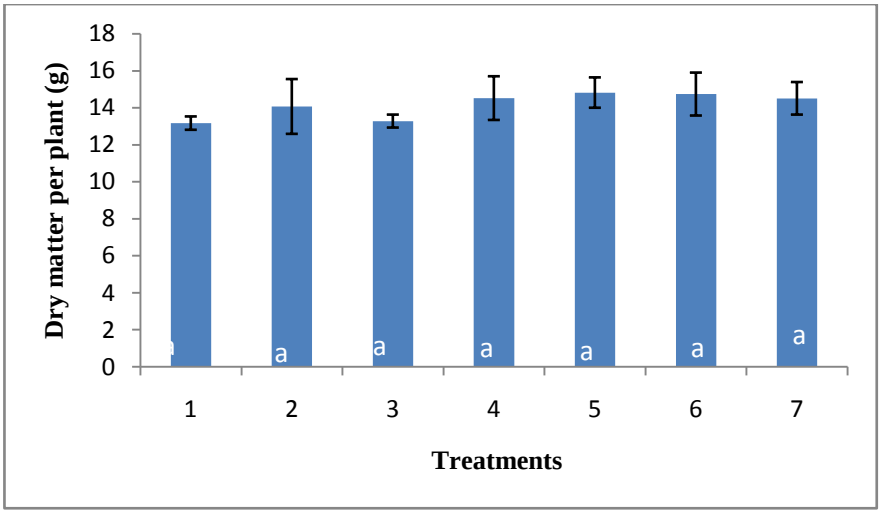


Fig 4.2 Effect of Treatments on Dry Matter Per Plant

Table 4.2 Effect of Treatments on different yield parameters of field pea crop

Treatments	Straw Yield(t/ha)	Grain Yield(q/ha)	No of pod	Pod length(cm)	Seed per pod
T 1. Control(NPK)0%+No Biofertilizers	1.98a±0.08	4.75c±0.02	6.22d±0.11	6.22c±0.11	5.22d±0.22
T2. NPK100% + No Bio fertilizers	3.62a±0.27	5.64bc±0.03	6.89cd±0.11	6.77c±0.22	5.89cd±0.11
T3. RHZ + N50%+ P 100% + K100%	2.47a±0.09	5.78bc±0.04	6.78cd±0.11	7.44b±0.29	5.66cd±0.33
T4.RHZ + N75%+ P 100% + K100%	3.13a±0.34	6.84b±0.02	7.66bc±0.19	7.89b±0.22	7.0b±0.00
T 5. AMF+ N100%+ P50% + K100%	3.06a±0.20	7.58ab±0.01	7.22bc±0.58	7.78b±0.11	6.0c±0.19
T6. AMF+ N100%+ P75% + K100%	2.80a±0.29	6.52bc±0.04	8b±0.38	6.22c±0.29	5.77cd±0.29
T7.RHZ +AMF+ N50%+ P50% + K100%	3.57a±0.27	8.84a±0.01	9.88a±0.29	8.66a±0.19	7.67a±0.0

4.2 Effect of Different Treatments on Yield Parameters

4.2.1 Straw Yield

The data presented in table 4.2 revealed that highest straw yield was registered under T2 “NPK100% + No Bio fertilizers” followed byT7“RHZ +AMF+ N50%+

P50% + K100%" ,T4 "RHZ + N75%+ P 100% + K100%"and T5"AMF+ N100%+ P50% + K100%", all of which were found statistically non significant to one another. The minimum straw yield was gained under absolute control T1 "NPK 0% + No Bio fertilizers". Our results are supported by findings of Singh *et al*. (2009) observed the increase in grain yield and straw yield of green gram with inoculation of *Rhizobium,* VAM and N levels over the control under temperate conditions.

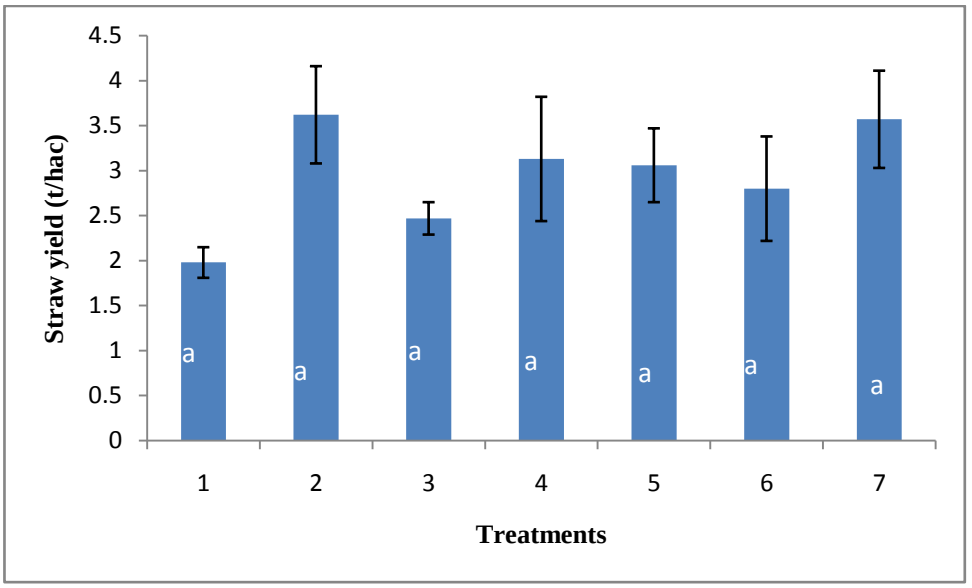


Fig 4.3 Effect of Treatments on Straw Yield of Field Pea crop

4.2.2 Grain Yield

The highest magnitude of increase in field pea grain yield was registered under treatment T7 "RHZ +AMF+ N50%+ P50% + K100% "followed by T5 "AMF+ N100%+ P50% + K100%" and T4 "RHZ + N75%+ P 100% + K100%", all of which were observed statistically significant at par with one another (Table 4.2). Moreover, all above treatments were found statistically at par in table. Likewise, other parameter, lowest seed yield was registered under absolute control T1. However, treatment T7 "RHZ +AMF+ N50%+ P50% + K100%" involving AM fungi and *Rhizobium* inoculation gave higher yield then all the other treatment as shown in (fig 4.4).The treatment T7 "RHZ +AMF+ N50%+ P50% + K100%" gave

yield of 8.84 q from a hectare .which mean 3.53 q from a acre.. Increased yield of fieldpea under *Rhizobium* and AM fungi is owing to improvement of yield components such as in term of quality and productivity. Moreover, *Rhizobium* has ability to fix atmospheric N and make it available to plants, where as AM fungi has ability to provide all insoluble P in avaible form to the plants, which in turn enhance soil fertility. The present results are in conformity with the findings of Moradi *et al*. (2010) and Darzi *et al*. (2012).

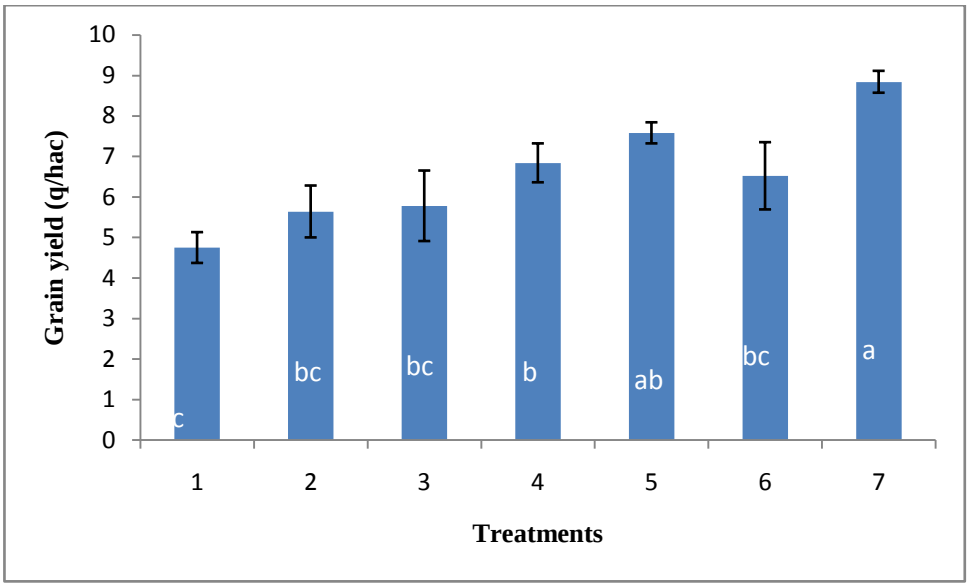


Fig 4.4 Effect of Treatments on Grain yield of Field pea crop

4.2.3 Number of Pods per Plant

The highest number of pod per plant was noted under T7"RHZ +AMF+ N50%+ P50% + K100%" followed by T6 "AMF+ N100%+ P75% + K100%" and T4 "RHZ +AMF+ N50%+ P50% + K100%" (Fig 4.5). The above treatments were statistically significant to each other from the rest of the treatments and the number of pod /plant of above treatments are recorded as 9.88, 8 and 7.66. The minimum number of pod/plant was noted under absolute control 6.22. Our results are supported by findings of Khan et al. (2005) who reported the effect of seed inoculation with *Rhizobium* along with various levels of phosphorus on chickpea (cv. Karak-1) growth produced significant increase in pods per plant.

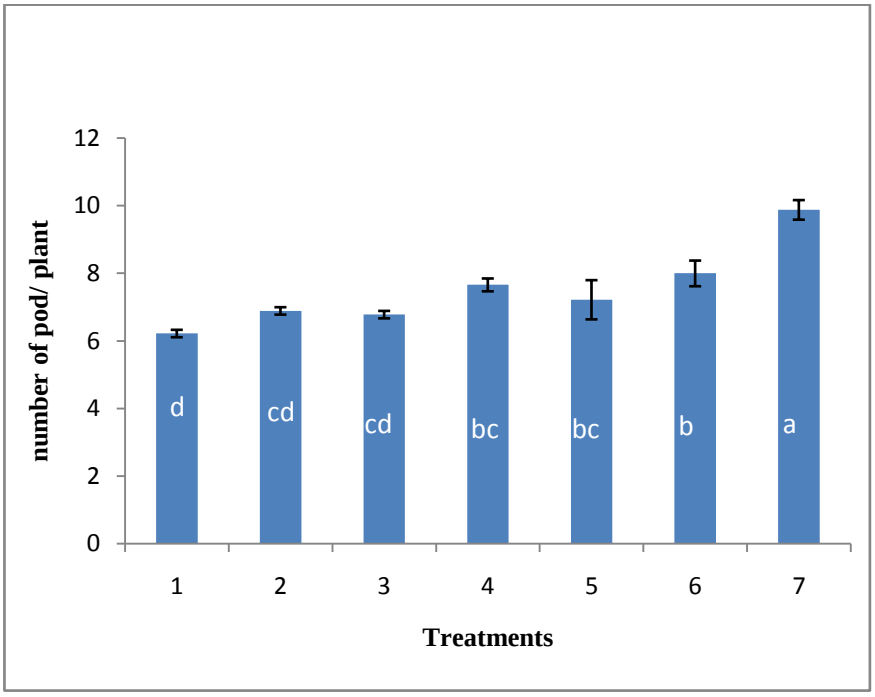


Fig 4.5 Effect of Treatments on Number of Pod per Plant

4.2.4 Pod Length (cm)

In general, treatment T7 "RHZ +AMF+ N50%+ P50% + K100% involving AM fungi and *Rhizobium* inoculation gave 8.66 cm as a significant larger pod length in field pea crop comparison with recommended dose of fertilizer i.e. T2 "NPK100% + No Bio fertilizers" as 6.77cm and 6.22 cm in absolute control treatment T1 "NPK 0% + No Bio fertilizers"(Table 4.2).where as significant increase in pod lenght in above parameter was observed under treatment T4"RHZ + N75%+ P 100% + K100%" and T5"AMF+ N100%+ P50% + K100%" as 7.89cm and 7.78cm. Similar results of significant differences for pod length have been reported by Jagvir et al. (2004) mentioning dual inoculation of *Rhizobium* and VAM result in increase in grain yield, over no inoculation for the yield of green gram, viz., pods per plant, pod length, seeds per pod, 1000-seed weight and seed yield per plant.

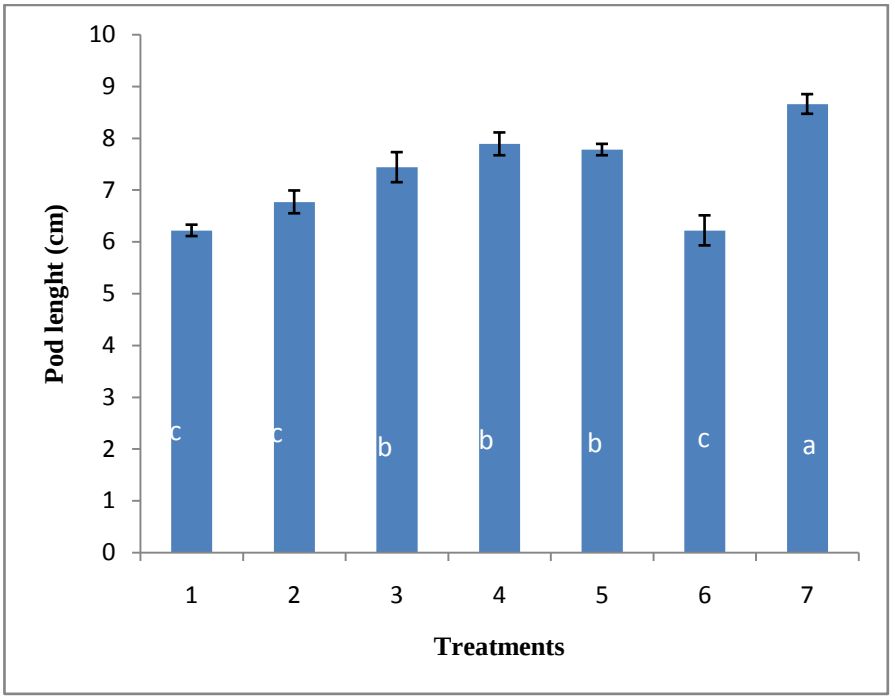


Fig 4.6 Effect of Treatments on Pod Lenght of Field Pea

4.2.5 Number of Seeds per Pod

The data presented in Fig 4.7 revealed the number of seeds per pod. The magnitude of increase higher number of seed per pod was registered under treatment T7 "AMF+ N100%+ P50% +K100%" followed by T4 "RHZ +AMF+ N50%+ P50% + K100%" as 7.67 and 7(no of seed/pod). However, above treatments gave significant increases of 23.21% and 15.86%, respectively over RDF. The lowest nodules were registered under absolute control T1 "NPK 0% + No Bio fertilizers". Our results are also supported by Meghvansi and Mahna (2009), who found that dual inoculation of *Rhzobium* + VAM was superior over single inoculation.

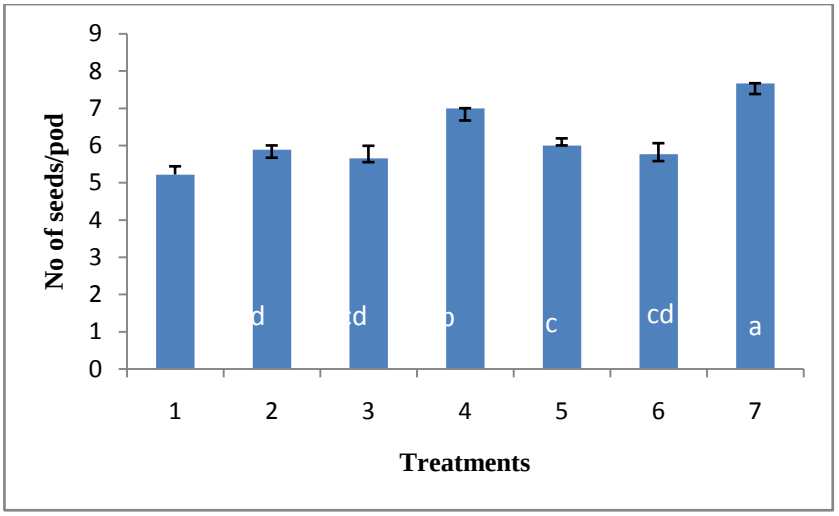


Fig 4.7 Effect of Treatments on Number of seed per pod

Table 4.3 Effect of different treatments on quality parameters in field pea seed

Treatments	N (%)	P (%)	K (%)	Crude Protein (%)
T1. Control(NPK)0%+No Biofertilizers	2.04d±0.07	0.22d±0.01	1.11b±0.05	12.33d±0.66
T2. NPK100% + No Bio fertilizers	3.19c±0.04	0.316c±0.003	1.77a±0.008	20c±0.0
T3. RHZ + N50%+ P 100% + K100%	3.56ab±0.18	0.33b±0.003	1.82a±0.005	22.66bc±0.33
T4. RHZ + N75%+ P 100% + K100%	3.60a±0.02	0.33ab±0.003	1.82a±0.003	23.33ab±0.33
T5. AMF+ N100%+ P50% + K100%	3.51ab±0.02	0.33ab±0.003	1.82a±0.0	22c±0.0
T6. AMF+ N100%+ P75% + K100%	3.46b±0.03	0.35a±0.003	1.81a±0.006	21.66c±0.33
T7.RHZ +AMF+ N50%+ P50% + K100%	3.62ab±0.02	0.34ab±0.003	1.79a±0.03	24a±0.57

4.3 Effect of Different Treatments on Quality Parameters.

4.3.1 Crude Protein Content

In general, treatment T7 “RHZ +AMF+ N50%+ P50% + K100% involving AM fungi and *Rhizobium* inoculation gave a significant higher protein content in

field pea seed comparison with recommended dose of fertilizer i.e. T2 “NPK100% + No Bio fertilizers “ and absolute control (Table 4.3).A significant respective increases (from RDF) of 13.95% and 4.77% in above parameter were observed under treatment T4“RHZ + N75%+ P 100% + K100%” and T5“AMF+ N100%+ P50% + K100%”(Fig 4.8) .Thus the crude protein content depends upon the plant nitrogen concentration. Hence AM and *Rhizobium* inoculation improved nitrogen concentration thereby enhancing the protein content of field pea pods. Above results are in conformity with the findings of Bagyaraj et al. (1979). Rao et al. (1986) also suggested that seed inoculation with AM and *Rhizobium* enhanced protein content of black gram and green gram. Sajitha *et al.* (2007) also reported that inoculation *Rhizobium* and VAM registered higher protein content.

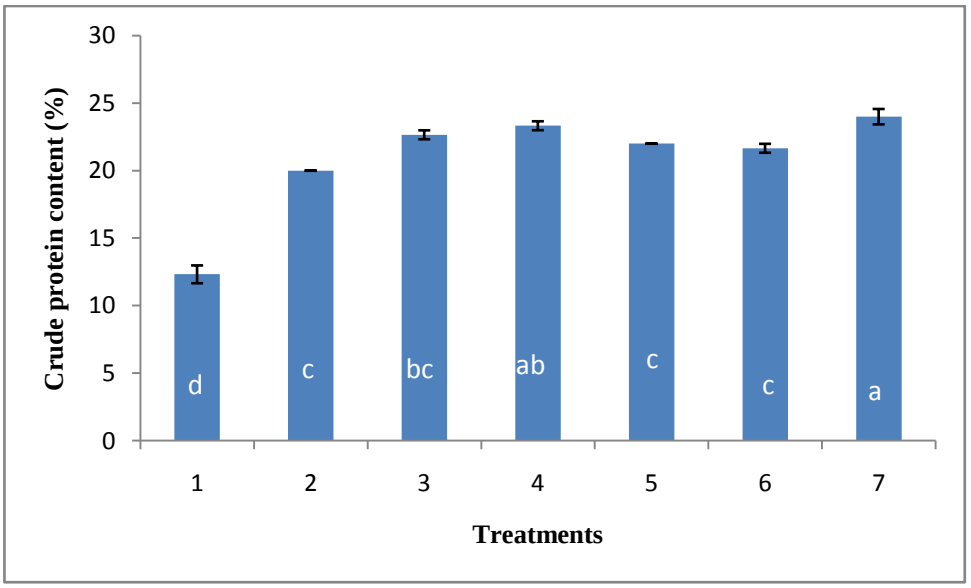


Fig 4.8 Effect of Treatments on Crude Protein content in seed

4.3.2 Seed Analysis

4.3.2.1 Nitrogen Concentration

In general, treatments involving AM fungi and *Rhizobium* inoculation gave significant higher N concentration in field pea seed comparison with recommended dose of fertilizer i.e. RDF (100% NPK) and absolute control (Table 4.3). A significant respective increases of 15.16% and 11.88% in above parameter were

observed under treatment T4 “RHZ + N75%+ P 100% + K100%” and T7 “RHZ +AMF+ N50%+ P50% + K100%” in comparison with RDF (T2). Similarly treatments T3 “RHZ + N50%+ P 100% + K100%”and T5 “AMF+ N100%+ P50% + K100%” gave non-significant increases of 10.04% and 9.12%, respectively in N concentration over RDF.The higher N concentration in inoculated treatments might be due to higher nitrogen’s enzyme activity and enhanced N_2 fixation (Islam, 1990). Our findings are in agreement with the observation of Tarafdar and Rao (2001) found that the nitrogen’s activity in *Rhizobium* inoculation involving treatment was 71 per cent. Hence treatment T4 “RHZ + N75%+ P 100% + K100%”gave higher nitrogen content in seed.

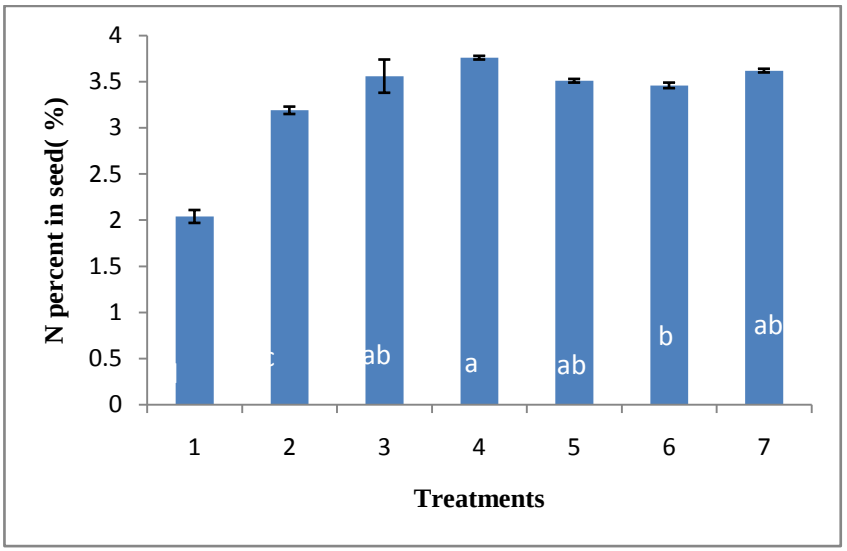


Fig 4.9 Effect of Treatments Nitrogen Concentration in Seed

4.3.2.2 Phosphorus Concentration

None of the treatment influenced P concentration in field pea seed except absolute control, which was found significant inferior to all other treatments (Table 4.3). However, treatments T6 “AMF+ N100%+ P75% + K100%” showed higher P concentration due AM fungi and 75% of P .which increased phosphorus in seed content and followed by T7 “RHZ +AMF+ N50%+ P50% + K100%”(Fig 4.10) involving AM fungi and *Rhizobium* inoculation gave nominally 5higher value then other treatment (but non-significant) indicating improvement in quality of field pea in long term or following its continuous use. our result is supported by Stancheva

et al. (2006) reported that co-inoculation of pea plant with VAM and *Rhizobium leguminoserum* cv. Viciae strain D 293 increased significantly total P content in plant tissue and percentage of root colonization.

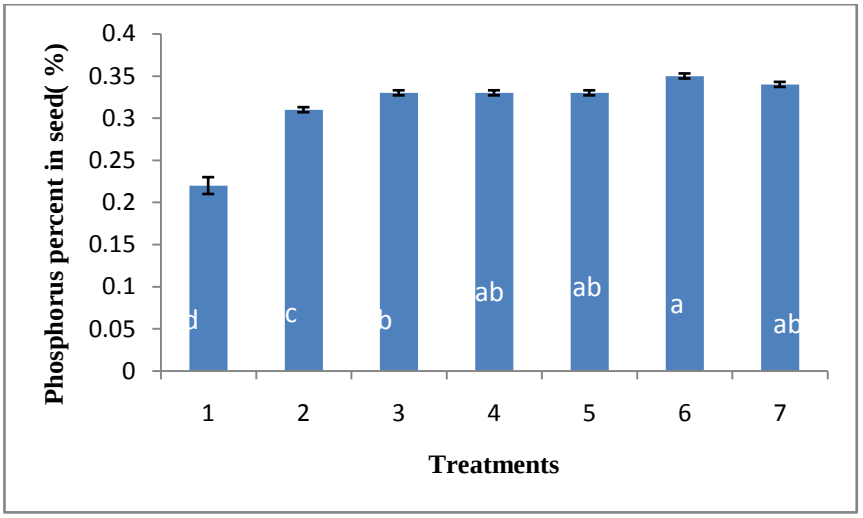


Fig 4.10 Effect of Treatments on Phosphorus Concentration in Seed

4.3.2.3 Potassium Concentration

The different treatments did not influenced K concentration significantly barring absolute control, which gave lowest value of K in field pea seed due to obvious reason (Fig4.11). However, treatments involving AM fungi and *Rhizobium* inoculation gave nominally higher (but non-significant) magnitude of K concentration in comparison with other treatments, indicating improvement in quality of field pea in long term or following its continuous use. Achieved results are in harmony with the investigations results obtained by many authors (Martin and Jamieson 1996, Douds and Nagahashi 2000) who reported that plants inoculated with mycorrhizal fungi, thus, obtaining higher green mass and dry matter yield as well as higher potash, phosphorus and nitrogen content in plant mass and grain.

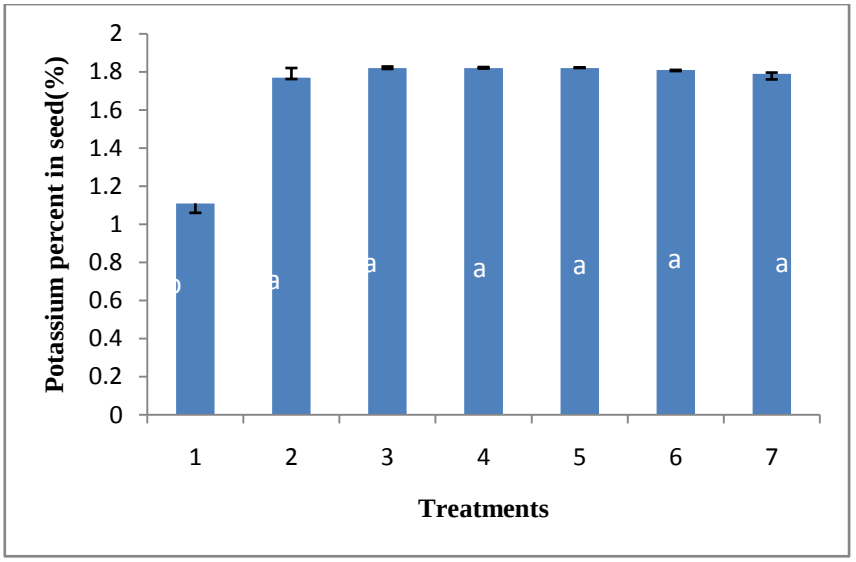


Fig 4.11 Effect of Treatments on Potassium concentration in seed

Chapter V

5. CONCLUSION

On the basis of one year experiment results, it is concluded that the combined application of AM fungi and *Rhizobium* inoculation in Field pea crop was found most suitable in terms of Quality and yield (8.84q/hec). Thus the *Rhizobium* + AM fungi as seed inoculation to field pea crop has proved to be most superior treatment combination in terms of quality and yield parameters as protein content, number of pods per plant, seeds per pod , pod length and finally grain yield (kg/plot and q ha^{-1}).Thus, the application of *Rhizobium* + AM fungi as a seed inoculation along with application of 50 % RD of Nitrogen, 50 % RD of phosphorus, 100 % RD of potash to Field Pea crop is recommended. The results are only indicative and require further experimentation to arrive at more consistent and final conclusion.

BIBLIOGRAPHY

Abdul Mallik,M (2000). Association of Arbuscular Mycorrhizae with some varieties of Tobacco(Nicotiana tobacum *L*.) and its effect on their growth, nutrition and certain soil borne diseases, Ph.D. Thesis, Bharathidasan University, Tiruchirapalli, S. India, pp 104.

Adholeya A, Johri RN (2003). Effect of Glomus coledonium and Rhizobium inoculation on greengram (*Vigna radiata* L. Wilczek). In: Singh R, Nainawatee HS, Sawney SK (eds) Proceedingsof national symposium on current status of biological Nitrogen fixation research, HaryanaAgricultural University, Hisar, India, pp 173.

Ahmad F, Ahmad I, Khan MS (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* 163: pp 173–18.

Bahadur, A singh, J Singh,K.P and Rai, M. 2006. Plant growth, yield and quality attributes of garden pea as influenced by organic amendments and Biofertilizers . *Indian Journal of Horticulture,* 63 (4): 464-466.

Bhardwaj, S.K .; Kaushal, R.; Sharma, Y. and Chaudhary, V. 2010 . Effect of conjoint use of inorganic fertilizers and organics on soil fertility and growth parameters of French bean and tomato crops in mid hills of Himachal Pradesh . *Progressive Horticulture,* 42 (1)

Bethlenfalvay, G.J, M.S. Brown, R.L Franson, 1990. *Glycin–Glomus Bradyrhizobium* symbiosis, Plant Physiol., 94, 723-728

Biswas, A. and Patra, A.P. 2007. Study on the effect of phosphorus, Vesicular Arbuscular Mychorryzae (VAM) and Phosphate Solubilizing Bacteria (PSB) on the performance of summer green gram. (In) *National Symposium on Legumes for Ecological Sustainability: Emerging Challenges and Opportunities* held during 3-5 Nov. 2007 at Indian Institute of Pulses Research, Kanpur.

Choudhary, S.K.; Jat, M.K.; Sharma, S.R. and Singh, P. 2011. Effect of INM on soil nutrient and yield in groundnut field of semi-arid area of Rajasthan . *Legume Research*, 34 (4) : 283-287.

Chapman HD (1965). Cation-exchange capacity. In: Black CA (ed.). Methods of soil analysis - Chemical and microbiological properties. Agronomy 9: pp 891-901.

Dadhich, L.K Gupta, A.K and Sharma , H.S. 2001. Yield and quality of clusterbean as influenced by molybdenum and phosphorus.*Advances in Plant Sciences*, 14 (1): 205-208.

Douds D.D., Nagahashi G. (2000): Signaling and recognition events prior to colonization of roots by arbuscular mycorrhizal fungi. In: Podila G.K., Douds D.D. (eds.): Current advances in Mycorrhizae research. St. Paul,

Dadhich, S.K.; Somani , L.L. and Verma, A. 2006. Improved soybean yield, nutrient uptake and P enrichment in soil due to co-inoculation of phosphate solubilizing bacteria and VAM fungi in a clay loam soil . *Indian Journal of Microbiology*, 46: 4.

Dania, S.O; Fagbola O and Dania, M.I. 2013. Response of maize-pigeonpea intercrop to arbuscular mycorrhizal fungi in nutrient depleted soil. *Annual Review and Research in Biology*, 3(3): 232-245.

Djebali, N.; Turki, S.; Zib, M and Hajlaoui, M.R. 2010. Growth and development responses of some legume species inoculated with a mycorrhiza based biofertilizer. *Agriculture and Biology Journal of North America*, 1(5): 748-754.

El-Shaikh, K.A.A; El-Dakkak, A.A.A. and Obiadalla-Ali, H.A. 2010 . Maximizing productivity of some garden pea cultivars and minimizing chemical phosphorus fertilizer via VAM mycorrhizal inoculants. *Journal of Horticultural Science and Ornamental Plants*, 2 (3) : 114-122.

Jagvir, S., M. S. Deshmukh and N. R. Tandulkar. 2004. Direct and Residual effect of sulphur in cotton-wheat cropping system in sandy soil. Fertilizer News 49: 61-63.

Jackson M.L. (1973). Soil Chemical Analysis.Prentice Hall.Inc. Englewood Chiffs.New Jersey, USA. *Journal of Ecology* 63: pp 995-1001.

Khan, M. S. S., A. Latif, M. Rafique, T. Sajjad and L. Perveen. 2005. Effect of seed inoculation and phosphorus fertilizer on yield of chickpea in semi-arid areas of NWFP. Indus J. Plant Sci. 4(4): 591-594.

Kristek, S.; Kristek, A and Pavlovic, H (2005). The influence of mycorrhizal fungi (*Glomus sp*.) on field pea plant survival along with in drought caused stress conditions. *Plant, soil and Environment,* 51(9): 385-389.

Mahanta, D.L. and Rai, R.K.(2008). Effect of sources of phosphorus and fertilizers on productivity and profitability of soybean (*Glycine max*) and wheat (*Triticum aestivum*) system . *Division of Agronomy,* IARI, New Delhi . 110012.

Mali, B.L. and Shah, R. 2010. Effect of phosphorus and potassium in combination with VAM fungi on growth, root colonization and uptake of NPK in soybean . *Journal of Plant Disease Sciences* , 5(1).

Meghvansi, M. K. and S. K. Mahna. 2009. Evaluating the symbiotic potential of *Glomus intraradices* and *Bradyrhizobium japonicum* in vertisol with two soybean cultivars.Ameri. Eurasian J. Agron. 2 (1): 21-25.

Negi , S Singh, R.V and Dwivedi, D.K. 2006. Effect of biofertilizers , nutrient sources and lime on growth and yield of garden pea. *Legume Research,* 29 (4): 282-285.

Pramanik, K and Bera, A.K. 2012.Response of biofertilizer and phyto hormone on growth and yield of chick pea (*Cicer arietinum* L.).*Journal of Crop and Weed,* 8 (2): 45-49.(*Cicer arietinum* L.) in rainfed area under Bundelkhand conditions.*Plant Archives,* 7(1) : 237-239.

Ramana,V.; Ramakrishna, M.; Purushotham, K. and Reddy, K.B. 2010.Effect of biofertilizers on growth, yield attributes and yield of French bean (*Phaseolus vulgaris* L.).*Legume*

Research,33 (3).

Robie, G.H. and Humiany, A.A. 2004. Role of VA mycorrhizae on the growth of cowpea plant and their associative effect with N_2-fixing

Sajitha , J.P.; Haripriya, K.; Maheswari, T.U. and Stella, D. 2007. Impact of organic nutrients on yield and quality of garden bean . *Acta Horticulture*, 752: 485-487.

Sarawgi, S.K.; Shrikant, C.; Alok, T and Sandeep B. 2012. Effect of phosphorus application alongwith PSB, *Rhizobium* and VAM on P fraction and productivity of soybean (*Glycine max*) . *Indian Journal of Agronomy*, 51 (1): 55-60.

Sepat, S. and Yadav, S.S.(2008) Phosphorus and sulphur management in mothbean [*Vigna aconitifolia* (Jacq.) Marechal] under rainfed condition. *Annals of Agricultural Research*, 29: 38-42.

Sharma, S.K. and Singh, H (2003) . Effect of seed rate and fertilizer doses on growth, yield and seed quality of pea (*Pisum sativum* L.) c.v . Arkel. *Seed Research*, 31: 43-46.

Singh, D.; Singh, D.R.; Singh, S.K. and Tiwari, P.K. (2007) . Effect of phosphorus levels on growth, yield and quality of garden pea (*Pisum sativum* L.) . *Haryana Journal of Horticultural Sciences*, 36 (3/4): 386-387.

Singh,D.K.; Lekh Chand; Singh, K.N. and Singh, J.K. 2006. Effect of different biofertilizers in combination with chemical fertilizers on pea (*Pisum sativum* L.) under temperate Kashmir conditions . *Environment and Ecology*, 24 (3): 684-686.

Singh, D.P.; Prakash, H.G. and Singh, R.P. 2005 . Effect of phosphorus and cycocel with and without *Rhizobium* inoculation on green pod production of pea . *Indian Journal of Pulses Research*, 18 (1): 43-45.

Singh, G. (2013). Effect of phosphorus application and urea spray on growth and yield of summer urdbean [(*Vigna mungo* (L.) Hepper] genotype . *The Journal of Plant Science Research*, 29

(1): 125-128.

Singh, M.; Sekhon, H.S. and Jagruo, Singh 2004 . Response of summer mungbean (*Vigna radiata* L.Wilczek) genotype to different phosphorus level . *Environment and Ecology*, 22 (1): 13-17.

Singh, S.R Bhat, M.I.; Wani, J.A. and Najar, G.R. 2009. Role of*Rhizobium* and VAM fungi for improvement in fertility and yield of greengram under temperate conditions . *Journal of the Indian Society of Soil Science*, 57 (1): 45-52.

Singh, S.R.; Najar, G.R. and Singh, U. 2012. Phosphorus management in field pea (*Pisum sativum* L.) - rice (*Oryza sativa)* cropping system under temperate conditions. *Indian Journal of Agricultural Sciences,* 82 (6): 494-499.

Singh, T.R.; Singh , S.; Singh, S.K.; Singh, M.P. and Shrivastava, B.K. 2004 . Effect of integrated nutrient management on crop nutrient uptake and yield under okra-pea-tomato cropping system in a mollisol . *Indian Journal of Horticulture*, 61 (4): 312-314.

Stancheva, I.; Geneva, M.; Zehirov, G.; Tsvetkova, G.; Hristozkova, G. and Georgiev , G. 2006 . Effect of combined inoculation of pea plants with arbuscular mycorrhizal fungi and *Rhizobium* on nodule formation and nitrogen fixing activity . *General and applied plant physiology* . 32: special issue, 60-66.

Tabassum Yaseen, Tanvir Burni and farrukh Hussain 2011. Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth, and productivity of cowpea (*Vigna unguiculata*) varieties . *African Journal of Biotechnology*, 10 (43): 8593-8598.

Tarafdar, J.C. and Rao, A.V. 2001. Response of clusterbean to *Glomus mosseae* and *Rhizobium* in arid soil fertilized with nitrogen, phosphorus and farm yard manure . *Journal of the Indian Society of Soil Science*, 49: 751-755.

Tomar, A.; Kumar, N ; Pareek, R.P. and Chaube , A.K. 2001. Synergism among VAM , phosphate solubilizing bacteria and *rhizobium* for symbiosis with black gram (*Vigna mungo* L.) under

field conditions . *Pedosphere*, 11 (4): 327-332.

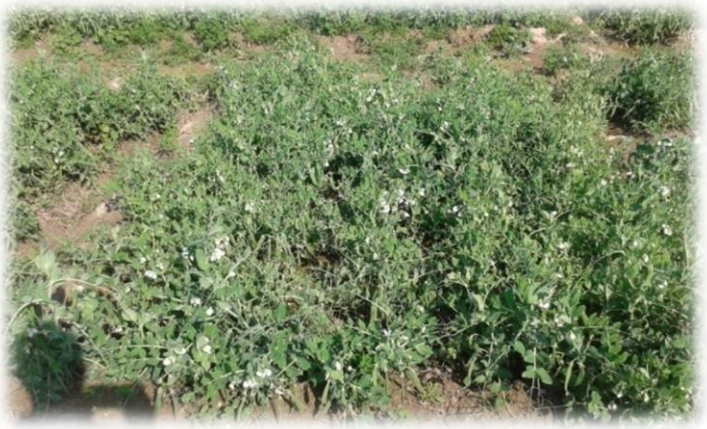
Vijaykumar, B.S. and Lakshminarasimhan, A.V. 2004. Mycorrhizal technology for increasing the yield of groundnut crop in semi-arid soils of Puttaparthy . *Journal of Ecobiology,* 16: 191-195.

Yadav, S.S. 2009. Studies on the effect of single and c ombined use of phosphorus and PSB on the vegetable pea (*Pisum sativum* L.) . *Bhartiya Krishi Anusandhan Patrik a,* 24: 3-4.

Appendix

Growing Field Pea at 10 DAS

Growing Field Pea Crop at 30DAS



Growing Field Pea Crop at 90 DAS

Growing crop at 110DAS and after heavy rainfall

Plot wise harvesting and packing