Nitrogen and Phosphorus Economy Through Dual Inoculation of *Rhizobium* and Arbuscular Mycorrhizal Fungi in Field Pea

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

Erneste Havugimana
Registration Number: 11313313

Under the supervision of
Dr. Balkrishna Sopan Bhople

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

Certification

This is to certify that the thesis entitled "**Nitrogen and Phosphorus Economy Through Dual Inoculation of *Rhizobium* and AM Fungi in Field Pea"** submitted in partial fulfilment of the requirements for the degree of Master of Science in Agronomy, Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, is a record of bona-fide research carried out that **Erneste Havugimana** Registration **no.11313313**, 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 Supervisor

Dr. Balkrishna Sopan Bhople

Designation: Assistant Professor

ID no. 16709

School of Agriculture

Lovely Professional University

Signature of co-supervisor

Dr. Arun Kumar

Designation: Assistant professor

ID no 18703

School of Agriculture

Lovely professional University

Signature of co-supervisor

Dr. Chandra-Mohan Mehta

Designation: Assistant professor

ID no. 18376

School of Agriculture

Lovely professional University

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.

Name of student: Erneste Havugimana	**Dr. Balkrishna Sopan Bhople**
Reg. number: 11313313	**ID No. 16709**

Acknowledgements

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

I would like to express my gratitude to Dr. Balkrishna S. Bhople (HOD, Agronomy) as my supervisor, I am greatly indebted to him for his stimulating guidance, constructive criticism and encouragement during the entire course of my research. I would like to thank Dr. Anil Kumar as my formal supervisor for critical scientific discussions at the beginning of this study. Furthermore, I would also like to thank Dr. Arun Kumar not only for his help in statistical analysis part but also his moral philosophy support and encouragement.

I would like to take this opportunity to express my cordial thanks and sincerest respect to government of Rwanda through Rwanda Education Board (REB) that granted me a study loan and other financial assistance, without which none of this would have been possible.

Special thanks to all the present and past members of school of agriculture, laboratory technicians and field workers in general lovely professional university for providing their full help and co-operation during the present study.

Last but not least, I thank my mum, brother and sisters for all the motivation and encouragement to attain this stage in my life. I extend my heartfelt thanks to my friends, colleagues for their timely help in numerous occasions during this program.

Table of contents

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

List of tables

List of figures

Abbreviations

%	percentage
AMF	Arbuscular Mycorrhizal Fungi
B:C	Benefit Cost Ratio
BNF	Biological Nitrogen Fixation
cm	Centimeter
DAS	Days After Sowing
DMRT	Duncan's Multiple Test
Fig	figure
ha	hectare
INM	Integrated Nutrient Management
K	Potassium
Kg	kilogram
N	Nitrogen
NUE	Nitrogen Use Efficiency
^{O}C	degree Celsius
P	Phosphorus
PCA	Principal Component Analysis
PSM	Phosphate Solubilizing Microorganisms
PUE	Phosphorus Use Efficiency
R	Replication
RDB	Randomized Brock Design
Rs	Rupees
SSP	Single Superphosphate
T	Treatment

Abstract

The study" **Nitrogen and Phosphorus Economy Through Dual Inoculation of Rhizobium and Arbuscular Mycorrhizal (AM) Fungi in Field Pea"** was carried out in experimental field of lovely professional university during Rabi season 2014-2015. The aim of this research was to test nitrogen and phosphorous saving due to dual inoculation of Rhizobium and AM fungi in field pea crop this was achieved by assessing the effect of combined AM fungi and rhizobium on nutrient use efficiency and nutrient uptake (N, P). The experiment was laid in Randomized Brock Design (RDB) with tree replications and seven treatments (Control: N0%P0%K0% + No Bio-fertilizers, NPK100% + No Bio fertilizers, RHZ + N50%+ P 100% + K100%, RHZ + N75%+ P 100% + K100%, AMF+ N100%+ P50% + K100%; AMF+ N100%+ P75% + K100%, RHZ +AMF+ N50%+ P50% + K100%) were used to compare effect of rhizobium and AM fungi on vegetative parameters, Yield parameters, quality parameters and Economic analysis part. The result showed that combined AM fungi and Rhizobium enhance vegetative growth. T7 (RHZ +AMF+ N50%+ P50% + K100%) lead to significant heights plant number of leaves and number branches as compared to single inoculation or recommended mineral fertilizer dose and control . Result revealed that yield parameters was significantly influenced by bio fertilizer application with different level of percentage of recommended dose over control but more pronounced was observed in dual inoculation treatment , T7 recorded the maximum grain yield 8.8q/ha versus 4.75q/ha for control. Nutrient uptake and nutrient use efficiency perfumed well in plots treated with bio fertilizers nitrogen and phosphorus uptake was 18.29 kg/ha and 1.92kg/ha respectively in T7 over 6.0 kg/ha and 0.52 kg/ha in control. The maximum NUE and PUE was recorded in T7 75.51% and 53.25% respectively over recommended dose in T2 46.22% for NUE and T3 4.69% for PUE. Benefit cost ratio (B:C) maximum profit was recorded under T7 with a ratio of 1.00 The dual inoculation of am fungi and rhizobium along with 50% of recommended mineral fertilizers recorded higher productivity and profitability due several reasons Rhizobium and AM fungi application reduce the cost of cultivation through to minimization of nitrogen and phosphorus fertilizer and in return improve productivity by its ability to increase nutrient uptake by the plant and nutrient use efficiency.

Keywords: Rhizobium, AM fungi, field pea, dual inoculation, mineral fertilizer

Chapter I

1. Introduction

The scarcity of agriculture food containing high nutrients values, high level of proteins, micronutrients, and the imbalance between the world population and food production are the increasing crucial problems affecting millions of people in developing countries (Burchi *et al.*, 2011*).* This low agriculture performance is caused by the several raisons including arable land scarcity following high demographic pressure, poverty of farmland due to erosion , lack of organic and mineral fertilizers, over use of soil, lack of knowledge of farmers in improved farming practice (Kumar *et al*., 2002). The major raison of poor agriculture production caused by insufficiency and imbalanced fertilizer application, to overcome these above constraints, farmers use excessive commercial fertilizers, pesticides and insecticides regardless their detrimental effects on soil and environmental health, as well as their increase of production cost. Incorporation of bio-fertilizers as integrated nutrient management is the most technology which should be adopted for soil fertility restoration in turn to improve crop production and productivity for sustainable agriculture.

Nitrogen, phosphorus and potassium are the primary nutrients for plant growth known collectively as NPK (borch and Lynch, 1998). Plant growth is a very complex process whereby the crops synthesize its needed food through solar energy, carbon dioxide, water and nutrient from the soil. The insufficiency of these primary nutrients limits the crop growth and its yield productivity (Andrade *et al.,* 1998). Nitrogen as the most used element is in unlimited quantity in the atmosphere, however is unavailable in the correct form for absorption and synthesis by the crop plants, to overcome its insufficiency in the soil the most of the farmers use commercial fertilizers to enhance agriculture productivity and get more yield, consequently the excessive use of chemical fertilizers lead to serious environmental contamination which often have negative effect on human and animal health. (World Bank, 1996).

Bio-fertilizers are the best tool for modern sustainable agriculture and the use of bio-fertilizers along with other different fertilizers organic or mineral fertilizers may be a very important in integrated nutrient management as long as this combination reduce input cost, improve soil heath through the minimum use of chemical fertilizers and become environmental friendly agriculture technology (Mia *et al*,. 2010). The role of legumes on soil heath and fertility improvement is known since the beginning of research in agriculture in the ancient time and Rhizobium is the most efficient bio-fertilizer according to the quantity of atmospheric nitrogen fixed in the soil. Rhizobia microbe infect root of legume plants and form nodules within which reduce atmospheric nitrogen into ammonia which is absorbable form of nitrogen and used by the plant to produce proteins and other nitrogen containing compounds. Arbuscular Mycorrhizal fungi are a type of Mycorrhizae in which the fungus penetrate and invade the cortical cells of the vascular plant roots (Xavier and Germida, 2002).

Legume crops have ability to form symbiotic association with two types of micro-organisms, one is Rhizobium bacteria through their symbiotic association they fix atmospheric nitrogen and other is arbuscular mycorrhizal (AM) fungi which is involved in availability and the uptake of phosphorus, many researchers have reported the enhancement of phosphorus nutrient availability and growth of leguminous plant by AMF (Arihara and Karasawa 2000, Atimanav and Adholeya 2002). Thereafter the simultaneous inoculation of legumes crops with rhizobium and arbuscular Mycorrhizal fungi showed the synergetic potential effect , there are evidences that dual inoculation increase growth and nutrient uptake in pigeon pea, cow pea, field pea and soybean.

Pulses are the main source of protein for millions of people and have been blessed to be wonder crop to humankind for many reasons; they are excellent natural resource management crops, very important environmental security, and crop diversification for viable agriculture (Schubler and walker, 2001). Although India is largest producer in the world, is short in supply of pulses. Pulse crops production and productivity have declined resulting in reduction of per capita availability of pulses. Field pea is one of the most popular important pulses crops of India, field pea belong to the family of leguminosae, confined to cool climate largely grown in temperate region; high altitude region or during cool season . Field pea occupy about 3% of total pulses' area and 5% in total pulses production of India. It is grown over an area of 0.7 million ha with 0.6 million tons of production (Clark, 2007).

The main constraint mitigating above crop production is lack or inadequate fertilizers especially NPK constitute the most limited factor for vegetative and reproductive growth. According to the current findings on the role of bio-fertilizers rhizobium and AMF in enhancing nitrogen and phosphorus use efficiency respectively and their tripartite mutualistic symbiosis to increase vegetative growth so that all these current information should be disseminated to the farmers. Based on these interesting reviews this research was conducted in the experimental field, department of Agronomy , school of Agriculture, Lovely Professional University to investigate **”Nitrogen and Phosphorus Economy Through Dual Inoculation of Rhizobium and Arbuscular Mycorrhizal Fungi (AMF) in Field Pea**.”

1.1. Objectives of the study

The main objective for this research was to test the Nitrogen and Phosphorus economy or saving through to dual inoculation of rhizobium and Am fungi which would be achieved through the followings objective:

1. To assess the effect of combined AM fungi and *Rhizobium* inoculation on nitrogen and phosphorus use efficiency
2. To assess the effect of combined AM fungi and *Rhizobium* inoculation on nutrient uptake

Chapter II

---

2. Review of Literature

2.1. Rhizosphere

Zone of Soil volume surrounding and influencing the plant roots is called rhizospheric zone and the plant root surfaces strongly adhering soil particle is rhizoplane, The rhizospheric includes the ectorhizosphere refers to the soil adhering directly to the roots of plant, rhizoplane or root surface and endorhizosphere which is the interior of the root mostly the studies of ecology of rhizospheric soil microbes include also rhizoplane (Kemery and Dana, 1995). The Rhizosphere includes the ectorhizosphere refers to the soil adhering directly to the roots of plant, rhizoplane or root surface and endorhizosphere which is the interior of the root. Very important interactions of plants, soil micro fauna, soil and microorganisms take places in the rhizospheric zone. Pinton et al in 2001 described exchanges of signal molecules and biochemical interactions between plants and soil microorganisms, these different interactions significantly can influence plant growth, plant disease resistance, and nutrient uptake by plants and finally crop yield. Bacteria are the most abundant microorganisms in the rhizosphere, and rhizobacteria are the consistent bacteria that colonize roots of different plants in the rhizosphere, they multiply rapidly and colonize ecological niches of roots at any stage of plants growth (Nehl *et al.*, 1996).

The beneficial microorganisms respond to chemicals released by plant roots and colonize actively the rhizosphere and endorhizospehre . for example the members of rhizobiacea colonize most leguminous and enhance plant growth by fixing atmospheric nitrogen similarly the arbiscular micolozer fingi (AM fungi) colonize most of land plant roots and influence plant growth(Allen and Allen, 1981; Brockwell *et al.*, 1995)

2.2. Rhizobia

Among the soil bacteria, there is one group which is genetically diverse and heterogeneous in its beneficial effect on legume plants growth and their productivity as they are able to draw out nodule formation on legumes called rhizobia (Denarie et al., 1996). Rhizobia consistent of the genera of Rhizobium, Bradyrhizobium and Azorhizobium, they are free- living ubiquitous soil micro-flora in the rhizosphere of legumes until the point where nodulation become possible (Allen and Allen 1992). The bacteria belonging to the family of rhizobiaceae have a unique feature to form a symbiotic relationship with the legumes plants of fabaceae family. The contribution of soil bacteria in their interaction with plant in the rhizosphere has been studied, but the concept of progressive interactions remains unclear. Rhizobium- legumes symbiosis is the most important mutualistic interactions hence contribute about 65% total nitrogen available in the biosphere, which is taken as the largest single input of in the nitrogen cycle (Batut *et Al.*, 2004; Newton, 2000). The rhizobium- legume interaction is a complex process which require different completely successful steps, the rhizobia are attracted by to flavonoid and chemicals mostly organic acids secreted by the plants roots in the rhizospheric zone , its response to these exudates is to induce node genes . Node genes are responsible to encode 25 proteins approximately required for synthesis and export of bacterial node factors. Node factors are lipochitooligosacharides and stimulate root cell division in the rhizosphere, at that stage the rhizobia attach to the under developing root hairs before penetrating to the growing hairs of roots (Gage, 2004; Walker *et al*., 2003).

2.2.1. Rhizobium

It belongs to bacterial group family *Rhizobiaceae*, and is a symbiotic nitrogen fixer in association with legumes only. They are the most efficient Bio-fertilizer as per quantity of nitrogen fixed concerned. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. Rhizobium become very useful in pulse crop production such as chickpea, lentil, red gram and black gram and in oil seed crop and forage crops (Anand and Dogra, 1997). *Rhizobium* has ability to fix atmospheric nitrogen in the soil, and its population in the soil depends on the presence of legume crops in the field. If rhizobium strain is compatible with legume crop present in the field, the specific legumes roots are colonized by rhizobium to form tumor shape like growth which are called nodules and act as industries or factories for production of Ammonia (Knee *et al.,* 2001). Seed inoculation with specific strain of rhizobium before sowing is always needed to restore and increase the population of Rhizobium near the rhizosphere to hasten N-fixation. Each legume requires a specific species of *Rhizobium* to form effective nodules.

2.2.2. Nitrogen fixation

Nitrogen fixation is a process by which nitrogen molecule (N_2) in the atmosphere is converted into ammonia (NH_3). Atmospheric nitrogen or molecular nitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds. Fixation processes free up the nitrogen atoms from their diatomic form (N_2) to be used in other ways. Nitrogen fixation is essential for all form of organisms on the earth. For example the nitrogen is taken as basic element in biosynthesis of nucleotides for Deoxyribonucleic acid (DNA) and Ribonucleic acid (RAN) and also Amino acids for proteins. Therefore process of nitrogen fixation is essential for agriculture and the manufacture of fertilizers.

2.2.3. Rhizobium-legume symbioses

The symbiosis association between *Bradyrhizobium* or *rhizobium* and legumes crops is a very effective and cheaper agronomic practice which ensuring the availability of nitrogen to legumes crops and others surrounding plant within the same field rather than fertilizer nitrogen application. Introduction of leguminous fodder crops in the pasture found as the practice technique to improve nitrogen value in the grasses and it can contribute 75 to 97 Kg of Nitrogen per ha. (Viera-Vargas *et al.,* 1999). The recent study done by Mandimba (1999) revealed that the nitrogen fixed by groundnut and contribute to the maize in intercropping system was equivalent to the application of 96kf of Nitrogen fertilizer per ha at the ratio of one maize plant to four groundnut plants as plant population in intercropping system. Frankia- non legume symbiosis or Actinorhizal interaction with no legume plants are major contributors to nitrogen supply in forests, wetland areas (Tate, 2003). More than 160 species of angiosperms classified among six to seven orders are involving in this association and contribute to nitrogen fixation as well as managing ecosystem balance. The above overview clearly indicates the significance of symbiosis of rhizobium and legume as main contributor of natural atmospheric nitrogen fixation.

2.3. Phosphate solubilizing microorganisms (PSM)

Phosphate solubilizing microorganism has ability of solubilizing phosphate inorganic compounds which are insoluble in soil like rock of phosphate, tricalcium phosphate, and hydroxyapatite and dicalcium phosphate. The species of *Pseudomonas, Bacillus, Aspergillus* *etc.*, secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil (Wani and Lee, 2002). An arbuscular mycorrhizal fungus (AMF) is a type of mycorrhizal in which the fungus penetrates the cortical cells of the roots of a vascular plant.

The term Mycorrhizal denotes “fungus roots”. It is a symbiotic association between host plants and certain group of fungi at the root system, in which the fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host and the host in turn is benefited by obtaining the much needed nutrients especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus (Subba Roa, 2001).

2.3.1. Arbuscular Mycorrhizal (AM) fungi

As members of the Glomeromycota, Arbuscular mycorrhizal fungi (AM fungi) are the most widespread of mycorrhizal fungi, occurring near about 80% of all plant species (Smith 1997). They are formed by a network of hyphae grown within the plant roots and reach out into the soil. Unlikely to the ectomycorrhizal fungi, arbuscular mycorrhizal fungi after penetrating into cellar wall of plants roots form intracellular structure (Kytoviita, 2000). AM fungi produce two distinct structures: the sac like vesicles which acts storage of lipids and dumbly branched masses called arbuscles, which act as the site of exchange nutrient between plants roots and fungus (Morton and Benny, 1990). Previously these groups of fungi were known as vesicular arbuscular Mycorrhizal however with the recent researches, it has been shown that the vesicles can be raised by non Mycorrhizal fungi but only arbuscular structure is unique to this group of fungi (McGonigle *et al.*,1990). the research carried out by Johnson et al in 2003 have shown that the proportion of vesicles and arbuscles is influenced by soil nutrient levels and can indicate the level of benefit received by each partner side of symbiosis. Although this group of fungus is considered as obligate symbiost, they have shown the capability of saprophyte (Hodge *et al.*, 2001) and at the absence of live host their viability and spread is very limited (Warner and Mosse, 1980). The infection of plant roots by arbuscular Mycorrhizal fungi instills benefits to the plant host including direct benefits such as increase growth (Kemery and Dana, 1995), increase seedling establishment (Van der Heijden, 2004), reduce water stress (Allen and Allen, 1986) and indirect benefits such as protection from pathogens, increase pollination process, and increase interactions with other mutualistic soil microorganisms like rhizobium bacteria (Hartnett and Wilson, 2002). The direct effect of increasing crop yield is due to the increase of nutrient uptake especially phosphorous after infection (Bolan *et al.*,1987) and water stress resistance is associated with network of hyphae which are more drought resistant than fine roots and have ability to resume growth rapidly after the cessation of drought conditions (Miller *et al.*,1995).

2.3.2. Factors influencing AM fungi development

Arbuscular Mycorrhizal fungi sporulation and root colonization has seasonal dynamics, which is either directly or indirectly influenced by different intervals of year, soil temperature, and soil moisture and soil pH. The seasonal change, spore density variability and root colonization difference are due to a wide range of plant hosts and its environmental condition (De Oliveira, A.N and L.A de Oliveira, 2005). The level of AM fungi multiplication depend upon of root morphology, metabolism rate of plant growth and root morphology within specific soil plant system in form of chemical composition of roots exudates (Koshe, 1981) . AS it was reported by Lugo and Cabello (2002) in the study of seasonal variation of Am fungi in mountain glass land, the higher number of vesicles, arbuscles and maximum root colonization was observed during summer season and this may be attributed to the capacity of arbuscular mycorrhizal fungi to obtain maximum profit and growth at high temperature and abundant rainfall. The intensity level of AM root colonization and spore density has shown significantly depend to the soil pH (Sylvia and Williams, 1992). Daniel and Trappe (1980) concluded that soil pH induced the availability of plant nutrients in soil which is responsible for stimulation or inhibition of AM fungi spores' germination. The maximum number of AM fungi spores and root colonization was reported in range of pH 7.2-7.4 whereas pH 6-7 was reported as the best for maximum Mycorrhizal development. (Sankaranaranyan and Sundarababu, 2001). Temperature and soil moisture fluctuations within different seasons influence directly or indirectly their spore population and root colonization. The count of spores has shown the significant correlation with the temperature and soil moisture in the study carried out by Staddon *et* al. (2002).

2.4. Interaction of bacteria and AM fungi

Bacteria and arbuscular mycorrhizal (AM) fungi are known to be able synergistically to interact and boost plant growth through different mechanisms that include nutrients availability and uptake to the plants; preventing fungal disease pathogen and plant resistance to soil moisture tress (Biró et al ., 2000). These interactions may be of essential important within sustainable agriculture as low input cropping system that rely on natural biological process rather than agrochemical fertilizers to increase soil fertility and maintain plant and soil health. . Many studies concerning AM fungi and bacteria interactions had been done but the mechanisms behind these interactions are in general not well understood, (Garbaye, 1994). And their functional properties are still requiring further experimental researches for confirmation. However both ectomycorrhizal and endomycorrizal fungi can interact with different bacterial species (Meyer and Linderman, 1986), these interactions occur in the soil zone surrounding roots of the plants hosts and fungal hyphae commonly referred as mycorrhizosphere (Rambelli, 1973).

The beneficial bacteria that interact with AM fungi include PGPR and N_2- fixing Bacteria Rhizobium and these may increase direct benefits of mycorrhizal relationship and/ or plant growth (Vivas *et al.,* 2003). AM fungi together with specific bacteria may create an indirect synergism that support vegetative plant growth (Barea, 2002), that include nutrient acquisition, root blanching enhancement, inhibition of plant pathogen (Gamalero et al., 2004). Artursson et al. (2005) declared the impact of Am fungi on the composition of bacterial communities, because establishment of mycorrhizae in the rhizospheric zone has been shown the change in composition of chemical compounds secreted by roots and these roots exudates are often the source of nutrients to the associated bacteria in the mycorrhizosphere (Barea, 1997; 2002; Gryndler, 2000; Linderman, 2000).

2.4.1. Dual effect of AM fungi and Rhizobium as bio-fertilizers

Dual Effect of fungi and rhizobium has been reported by Mukherjee and Rai (2000) where the highest grain yield in *Triticum aestivum* at phosphorus levels by inoculation of *Pseudomonas striata* followed by *Glomus fasciculatum* bio-fertilizers. The studies on the yield of chick pea were carried out at field research, Faisalabad, university of agriculture, results revealed that the highest 1000 seed weight, seed yield and biological yield (256.11g, 3087.6 and 7497 kg/ha, respectively) were obtained with seed inoculated with rhizobium along with endomycorrhizal application (Hakoomat et al., 2004). Rajendran and Jayasree (2007) studied the effect of Rhizobium and AM fungi bio-fertilizer on seedling production of *Acacia nilotica,* they showed that the length of seedlings and the biomass were significantly increased in all treatments compared to the control. The maximum growth and biomass were recorded in plot received combination of Rhizobium +AM fungi (azospirillum) it was 156.9% more than the control. The effectiveness of microbial bio agents to control collar rot disease in chick pea was studied (Ashraf Zahid et al., 2007) the high reduction in seedling mortality was obtained in treatments where rhizobium and VAM comparing to the control (100% mortality) and in this experiment was shown that both amendments used have effectively improved various yield parameters by controlling the disease and reducing seedling mortality.

The dual application of Rhizobium and arbuscular mycorrhizal fungi showed a synergistic effect in all mung bean varieties. Among these cultivars tested Vaibhav variety was shown the highest response to growth parameters, root nodulation, and grain yield production (Manke *et al.,* 2008). Under field condition, the effect of rhizobium and phosphate solubilizing microorganisms *(Glomus intraradices, Pseudomonas putida*), on growth, nodulation and root rot disease of chickpea was studied (Mohd and Siddiqui, 2009) the result showed that the number of nodules was significantly higher in all plants inoculated with rhizobium when compared to the control. The effect of vesicular arbuscular Mycorrhizal fungi and rhizobium co-inoculation in green gram (*Vigna radiata* L.*).* was studied under temperate conditions (Bhat *et al.,* 2010), the result showed that the significant effect of rhizobium and AM fungi on nitrogen level, number of nodulation, yield , NPK content in grain as well as in straw and crude protein content in grain. Based on its quality facilities as it is very cheap, easy for handling and plant growth enhancement subsequently seed quality, rhizobium is considered as promising fertilizer in sustainable agriculture and the ubiquitous occurrence and role of AM fungi for plant growth is now a well-known fact (Chaurasia and Khare, 2001).

2.4.1.1. Enhancement of Nitrogen availability to plant

Nitrogen-fixing bacteria improve availability of nitrogen to plant in soil, and that availability may be more improved when plants also are colonized by Arbuscular Mycorrhizal fungi (Barea *et al.*, 2002). Nitrogen fixing rhizobia, the Mycorrhizal fungi and plant root nodules symbioses improve mineral availability , and impart nutrition and growth of the plant., AM fungi may participate to increase nutrient status in the mycorrhizosphere, by organic Nitrogen compounds decomposition, plants may have a greater benefit through additional nitrogen provided through N 2 fixation (Toro *et al.*, 1998). Increased N fixation within Mycorrhizal plants was explained that when nitrogen and phosphorus are both limiting, AM fungi can improve phosphorus uptake by the plant which in turn would provide energy for nitrogen fixation by rhizobia (Fitter and Garbaye, 1994). This hypothesis has been supported by Rajendran and Jayasree (2007). It has been found that the enhanced N2 -fixing ability in Mycorrhizal plants compared with non-mycorrhizal plants, usually disappears if the non-Mycorrhizal plants are supplied with a readily available Phosphorus source. The uptake of other essential nutrients from the soil by arbuscular mycorrhizal fungal hyphae may also play a role in plant growth improvement as well as in more indirect effects upon the N2- fixation mechanism (Barea *et al.,* 1992). nitrogen in Organic forms may also be made more available to plants by bacteria association with hyphae of mycorrhizal fungal as the experiment done by Hedge *et al.*(1999) shown that the AM fungus was able to enhance decomposition and increase plant N capture from grass leaves.

2.4.1.2. Enhancement of phosphorus availability

On the other side rhizobacteria may support Arbuscular Mycorrhizal fungi to increase Phosphate bioavailability through their symbiotic association with plant roots. Soil with low Phosphorus availability, free-living phosphate-solubilizing bacteria are able to release phosphate ions from sparingly soluble inorganic and organic P compounds presented in soil (Kucey and Bonetti, 1988), thereby contribute to increase available soil phosphate pool for AM fungal hyphae to pass on the plant (Smith and Read, 1997). The inorganic phosphate may be in form of either crystal lattices of largely insoluble forms or chemically bonded to the clay minerals surface strictly unavailable to plants. Organic Phosphate is largely unavailable to plants unless it is converted to inorganic form, by phosphate solubilizing bacteria. Soluble Phosphate enter in the soil after its mineralization by bacteria which are localized in rhizospheric zone and increase the concentration of phosphate ions in the soil, subsequently plants can benefit from. Organic P may be mineralized by bacteria that secrete phosphatases whereas inorganic P may be released by bacteria that excrete organic acids (Smith and Read, 1997).

Different studies have shown the synergistic positive interactions between AM fungi and phosphate-solubilizing bacteria (Barea *et al*., 1987) the study carried out by Toro *et al*. (1998) on phosphate limited systems containing plants, AM fungi and phosphate-solubilizing revealed that the bacteria promoted Mycorrhizal development and establishment whereas Mycorrhizal symbiosis increase the size and population of rhizobium bacteria. The treatments inoculated with both AM fungi and bacteria significantly increased plant biomass and N and P accumulation in plant tissues, compared with their controls which were not dually inoculated (An and Kim , 1998).

2.5. Field pea description

Common Alternate name as garden pea, common pea, spring pea, *pisum sativum L.* belongs to the Legume family of fabaceae. Field pea is cool season annual legume crops, even if the modern variety have shorter vines around 2 fit long but the vines can be up to 9ft long with the hallow stem and the taller cultivars will need support for climbing (Elzebroek and Wind, 2008). The field pea plant has alternate pinnately compound leaves. Many modern cultivars have a semi-leafless or 'afila' leaf type in which the leaflets are converted into additional tendrils (McGee, 2012). Each inflorescence consists of racemes with one to four flowers and occurs in the leaf axils of the plants. The flower consists of five green sepals which are much fused and five pulp or white petals with different sizes. The ovary may have up to fifteen ovules, and fruit in closed pod, the round smooth wrinkled seed may be green, yellow, beige, blue-red or brown. The self-pollinating of field pea flowers enables the breeders to create the true breeding lines (Gill and Vear, 1980).

2.5.1. Origin history and distribution

The centers of origin of *Pisum sativum* are Ethiopia, the Mediterranean region, and central Asia, with a secondary center of diversity in the Near East (Vavilov, 1951). Across the world human has been eating field peas for 9,500 years old approximately (Elzebroek and Wind, 2008). *Pisum sativum* is currently grown in temperate regions, at high elevations, or during cool seasons in warm regions throughout the world (Elzebroek and Wind, 2008). Major pea producers are China, India, Canada, Russia, France and the United States (FAO, 2012).

2.5.2. Pea Varieties

The varieties grown in different regions of the world exhibit a wide range of variation in stem height, branches, pod size, number of seeds per pod, smoothness of seeds i.e smooth or wrinkled etc. The cultivars are classified based on different characteristics as given below:

Based on maturity period

- Early season types: 65 days after sowing green pods will be ready for first harvesting
- Mid-season types: pods are ready for harvesting in 85-90 days after sowing

- Late season types : for this type pod will be ready for harvesting in 110 days after sowing

Based on height of plant

The field pea cultivars can be grouped in:

- Bush or dwarf types
- Medium tall and
- tall

2.5.3. Climate and soil

Field pea is a cool season crop and grows well in cool weather. The optimum temperature for good seed germination is 22°C, but seeds can germinate at 5°C, with low of germination speed. The high temperature increase decay of seedlings. Even though early stage of crop is tolerant to the frost, flowering and fruit development stages are adversely affected by frost. Optimum mean temperature for crop growth is 10-18°C. The increases of temperature hasten the maturity and reduce yield. Pods Quality produced at high temperature is also low due to conversion of sugars to hemicellulose and starch.

Well drained, loose and friable loamy soil is ideal for early crop and clayey soil for high yield. Ideal pH is 6.0-7.5 and it grows under alkaline soil, Liming is recommended in acidic soil.

2.5.4. Season

Most of the North India, field pea is sown from beginning of October to middle of November in the plains regions, Yield is drastically reduced when crop is sown after 5th December (Kumar and Sharma, 2006). And Crop sown in September will be susceptible to wilt disease. In hills, pea is sown in March for summer crop and in May for autumn crop.

3. Rationale and Scope of the Study

In India majority of the people are vegetarians, thus the main source of proteins in their diary diet is leguminous vegetables. The most third world countries' people take vegetable legumes as their major source of proteins especially poor people, the vegetable crops such as common beans, soybean, groundnut, and pea are rich in phosphorus, calcium, iron, and different essential vitamins (Zuccami, 2007). According to the current statistics, revealing that the world population is increasing continuously whereas, on the other hand, food grain production is not increasing proportionally, which is the main cause of scarcity of food containing high levels of protein, micronutrients and various vitamins sources to millions of people in developing countries (Burchi *et al*., 2011).

Innovative technologies to meet the growing challenges of scarcity of food and malnutrition in the poor and hungry parts of the world must be addressed (Clugston, 2002). To increase the quantity and yield of economically important legumes, particularly field pea, farmers apply large quantities of chemical fertilizer, which has detrimental effects on the soil, water and atmosphere such as the accumulation of toxic salts, greenhouse effect, on the other hand increase cost of production while integrated nutrient management (INM) technology for field pea involving bio-fertilizer i.e. Rhizobium and AMF which is environmentally friendly and potentially cost effective could be a better solution (Mia and Shamsuddin, 2010). The above technology would increase N and phosphorus use efficiency and thus economize fertilizer doses. The occurrence and activity of soil microbes have their bearing on soil fertility as they help in nutrient mineralization in soil and in turn maintaining soil health.

4. Materials and Research Methodology

The study was carried out at the Experimental field of the Department of agronomy, school of agriculture Lovely Professional University, Jalandhar, Punjab (India) during 2014-2015 with the main objective to investigate nitrogen and phosphorous economy through dual inoculation of rhizobium and arbuscular Mycorrhizal fungi in field pea. This chapter describe in details materials, methods and techniques used in this study.

4.1. Description of experimental site

The experimental site is geographically located at 31° 15’ North latitude and 75^0 41’ East longitudes at elevation of 245 m above mean sea level. This experimental site fall in agro climatic zone of “*Central Plain Zone (PB-3)”* of Punjab.The major crops grown in the region are mainly wheat, rice, maize, groundnut, cotton, gram, barley, pear and guava.

4.1.1. Climatic and weather condition of the site

The climate of the experimental area is characterized by hot and dry summer and wet and humid monsoons, distinctly experiences all the four seasons. The annual temperatures range from 1 to 46 ^{o}C and can reach 49 ^{o}C in summer season and 0^{o}c in winter. The rain fall varies from 460 mm in plain to 960 in the sub mountain region and about 80% of total rain fall is received in short period of 3 months (*mid-June to mid-September*). It experiences also three seasons: Summer season from April and June and it is characterized by the rise in temperatures up to 38^{o}C; Monsoon season from July to September and it is during this period when the majority of rain occurs and Winter season from December to February with typical fall of temperatures up to 0^{o}C. Major constraints of the region are declining water table and soil sodicity and salinity.

4.1.2. Soil status of the site

To know physical and chemical characteristics of experimental soil before sowing, the top soil (rhizospheric zone) samples were collected from different corners and center areas within the field, and mixed them together. The dried experimental soil was subjected to both chemical and physical analysis. The results in Table 4.1 of soil analysis showed that the soil is predominantly belong silt loam, slightly alkaline in reaction, non-saline, low in organic carbon, low in available nitrogen and potassium and medium in available phosphorus status. The table below contains details on experimental soil status before sowing

Table 4.1: soil status and analytical methods used for soil analysis before sowing

Number	Parameter	Status/ Value	Method
Physical properties			
1	Textural class	Silt loam	Triangle Method
2	Particles analysis (%)		International pipette method (Piper, 1950)
	Sand	30	-do-
	Silt	64	-do-
	Clay	6	-do-
Chemical properties			
3	Soil reaction (pH)	Alkaline 7.7	Water suspension (Jackson, 1967)
4	Electrical Conductivity(ds/cm)	1.20	Water suspension (Hanlon et al., 1993)
5	Organic carbon (%)	0.40	Rapid titration method (Walkley and Black 1934)
6	Exchangeable Cations (meq/100 g)	0.347	Ammonium Acetate method (Chapman, 1965)
Available macronutrients (kg ha^{-1})			
7	N	150.52	Alkaline Permanganate Method (Subbiah and Asija, 1956)
8	P	14.1	0.5 M $NaHCO_3$, pH=8.5 (Olsen1954)
9	K	133	1 N Neutral ammonium acetate (Black, 1965)

4.2. Experimental details

4.2.1. Treatments and replications

The experimental design consisted of seven treatments and three replications, each replication received seven treatments randomly. Treatments are made by bio fertilizers (*Rhizobium* and AM fungi) along with different level of mineral fertilizers. Different treatments used are presented in table 4.2

Table: 4.2: Details on seven Treatments used in the experiment

Treatment code	Description
T1	Control : N0%P0%K0% + No Bio-fertilizers
T2	NPK100% + No Bio fertilizers
T3	RHZ + N50%+ P 100% + K100%
T4	RHZ + N75%+ P 100% + K100%
T5	AMF+ N100%+ P50% + K100%
T6	AMF+ N100%+ P75% + K100%
T7	RHZ +AMF+ N50%+ P50% + K100%

Where AMF: Arbuscular Mycorrhizal Fungi, RHZ: *Rhizobium*, N: Nitrogen, P: phosphorous, K: potassium

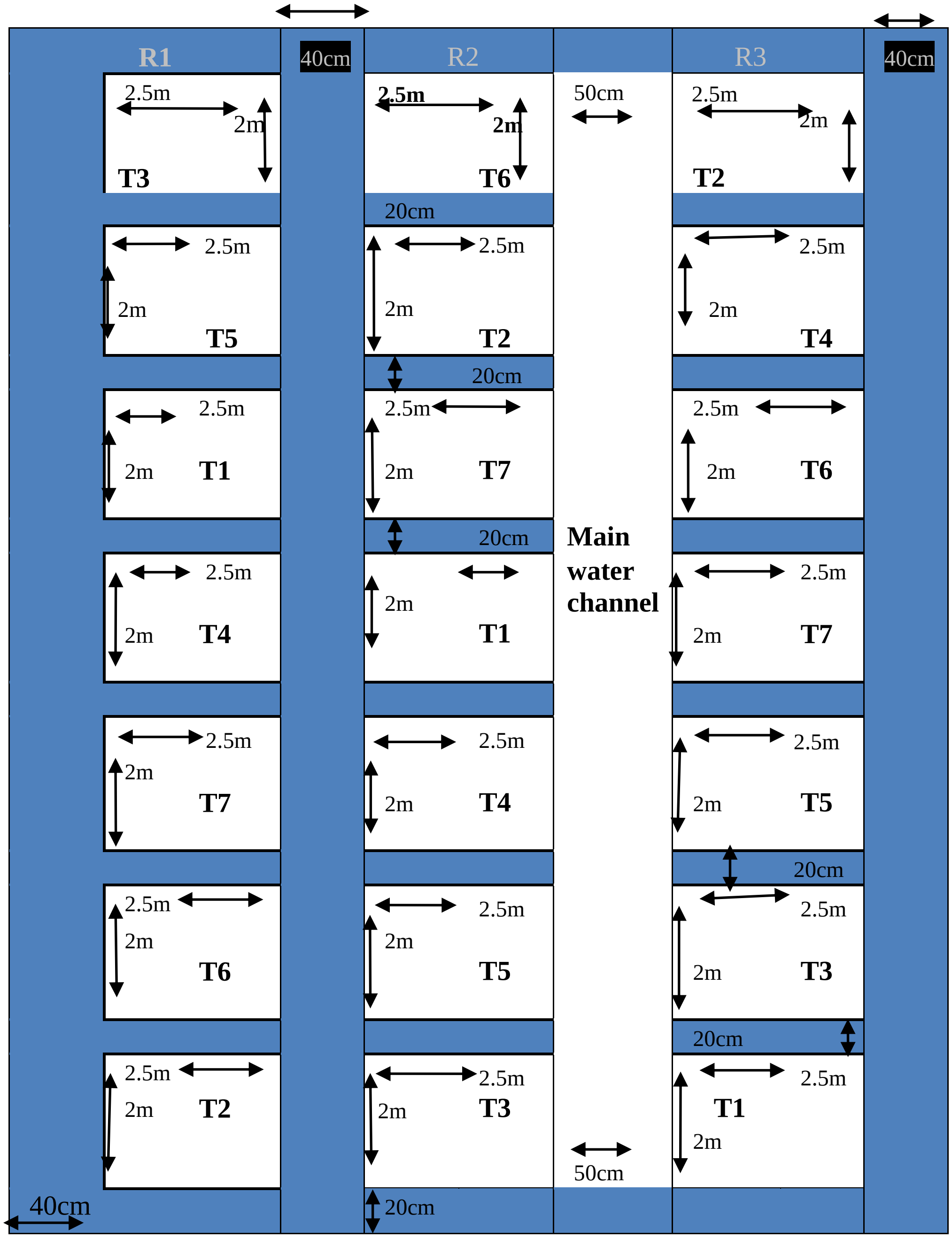
4.2.2. Field preparation

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. Once the field was leveled uniformly, the layout was carried out manually.

4.2.3. Design and layout

The experiment was laid in Randomized Block Design (RBD) and treatments were allocated randomly in each replication. Thereby seven treatments in three replications account 21 plots and the plot size was 2m x 2.5 m. the experimental details and field layout are shown below:

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



T: Treatment

R: Replication

Fig 4.1. Field Layout with treatments allocated randomly in each replication

4.3. Variety and bio fertilizer description

4.3.1. Seed

Seed of field pea used in this research was obtained from Punjab Agriculture University, Punjab 89, and (P89) early season variety developed through selection cross between Pusa2 x Morrasis 55 by Punjab Agriculture University. It is medium dwarf, vigorous, having more number of well filled pods. Average green pod is 60 quintal per acre and it can give the first picking in 83-90 days.

4.3.2. 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 (PAU).

4.3.2. Arbuscular Mycorrhizal fungi

Arbuscular Mycorrhizal Fungi culture used was *Glomus musseae* collected from International Biotech, Chak Kala Tibba, Sitto Road Abohar.

4.3.3. Mineral fertilizer

Macronutrients viz. N, P and K were applied in different plots as urea, SSP single superphosphate (P_2O_5) and Potassium (K) respectively. All these mineral fertilizers were obtained from the stock of lovely professional university (LPU), school of agriculture.

4.4. 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 water irrigation, canal cleaning were done. As detailed in table 4.3

Table: 4.3. The schedule of various agronomic operations done during experimental period

Sr. /No.	Operation	Date
1.	Ploughing and planking of the field	15th november, 2014
2.	Pre sowing irrigation	20th november, 2014
3.	Lay out of field experiment	25th november, 2014
4.	Seed Inoculation with *Rhizobium*	25th november, 2014
5	Basal application of AMF	25th november, 2014
6.	Sowing	25th november, 2014
7.	Fertilizer application (urea and SSP)	25th november, 2014
8.	1st weeding	9th January 2015
9	Thinning	9th January 2015
10.	Irrigation	10th february, 2015
11.	2and Weeding	3rd February,2015
12.	Harvesting	29th march, 2015

Note: the natural rainfall occurred 8 times during growth period and the number of irrigation was reduced 9/01, 3/02, 15/02, 1/03, 2/03, 08/03/, 15/3, 16/3 2015

Fig: 4.2. Crop view after germination

Fig: 4.3. weeding operation

Fig: 4.4. Plant view after tagging at 25DAS

Fig: 4.5. crop at flowerindg time

4.5. Data Collection

Before collecting data directly from the field, in each plot five field pea plants were randomly selected, tagged and used to record different vegetative parameters such as plant height , number of branches and number of leaves in known interval period.

4.5.1. Determination of vegetative parameters

4.5.1.1. Plant height (cm)

The height of 5 tagged plants in each plot was recorded three times during crop growth (30, 60 and 90 DAS) in interval of 30 days .using a meter scale from ground level to the upper youngest leaf of the plant.

4.5.1.2. Number of leaves per plant

Number of green leaves was accounted on five permanent tagged in 30, 60 and 90 DAS without considering the yellowish old and their average worked out.

4.5.1.3. Number of branches per plant

The average number of branches was calculated from the counted in five tagged plants within plot in different periods of 30, 60 and 90 days after sowing (DAS)

4.5.2. Determination of Quality parameters

4.5.2.1. Laboratory analysis

1. **Dry matter (%)**

The dry matter accumulation was estimated at flowering stage of field pea. The randomly selected plants were removed from each plot. The removed plant samples were dried in an oven at 72^{o}C until weight become constant. The dry matter Percent was measured and expressing the remaining weight of sample after drying of the wet sample.

$$\text{Dry weight \%} = \frac{(\text{oven dry weight})}{\text{wet weight sample}} \text{X } 100$$

2. **Plant analysis**

Randomly selected plants were removed from the field in each plots, dried in an oven at 72^{o}C for 72 hours, subsequently the oven dried sample were grounded into a kind of floor and passed through 1mm sieve and stored in plastic bags for analysis. The analytical procedures followed for estimation of N, P content in plant sample are described below:

Table: 4.4. Analytical methods employed for plant analysis

Sr. No.	Parameter	Method employed	Reference
1.	Nitrogen	Micro-kjeldahl method	Jackson (1967))
2.	Phosphorus	Vanado-molybdo-phosphoric acid yellow colour method	Richards (1954)

4.5.2.2. Procedures of plant analysis

1. **Nitrogen content**

One gram of prepared plant material wrapped in a piece of filter paper, to a 300ml Kjeldahl's digestion flask, 10g of catalyst mixture ($CuSO_4$, K_2SO_4, Se, HgO in ratio 20:480:1:3) and 30ml of conc.H_2SO_4 was added then the contents in the flask was mixed , then starting digestion of the contents in the flask first on the low heat on and gradually the temperature increased until acid reached a boiling point. And heating was continued until the organic matter was destroyed and solution became a clear greenish colour. The content was cooled and 100ml volume was made with distillated water.

10ml of 0.02N H_2SO_4 was pipetted out, in a 150ml of conical flask and 3 drops of methyl red indicator was, added; the conical flask was placed under the delivery tube of condenser. 5ml of the aliquot was taken in the distillated flask and connected to the mouth of the distillation flask. 25ml of 45% NaOH was poured in the distillation flask containing the aliquot through the funnel attached to the distillation apparatus, the tab was closed and starting distillation. 30ml of distillate was collected and excess of 0.02N H_2SO_4 in conical flask was titrated against 0.02N NaOH. . Change of colour from pink to yellow was end point. Nitrogen percentage in plant sample was calculated by the following formula:

$$\mathrm{N\%} = (\mathrm{X} - \mathrm{Y})\mathrm{x}\ 0.028\ \mathrm{x}\ 25$$

Where: X: volume of 0.02N H_2SO_4 Y: volume of 0.02N NaOH used

2. **Phosphorous content**

One g of plant sample was transferred in 250ml Erlenmeyer flask, 20 ml of triple acid mixture (HNO_3, $HClO_4$ and H_2SO_4 in the ratio of 9:3:1),was added after swilling the content and heated on hot plate. The heating was continued till the contents of flask gave a yellowish green appearance, then the contents was cooled and made the volume of 100ml with distillated water, then filtered it through whatman No.1 filter paper.

5ml of plant filtrate in 25ml of volumetric flask, 2-3 drops of 2-4 dinitrophenol indicator and 4N Na_2CO_3 solution drop till yellow colour appeared, and 6N HCl was added till disappearance of yellow colour, for getting pH of 4.8 2ml of 6N HCl in excess was added , 5ml of vanadate molybdate was added and the volume of 25ml was made and the colour was developed in 30 minutes , the intensity of yellow colour was read on spectronic -20 photoelectric calorimeter at a wavelength of 470 mµ. Phosphorous percentage in plant sample was calculated by the following formula:

$$\mathrm{P\%} = \frac{\text{ppm of p in given plant sample}}{10000}$$

3. **Nutrient uptake in kg/ha**

Nitrogen and phosphorous uptake in different plots was calculated by applying the following formula:

$$\text{nutrient uptake(kg/ha)} = \text{(nutrient\% plant sample)x yield biomas(kg/ha)}/100$$

Where: nutrient % was either N% or P%

4. **Nutrient use efficiency in %**

Nutrient use efficiency explain how efficiently nutrients in fertilizer have been used by the targeted plant and was calculated as the ratio of total nutrient uptake (N, P, K or micronutrient) to the nutrient supply or rate of fertilizer application. Nutrient use efficiency in each plot was computed based on formula described by Fageria *et al.* (1997)

$$\text{PUE (\%)} = \frac{\text{[Total P uptake (kg/ ha) in fertilized plot]} - \text{[Total P uptake (kg /ha) in control plot}}{\text{P dose applied (kg /ha)}} \times 100$$

$$\text{NUE (\%)} = \frac{[\text{Total N uptake (kg/ ha) in fertilized plot}] - [\text{Total N uptake (kg /ha) in control plot}}{\text{N dose applied (kg /ha)}} \times 100$$

4.5.3. Yield parameters

4.5.3. 1. Number of pods per plant

At harvesting time, 10 plants were selected randomly in each plot and used for counting number of pods per plant after their average worked out to present the whole plot.

4.5.3. 2. Number of seed per pod

Ten pods were also randomly selected from each plot at harvesting time and the total seeds were counted to calculate the average number of seed per pod in given plot.

4.5.3. 3. Biomass Yield (t/ha)

At crop maturity stage, whole biomass in plots were harvested manually then packaged in big polyethylene bags according to the treatments within replication. The biomass weight in each plot was weighed in kg/plot size (kg/5m^2). On the basis of biomass weight in a given plot, the biomass yield in (t/ha) was calculated by the following formula

$$\text{Biomass yield (t/ha)} = \frac{\text{Biomass yield plot (kg)}}{\text{Plot size (m}^2\text{)}} \times 10$$

4.5.3. 4. Grain yield (t/ha)

After weighing the biomass yield, the pods were threshed manually to collect the seeds and seeds yield was weighed in kg/plot size, then after plot yield was expressed in t/ha by this formula:

$$\text{Seed yield (t/ha)} = \frac{\text{Seed yield plot (kg)}}{\text{Plot size (m}^2\text{)}} \times 10$$

4.5.3. 5. Straw yield (t/ha)

The straw yield in each plot was the remaining part of biomass after removing seed yield. And the yield of straw in tone per hectare in each plot was obtained by subtracting the seed yield from biomass yield.

$$\textbf{Stray yield}(\textbf{t/ha}) = \text{biomassyield(t/ha)} - \text{seed yied (t/ha)}$$

4.5.4. Economic analysis

The economic analysis of the experiment was carried out by taking into consideration the prevailing market prices of produce and inputs used. Table: 4.5 describe the unit prices of different inputs and produce considered in this part. Using these data the total cost of production and gross return was calculated and the value each plot was expressed in Rs / ha

Table: 4.5. The prices of inputs and produces in Rs per kg

Inputs/produce	urea	SSP	AM fungi	rhizobium	Seeds	Dry grain yield
Rs/ kg	11.54	27.93	90	100	140	35

1. Net profit or net return

The net profit in each plot was estimated by making the difference between the gross return and total cost of production as it is shown by formula below:

$$\textbf{Net profit } (\boldsymbol{Rs/ha}) = \text{total return (Rs/ha)} - \text{total cost (Rs/ha)}$$

2. Benefit Cost ratio (B: C)

Benefit cost ratio B: C is a technique used to evaluate a project or any kind of investment by comparing benefits and economic costs of a project activities (boardman, 1996). In Agriculture benefit cost ratio is used for agriculture investment for justifying the opportunity cost in term of agricultural production and incomes. Calculation of **Benefit Cost ratio (B: C)** in each plot, the following formula was used

$$\text{Benefit Cost ratio (B: C)} = \frac{\text{Net profit } (Rs/ha)}{\text{Total cost (Rs/ha)}}$$

4.5.5. Statistical analysis

All data were statistically analyzed using SPSS 16 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 result graphically and past software was used for PCA ordination plot of all parameters.

Chapter V

5. Results and discussion

5.1. Growth vegetative parameters

5.1. 1. Plant height

Data presented in table 5.1 and fig: 5.1 showed that at early stage of plant development, there was no statistical difference in height among different treatment in period of 30 days after sowing; he mean height average was in between 17.33cm-18.66 cm. All treatments gave statistically similar plant height.

At 60 days after sowing (DAS), the highest plant height was recorded in T5 followed by T7 with the value of 30.5cn and 28.3 respectively and the lowest plant height was observed in control T1, (N0%P0%K0% + No Bio fertilizers) however T5 gave statistically significant higher plant height compared to other remaining treatments as it shown in fig 5.1.

At 90 days after sowing (DAS), the highest plant height was recorded under AMF+ N100%+ P50% + K100% followed by NPK100% + No Bio fertilizers, and Rh +AMF+ N50%+ P50%, K100% with 44.6cm, 43.2cm and 42.5cm respectively. However these above treatments gave statistically similar plant height and are significant compared to the other treatments. The minimum plant height was noted under control with mean plant height of 24.3 cm

5.1. 2. Number of leaves

Data presented in table 5.1 and fig.5.2 revealed that the different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer did not show any statistical difference in number of leaves in 30DAS. Highest mean number of leaves was observed in T5 (6.4 leaves) and the lowest was observed in T2 and T4 (both 5.8 leaves) but found statistically at par with T5. In 60 DAS the effect on number of leaves was observed to be significant T4 recorded the maximum effective number of leaves (12.9) but found statistically at par with T5 and T7 with (12.8 and 12.53 respectively) and found superior to the rest of other treatment. In 90 DAS the effect on number of leaves was observed to be significant (table 4.1 and fig.4.2) the highest mean number of leaves was noted under T5 (23.73) and found statistically at par with T7 (23.33) the minimum mean number of leaves was noted under control T1 (15.66).

5.1. 3. Number of branches

The data presented in table 5.1 and fig.5.3 reveal that the effect of different treatments both in 30 and 60 DAS was not found any statistical significant over control , T1 (control N0%P0%K0%+ no Bio-fertilizers) found at par with all remaining treatment on number of branches in period of 30 and 60 days after sowing. The effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer in 90 DAS on the effective number branches was observed to be significant over control. The highest mean number of branches was noted under T5 and T7 with (3.66 each) and lowest mean number of branches was found in T3 with 2.53

The result showed that the application of rhizobium and AM fungi along with 50% of recommended dose of nitrogen and phosphorous T7 and application of AM fungi with 100% , 50% of nitrogen and phosphorous respectively T5 had significant and favorable effect on growth attributes of field pea in this experiment . All vegetative growth parameters *viz* plant height, number of leaves and number of branches performed well either with dual inoculation of rhizobium and AM fungi or single bio fertilizer application with different dose of accompanying mineral fertilizers compared to the T1 control. The absence of difference significance on plant height; number of leaves and number of branches (Table 5.1) in 30 days after sowing may be caused by the early stage of root plants development; AM fungi colonization in the rhizospheric zone and N2-fixation system not yet development. In general it was the early stage of the plant development. The present result are in conformity with the findings of Geneva et al. (2006) showed that colonization of AM fungi occur in 15-20 DAS subsequently the increase in number and development of vesicles and arbuscules occur in 30 to 45 day after sowing. The nitrogenase activity in legumes crops found to be pronounced after full development of root system (Fujiyoshi *et al.,* 2000). The research carried out by Kumar et al. (2010) revealed that the plant height, number of branches, number leaves increase and nodules increased with the age of the plant and the maximum number of nodules was observed at 60DAS.

At 60 and 90 DAS the significance increase in plant height, number of leaves and number of branches in 90 days after sowing was observed due to dual inoculation of rhizobium and AM fungi as well as single inoculation of one of these bio fertilizers over control (Table 5.1.). The good performance of T5 and T7 observed in plant height, number of leaves and branches based on the important role of AM fungi in phosphate mobilization, phosphorus sulphur, zinc and water uptake by the plant. The relative high availability of phosphorus in rhizospheric zone of legume plant helped in rhizobium activities and result in high nodulation and more nitrogen uptake which influence vegetative growth of plant (Yawalkar *et al.,* 1996). Such kind of result was reported by Bahadur *et al*. (2006) in field pea , Raman *et al*.(2010) in common bean and Djebali *et al*.(2010) in French bean, pea and alfalfa.

As Phosphorous play important role not only in conserving energy but also transferring it in the different metabolic reactions of living cells such as transformation of biological energy, it has been considered as essential constituent of living organisms (Pandey *et al.,*2003). It helps in root development and proliferation also improves root nodulation and atmospheric N2 fixation. Phosphorous is the main constituent of co-enzymes, ATP and ADP as energy currency, thus it influence the photosynthesis, protein biosynthesis, phospholipids, nucleic acid synthesis and membrane cytoplasmic streaming. The energy obtained from these reactions cited above (photosynthesis, and metabolisms of carbohydrates) is stored in form of ATP and ADP and trans-located to different part of plants and promote meristematic tissues development in the apical buds and inter calary meristems which increase vigor growth of plants.

In general phosphorous taken as indispensable nutrient for pulse crops for its help in roots growth and make them more efficiency in BNF biological nitrogen fixation. These results are in nearly close conformity with findings of Sharma *et al*. (1995), Biswa and Patra (2007) 0n significance of plant height number of leaves and nodules of field pea.

Table: 5.1 Effect of different treatments on growth parameters (plant height, number of leaves and number of branches) at different stage of plant growth

Growth parameters at different stage of plant growth									
Tno	Plant height (Cm)			Number of leaves			Number of branches		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	17.6±0.11	24.3c±1.59	24.3c±1.76	5.8 ± 0 .13	10.6ab ± 0.59	15.66b±1.79	1.00±0.50	1.33±0.06	2.7ab±0.29
T2	17.4±0.29	25.7bc±0.57	43.2a±0.83	5.80 ± 0.23	10.2a±0.94	19.06ab±1.00	0.66±0.85	1.06±0.33	3.3ab±0.17
T3	17.5±1.47	25.4bc±0.46	37.3bc±1.04	6.26 ±0.06	10.7ab±0.52	16.20b±1.11	0.86±0.16	1.11±0.26	2.5b±0.17
T4	17.6±0.86	26.9bc±0.75	41.6ab±0.72	5.80 ±0.52	12.9a±0.99	18.33ab±1.64	1.40±0.53	1.66±0.17	3.26ab±0.35
T5	18.8±0.70	30.5a±0.56	44.66a±1.22	6.40 ±0.20	12.8a±0.41	23.73a±0.99	1.20±0.11	1.73±0.52	3.66a±0.24
T6	17.3±0.06	24.9c±0.88	40.4ab±2.10	6.20 ±0.11	11ab±0.40	20.53ab±2.95	1.33±0.10	1.66±0.29	3.35ab±0.43
T7	18.0±0.48	28.3ab±1.53	42.5a±1.18	6.40 ±0.11	12.5a±0.59	23.33a±1.83	1.53±0.77	1.86±0.17	3.66a±0.40

T1 (control NPK0% + no bio fertilizer); T2 (NPK100% + No Bio fertilizers); T3 (RHZ + N50%+ P 100% + K100%); T4 (RHZ + N75%+ P 100% + K100%); T5 (AMF+ N100%+ P50% + K100%), T6 (AMF+ N100%+ P75% + K100%); T7 (RHZ +AMF+ N50%+ P50% + K100%)

Data are average of three replications.
The mean followed by different letters are significantly different at $p>0.05$, according to DMRT (Duncan's Multiple Range Test) for separation of mean.

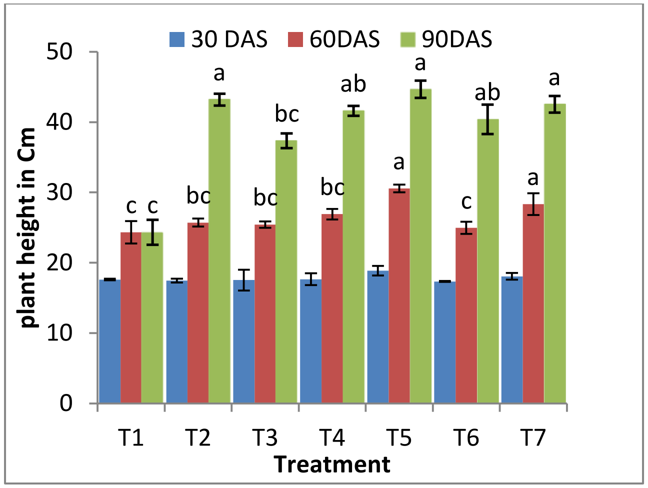


Fig5.1: Effect of different treatments on height at different growth stage

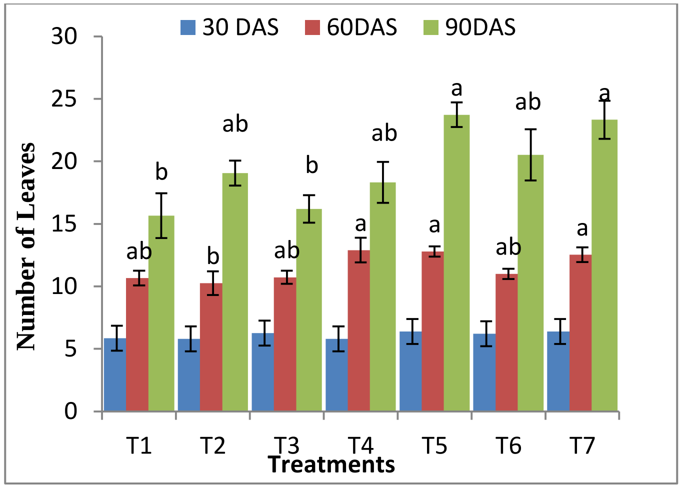


Fig5.2: Effect of different treatments on number of leaves at different growth stage

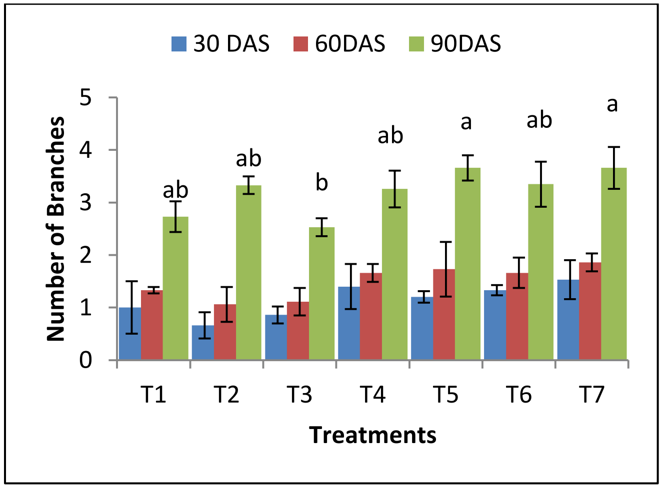


Fig5.3: Effect of different treatments on number of branches at different growth stage

5.2. Quality parameters

5.2. 1. Dry matter percentage

The effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer on dry matter content were not found to be significant (Table 5.2) however the highest dry matter content % was observed under T5 (14.8%) and found statistically at par with all treatment values of dry matter content in percentage and the lowest dry matter content was recorded in T3 and T2 NPK100% + No Bio fertilizers (12.28%).

5.2. 2. Phosphorous content (%)

Data presented in table 5.2 and Fig5.4.Indicated that application of AM fungi along with phosphorous fertilizer increase phosphorous content. The effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer on phosphorous content was found to be significant, the maximum phosphorus content was recorded under T7 (0.27%) followed by T6 (0.24%) Being at par with T5, T3 and T2 registered a significant increase over T1 control (0.16%)

5.2. 3. Nitrogen content (%)

A perusal of data (table 5.2 and Fig 5.4) revealed that The effect of different treatments of rhizobium and AM fungi along with different doses of inorganic fertilizer on nitrogen content was found to be significant, the maximum nitrogen content was recorded under T7 (2.79%) followed by T5 (2.58%) the later was found to be at par with T6, and T4 (2.46%) found to be at par with T2 and T3 which were statistically significant over T1 control (1.88 %).

Dry matter accumulation at flowering stage are presented in Table 5.2 , although the effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer on dry matter content were not found to be statistically significant . The highest value was observed in plot treated with AM fungi T5, T6 and T7. Both Nitrogen and Phosphorous % content in plant showed statistically a significant difference due to the effect of different treatments of rhizobium and AM fungi in T7 with content of (2.79%) and (0.27%) respectively. This study showed that Arbuscular Mycorrhizal fungi affect positively plant growth and nutrient mobilization in rhizospheric zone of pea due to extra radical hyphae of AMF lead to high root colonization and heavy nodulation (Makoi and ndakidemi, 2009). Seed Co-inoculation with rhizobium and compatible AMF strain could enhance pea plant in N and P content and definitely pea growth. These are in conformity with the findings of Bagyaraj *et al*.(1979). Moradi *et al*. (2000). The significance increase of Nutrient specifically nitrogen and phosphorous uptake due to dual inoculation of rhizobium and AM fungi as stated before their interaction may increase directly benefits of mycorrhizae and/ or plant growth relationship (Yadav, 2011), it can also create indirect synergism that support vegetative plant growth like nutrient acquisition, root blanching and inhibition of plant pathogen (Tomar *et al*., 2011). from the presented result in Table 5.2 it is evident that the AM fungi was influenced by rhizobium, the increase of both nitrogen and phosphorus content in field pea of T7 and other treated plots compared to the control, first rhizobium inoculation increase the legume nodulation and cause more nitrogen fixation and its availability to plants thus, it is used as alternative for urea to minimize cost of production (Karim *et al.*, 2001). second Am fungi increase the microflora population and their activities in rhizospheric zone through acid and phosphate solubilizers which lead to the availability and plant uptake of phosphorus otherwise was unavailable to the plants (Lipman and Conybeare, 1936) ; (Bai *et al.*, 2008).

AM fungi support nitrogen fixation by providing sufficient phosphorus and other essential immobile nutrients for atmospheric nitrogen fixation (Clark and Zeto, 2000). This present study of dual inoculation of rhizobium and AM fungi showed a synergistic effect of improvement on nitrogen and phosphorus plant content which was evident with the findings of Tavasolee *et al.*, (2011) who stated that a compatible AM fungi fortify the performance of rhizobium infection and vice versa. Many researchers reported that dual inoculation of AM fungi and rhizobium increase nitrogen and phosphorous content of legumes which support our findings (Talaat and abdallah, 2008) ;(Clark and Zeto, 2000) ;(Chakrabarty *et al*., 2007).

From these result we can assume that dual inoculation of rhizobium and Am fungi increase nitrogen and phosphorous content in field pea plant which indicate that this combination can enhance the productivity of crops.

5.2. 4. Phosphorous uptake

Comparison of various treatment means of phosphorous uptake based on the effect of rhizobium , AM fungi along with different dose's levels of mineral fertilizer indicated that the uptake of phosphorous by crop plant increased significantly over control as it was shown by pertained data of p uptake in given Table 5.2 and Fig 5.5. The maximum phosphorous uptake was recorded under T7 (1.92kg/ha) followed by T5 (1.38kg/ha) recorded statistically significant over other remaining treatments. The lowest result was recorded under T1 control NPK0% + no bio fertilizer (0.52kg/ha)

5.2. 5. Nitrogen uptake

Data presented in Table 5.2 and fig 5.5 revealed that the effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer were found to be significant on nitrogen uptake over control. The maximum Nitrogen uptake was recorded under T7 (18.29 kg/ha) and proved superior to others. The difference in nitrogen uptake in T4 (13.32kg/ha) was found statistically significant to T3 (9.64 kg/ha). The lowest result was recorded under T1 control NPK0% + no bio fertilizer (6.04 kg/ha).

Nutrient uptake by plants depend upon several factors such as fertilizer application methods, method of tillage, agricultural practices, environmental factors , soil properties and microbial activities, in the soil bio fertilizer also can play important role in nutrient uptake (Andrade *et al.,* 1998). Data in Table 5.2 and fig 5.5 revealed that the significance increase of nitrogen and phosphorus uptake (kg/ha) in dual inoculation T7 compared to single inoculation as well as recommended dose and more significant to control. this may be due to the ability of Am fungi to modify and increase phosphorous and other nutrients in rhizospheric zone which lead to increase nutrient uptake in plant, hence increase phosphorus uptake as the P provide energy to plant to capture more nutrient circulating in rhizospheric zone of the plant this was confirmed by Pandey *et al.* (2003) in field pea

5.2. 6. Phosphorous use efficiency

It is apparent from data (Table 5.2 and fig5.6) that effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer were found to be significant on phosphorous use efficiency. The maximum phosphorous use efficiency was recorded under T7 (53.25%) and proved superior to others. T5 (32.79) showed statistically significant difference over T6 (17.15%). The lowest result was recorded under T3 (4.89%)

5.2. 7. Nitrogen use efficiency

Data presented in in Table 5.2 and Fig 5.6. Revealed the effect of rhizobium and AM fungi along with different dose of mineral fertilizer indicated statistically significance difference in Nitrogen use efficiency. The maximum NUE was recorded under T7 (75.51%). T3 (60.32%) found to be at par with T4 (57.88%). The lowest result was recorded in T5 (42.61%) with no statistical difference with T2 and T6 (46.22%; 43.28 respectively).

Nutrient use efficiency explain how efficiently nutrients in fertilizer have been used by the targeted plant and is calculated as the ratio of total nutrient uptake (N, P, K or micronutrient) to the nutrient supply or rate of fertilizer. Maximum nitrogen and phosphorus accumulation found to occur at or near flowering time in the most cereal and legume crops (Moll *et al*., 1982). As they are presented in Table 5.2 and Fig 5.6 the highest phosphorous and nitrogen use efficiency (PUE and NUE) in the present work was obtained in the plot treated both by AM fungi and rhizobium T7 and was more significant compared to the nutrient use efficiency in recommended dose of inorganic fertilizer, this illustrate the positive relationship between nutrient use efficiency and bio-fertilizer synergistic action of symbiotic nitrogen fixation and phosphorous availability in soil. Tang *et al.* (2001) stated that mechanisms affecting phosphorous absorption and utilization efficiency are related to rhizospheric colonization of AM fungi. The shoot growth and Nitrogen fixation of legume plant are determined by phosphorous utilization efficiency and AM fungi can improve other plant growth parameters and nutrient uptake (Bucher *et al.,* 2001; Jia *et al*., 2004 and Rodino *et al*., 2009). Plants grown in soil where phosphorous nutrient is likely to be growth limiting factor benefit from AM fungi by increasing soil volume colonized by AM hyphae compare to that of non-AM plants root hairs, this explain why and how the nutrient uptake and use efficiency were more pronounced in treatments within 50%

recommended dose along with AM fungi than treatments with 100% without AM fungi. This would be in conformity with previous research studies showing potential benefits from Mycorrhizal to plant growth under moderate deficiency phosphorus especially for leguminous crops harboring harsh root system with less root hairs extension than cereals as AM fungi can improve phosphorus uptake by the plants which will result in more energy available for nitrogen fixation and uptake with other macro and micronutrients. (Tajini *et al.,* 2009; Mathimaran *et al*., 2005; Xiurong *et al*., 2011; Isobe and Tsuboki, 1998). Thus the result presented agrees with the study done by Demir and Akkopru (2007) that the dual inoculation of AM fungi and rhizobium in field pea improve nodulation; N2 fixation and phosphorus use efficiency, leading to augmentation of plant growth through to intimate interactions of all three partners during co-evolution. But this report disagree with the works that stated that AM fungus inhibit nodule development and nitrogen fixation leading to plant growth failure (Franzini *et al.,*2010). The dual inoculation of AM fungi and rhizobium in field pea crops increase accumulation of nitrogen and phosphorus which increase mineral nutrient uptake and photosynthesis of the plants leading to greater portion of photosynthates availability to the rhizobium nodules (Nautiyal *et al*., 2010)

Table: 5.2: Effect of different treatments mean on quality parameters dry matter %, N and P uptake (kg/ha), PUE and NUE (%) comparisons

	Quality parameters						
T no	dry matter %	P content (%)	Ncontent %	P uptake (kg/ha)	N uptake (kg/ha)	PUE (%)	NUE (%)
T1	13.17±0.36	0.16b±0.18	1.88c±0.10	0.52d±0.05	6.04c±0.59	N/A	N/A
T2	13.28±1.48	0.19ab±0.027	2.4b±0.05	1.07bcd±0.09	13.48ab±0.67	10.27bc±1.97	46.22b±4.77
T3	13.28±0.35	0.19ab±0.03	2.37b±0.02	0.76cd±0.11	9.64bc±1.18	4.69c±1.19	60.32ab±11.11
T4	14.52±1.18	0.18b±0.03	2.46b±0.08	0.96bcd±0.12	13.32ab±1.76	8.43c±2.83	57.88ab±5.24
T5	14.82±0.82	0.24ab±0.02	2.58ab±0.07	1.38b±0.18	14.72ab±2.24	32.79ab±9.02	42.61b±8.91
T6	14.73±1.15	0.24ab±0.02	2.57ab±0.08	1.20bc±0.13	12.81ab±1.95	17.15bc±4.53	43.28b±12.95
T7	14.72±0.68	0.27a±0.01	2.79a±0.10	1.92a±0.362	18.29a ±2.80	53.25a±15.85	75.51a±3.61

T1 (control NPK0% + no bio fertilizer); T2 (NPK100% + No Bio fertilizers); T3 (RHZ + N50%+ P 100% + K100%); T4 (RHZ + N75%+ P 100% + K100%); T5 (AMF+ N100%+ P50% + K100%), T6 (AMF+ N100%+ P75% + K100%); T7 (RHZ +AMF+ N50%+ P50% + K100%)

Data are average of three replications.
The mean followed by different letters are significantly different at $p>0.05$, according to DMRT (Duncan's Multiple Range Test) for separation of mean.

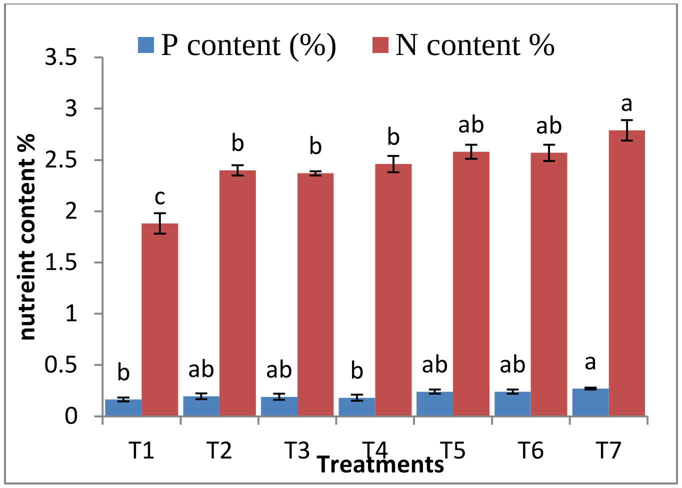


Fig: 5.4. Effect of different treatments on nutrient (P, N) content in %

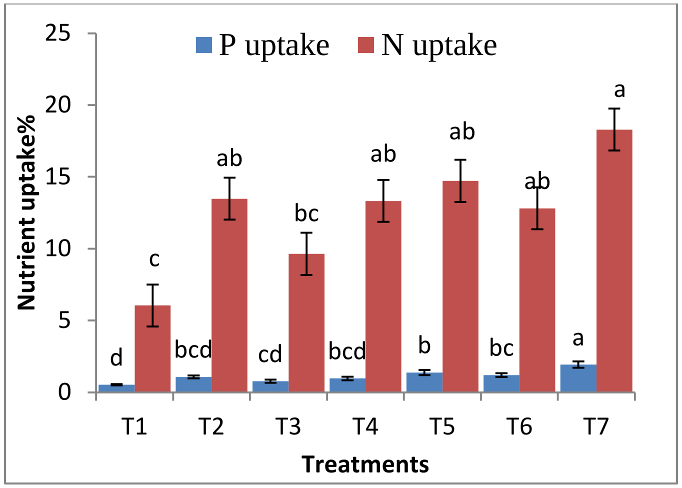


Fig: 5.5. Effect of different treatments on nutrient uptake (P, N) in kg/ha

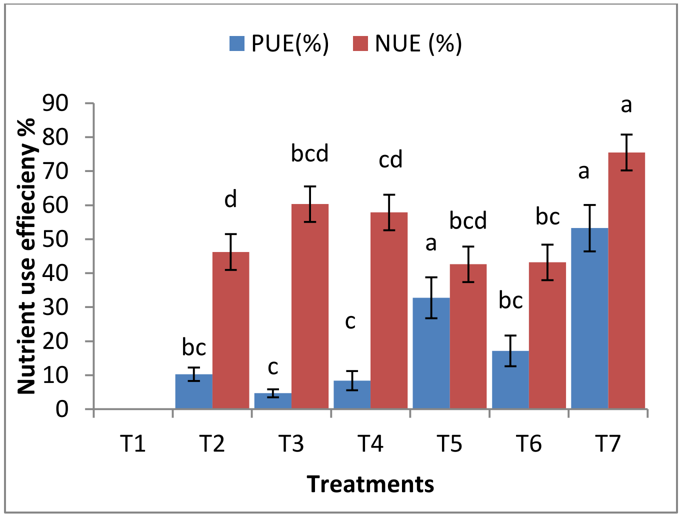


Fig: 5.6. Effect of different treatments nutrient use efficiency (P, N) in %

5.3. Yield parameters

5.3. 1. Number of pods per plant

Data in Table 5.3 and Fig 5.7 showed that the effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer were found to be significant. The T7 RHZ +AMF+ N50%+ P50% + K100% recorded the maximum number of pod (9.88) and found statistically significant over remaining treatments. Was followed by (T6) AMF+ N100%+ P75% + K100% which was statistically at par with others except T1 (control). The later showed the lowest value (4.22).

5.3. 2. Number of seeds per pod

Data presented in Table 5.3. And Fig 5.7 showed that the effect of different treatments, rhizobium and AM fungi along with different doses of mineral fertilizer were found to be significant. The T7 recorded the maximum number of seed per pod (8) and found statistically significant over other treatments. T4 with 7 seed per pod showed statistically superior to the rest of treatment. The lowest value was found in control T1 (3.48).

5.3. 3. Biomass Yield

The effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer on yield biomass were found to be significant (Table 5.3) T7 recorded the maximum biomass yield (4.43t/ha) followed by T2 NPK100% + No Bio fertilizers (4.11t/ha) which found statistically at par with other remaining treatments except T1 control NPK0% + no bio fertilizer (2.44 t/ha).

5.3. 4. Straw yield

The effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer on straw yield were not found to be significant anymore (Table 5.3) however the highest straw yield was observed under T2 NPK100% + No Bio fertilizers (3.61 t/ha) but found statistically at par with all treatment values of straw yield . And the lowest was recorded in T1 control NPK0% + no bio fertilizer (1.96 t/ha)

5.3. 5. Grain yield

Data in Table 5.3 showed that the effect of different treatments of rhizobium and AM fungi along with different doses of mineral fertilizer on grain yield was found to be significant. Application of NP 50% + AM fungi along with seed inoculated with rhizobium as in T7 recorded the maximum grain yield (0.88 t/ha) and was followed by T5 (0.758 q/ha). T4 (0.686 t/ha) was found statistically significant over other four remaining treatments among which T6, found statistically at par with T3, T2. The lowest yield was recorded in T1 control NPK0% + no bio fertilizer (0.475t/ha)

From the data presented in Table 4.3 and Fig 5.7 it has been observed that the significance increase in number of pods per plant and number of seeds per pod of field pea in the plot treated with rhizobium and AM fungi T7 9.8 pods/ par plant and 8 seeds per pod, T4 also showed good performance in number of seeds per pods. The good performance of these parameters subscribed above showed a positive correlation of synergic effect of rhizobium and AM fungi on yield of pea crop. The results are in agreement with others research works done by Shockly et al. (2004), Siviero et al. (2008) and Singh et al. (2007).

This study demonstrated how dual inoculation of rhizobium strain of pea and AM fungi affects positively biomass yield and grain yield when compared to the control as well as when compared to the yield from recommended dose of mineral fertilizers. T7 recorded the maximum biomass yield (4.43t/ha) and maximum grain yield (8.8q/ha) over other treatment (Table 4.3) this may be due to the fact that dual inoculation rhizobium and arbuscular Mycorrhizal fungi synergistically interact and improve growth plant through nutrient availability and uptake by the plant (Makoi and Ndakidemi, 2009). This result was in conformity with Aliasgharzadeh et al. (2001), Artursson and Jansson (2005) stated that the increase of seed and biological yield of dual inoculated plot is due to high reduction in seedling mortality, enhancement of nitrogen availability to pulse crops and phosphorus uptake improved by AM fungi which in return provide energy for nitrogen fixation and other chemical reaction and translocation of carbohydrates from the leaves to the sink sites. The increase in grain yield observed in treatment T7 might be due to the concomitant increase in number of pod per plant and number of seed per pods where more nutrients have been taken up by crops for high photosynthesis rate and storing of excess assimilates in the leaves subsequently its translocation into grain due to the adequate availability of nutrients, as it has been described by Kanaujia *et al.* (1999) and Mortimer *et al.* (2008)

Table 5.3: Effect of different treatments mean yield parameters (number of pod/plant, number of seed per pod, biomass, straw and grain yield) comparisons

Treatments	Yield parameters				
	Number of pods /plant	Number of seed / pod	Biomass yield T/ha	Straw yield t/ha	Grain yield t/ha
T1	4.2c±0.67	3.48d±0.59	2.44b±0.213854	1.96±0.17	0.475c±0.03
T2	6.88b±0.11	5.88c±0.11	4.11ab ±0.583771	3.61±0.54	0.564bc±0.06
T3	6.77b±0.11	5.66c±0.33	3.03ab ±0.259215	2.46±0.18	0.57bc±0.08
T4	7.66b±0.19	7.0b±0.00	3.81ab ±0.705382	3.13±0.69	0.686b±0.04
T4	7.22b±0.58	5.89c±0.19	3.82ab ±0.433948	3.06±0.41	0.758ab±0.02
T6	8.0b±0.38	6.0c±0.29	3.45ab ±0.66942	2.80±0.58	0.654bc±0.08
T7	9.88a±0.29	8.0a±0.33	4.43a ± 0.57644	3.57±0.54	0.880a±0.02

T1 (control NPK0% + no bio fertilizer); T2 (NPK100% + No Bio fertilizers); T3 (RHZ + N50%+ P 100% + K100%); T4 (RHZ + N75%+ P 100% + K100%); T5 (AMF+ N100%+ P50% + K100%), T6 (AMF+ N100%+ P75% + K100%); T7 (RHZ +AMF+ N50%+ P50% + K100%)

Data are average of three replications.

The mean followed by different letters are significantly different at p>0.05, according to DMRT (Duncan's Multiple Range Test) for separation of mean

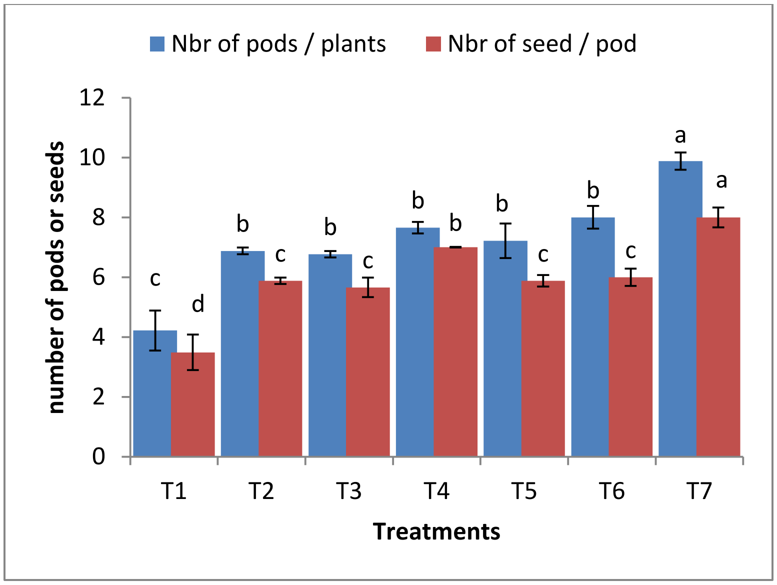


Fig 5.7: Effect of different treatments mean ± S.E number of pods/ plant and seed /pods comparisons

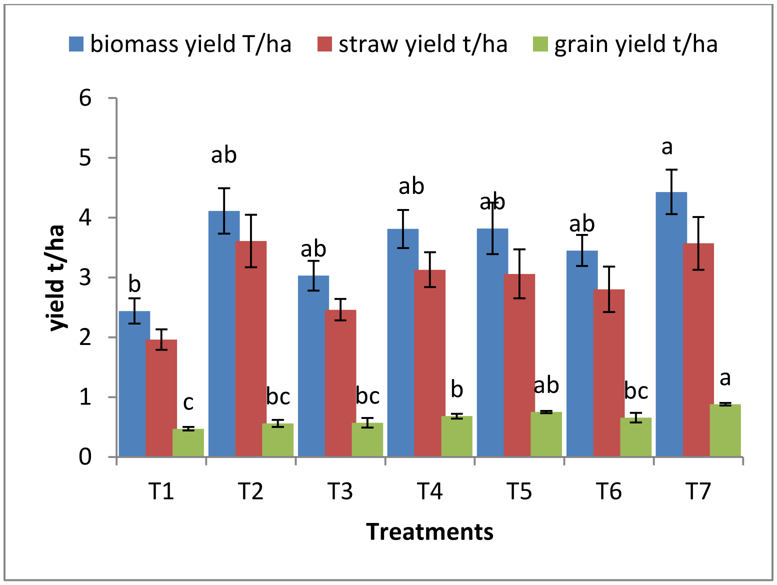


Fig5.8: Effect of different treatments means ± S.E biomass, straw and grain yield comparisons

5.4. Economic part

Referred to the data presented in table 5.4 showed that Treatment T7was statistically significance with a higher net return (Rs.13222 ha^{-1}) over control as well as plot within recommended dose of mineral fertilizer. The lowest net return was observed in control treatment with Rs.2260 ha $^{-1}$

Regarding benefit cost (B: C) ration, the highest maximum profit was recorded under T7 which was at par with T5 with a ratio of 1.00 and 0.72 respectively over the control 0.18

Table: 5.4. Effect of different treatments on Net profit and B: C ratio

Treatment	Total Cost (Rs/ha)	Total return (Rs/ha)	Net profit (Rs/ha)	B:C ratio
T1	12000	14260c±1156.3	2260c±1156.3	0.18c±0.09
T2	13976	17540bc±1408.9	3564.2c±1408.9	0.25c±0.10
T3	13926	17700bc ± 2101.1	3774.2c±2101.1	0.27c±0.15
T4	14001	20600b ± 1450.1	6599bc±1450.1	0.47bc±0.10
T5	13228	22760ab±781.02	9532.1ab±7810	0.72a±0.05
T6	13647	19640b±2500.9	5993.1bc±2500	0.44bc±0.18
T7	13178	26400 a ±827.7	13222a±827.7	1.00a±0.06

The mean followed by different letters are significantly different at p>0.05, according to DMRT (Duncan's Multiple Range Test) for separation of mean.

Benefit cost ratio B:C is a technique used to evaluate a project or any kind of investment by comparing benefits and economic costs of a project activities (Boardman, 1996). In Agriculture it is used for agriculture investment for justifying the opportunity cost in term of agricultural production and incomes. The dual inoculation of am fungi and rhizobium along with 50% of recommended mineral fertilizers recorded higher productivity and profitability due several reasons: Rhizobium and AM fungi application reduce the cost of cultivation through to minimization of nitrogen and phosphorus fertilizer and in return improve productivity by its ability to increase nutrient uptake by the plant and nutrient use efficiency. These result are in agreement with Bai (2014), who reported that the B:C ratio increase with rhizobium and AM fungi application in field pea.

5.5. PCA ordination plot

PCA(principle component analysis) ordination plot of different biofertilzer along with different level of mineral application as treatments on number of leaves and branches, plant height in Cm (30, 60 90DAS,) ;Dry matter (%), N, P content (%) , NUE(%), PUE(%) ; N uptake (Kg/ha); P uptake (Kg/ha) biomas yield (t/ha), grain yield (kg/ha) net profit (Rs/ha) and B.C ratio for field pea crops. Shorter distance between sites in PCA ordination indicates high degree of similarity between treatments. Component 1 and 2 represent 78.53% and 18.94% respectively.

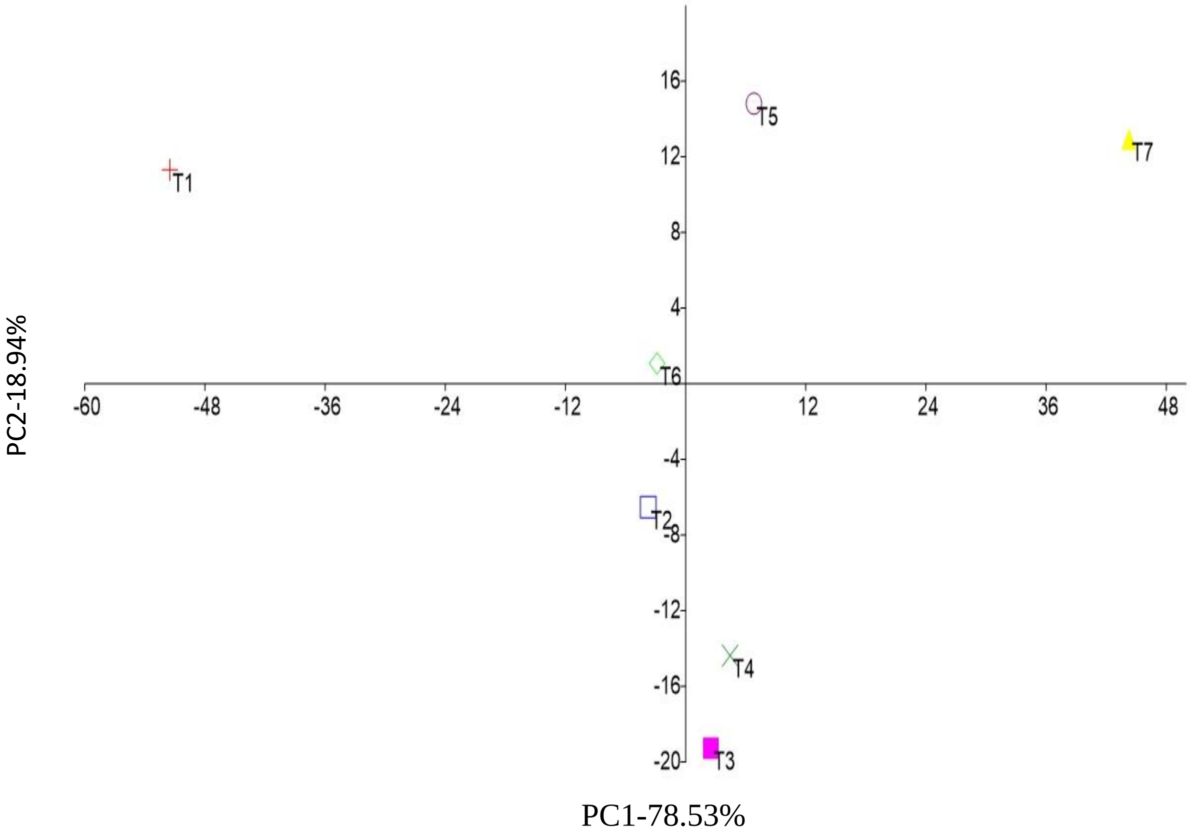


Fig 5.9: PCA plot diagram

Chapter VI

6. Summary and Conclusion

Results from the field experiment “Nitrogen and Phosphorus Economy Through Dual Inoculation of Rhizobium and Arbuscular Mycorrhizal (AM) Fungi in Field Pea” presented in chapter v are summarized:

1. Integration of dual bio-fertilizer, AM fungi and rhizobium along with inorganic fertilizer brought about a significant increase of number of leaves per plants, plant height at 60 and 90 DAS and also number of branches at 90 DAS.
2. T7 proved the most superior treatment in term of nutrient content, nutrient uptake, and nutrient use efficiency compared to other plots treated with single bio-fertilizer or control.
3. The integration of bio-fertilizer Rhizobium and Arbuscular Mycorrhizal (AM) Fungi) with 50% of inorganic fertilizer as in T7 showed a significant increase in number of pods per plant, number of seed per pod, biomass yield grain yield compared to the remaining treatments.
4. The highest net profit and benefit cost ratio (B:C) recorded under T7 and T5 and was significantly different from other treatments.

The results of current study work exhibited many beneficial effects of dual inoculation with rhizobium and arbuscular mycorrhizal fungi on growth, yield and profit of field pea crops along with low level of inorganic fertilizer rather than using a hundred percent recommended dose of mineral fertilizer. Seven treatments applied during experiment were studied to investigate the effect of dual inoculation of Rhizobium and AM fungi on Nitrogen and phosphorus economy in field pea. The result from this study suggested that the technology of applying Rhizobium and AMF along with half of recommended dose of mineral fertilizer as integrated nutrient management would increase productivity and profitability due several reasons Rhizobium and AM fungi application reduce the cost of cultivation through minimization of nitrogen and phosphorus fertilizer and in return improve productivity by its ability to increase nutrient uptake by the plant and nutrient use efficiency over recommended dose of mineral fertilizers.

Bibliography

Aliasgharzadeh, N., Rastin, S., Towfighi, H. and Alizadeh, A. (2001). Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza*, 11(3), pp.119-122.

Allen, E. and Allen, M. (1980). Natural Re-Establishment of Vesicular-Arbuscular Mycorrhizae Following Stripmine Reclamation in Wyoming. *The Journal of Applied Ecology*, 17(1), p.139.

An, J. and Kim, Y. (1998). A gene cluster encoding malonyl-CoA decarboxylase (MatA), malonyl-CoA synthetase (MatB) and a putative dicarboxylate carrier protein (MatC) in Rhizobium trifolii . Cloning, sequencing, and expression of the enzymes in Escherichia coli. *Eur J Biochem*, 257(2), pp.395-402.

Anand, R. and Dogra, R. (1997). Comparative efficieny of Rhizobium/Bradyrhizobium spp. strains in nodulating Cajanus cajan in relation to characteristic metabolic enzyme activities. *Biology and Fertility of Soils*, 24(3), pp.283-287.

Andrade, De Leij, and Lynch, (1998). Plant mediated interactions between Pseudomonas fluorescens, Rhizobium leguminosarum and arbuscular mycorrhizae on pea. *Letters in Applied Microbiology*, 26(4), pp.311-316.

Artursson, V., Finlay, R. and Jansson, J. (2005). Combined bromodeoxyuridine immunocapture and terminal-restriction fragment length polymorphism analysis highlights differences in the active soil bacterial metagenome due to Glomus mosseae inoculation or plant species. *Environmental Microbiology*, 7(12), pp.1952-1966.

Ashraf zahid, M., Muhammad Iqbal, S., Ali, A. and Hussain,S. (2007). Efficacy of microbial bioagents for the control of collar rot disease in chick pea. *Pak.J.Bot*, 39(7): pp.2667-2672.

BÃ¤ssler, M. (1984). Allen, O. N. & Allen, Ethel K., The Leguminosae. A Source Book of Characteristics, Uses and Nodulation. LXIX + 812 Pp., 132 figures, 12 photographs. The

University of Wisconsin Press. Madison, Wisconsin, USA, 1981. *Feddes Repertorium*, 95(1-2), pp.127-128.

Bagyaraj, D., manjunath, A. and patil, R. (1979). Interaction between a vesicular-arbuscular mycorrhiza and rhizobium and their effects on soybean in the field. *New phytologist*, 82(1), pp.141-145.

Bahadur, A., Singh, J., Singh, K. 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), pp. 464-466.

Bai B.(2014). Nitrogen and Phosphorus economy through inoculation with Arbuscular Mycorrhizal Fungi (AMF) and Rhizobium in garden pea (Pisum sativum L.), M.Sc. Thesis, CSK HPKV, Palampur (HP).

Bai J., Yin R., Zhang H., Wang H., Chen X. and Luo Y. (2008). The Influence of Arbuscular Mycorrhizal Fungi on As and p Uptake by Maize (*Zea mays* L.) from As-Contaminated Soils, *Applied Soil Ecology*, 38(2), pp. 137-145

BAREA, J., AZCON, R. and AZCON-AGUILIAR C., 2002 *Mycorrhizosphere interactions to improve plant fitness and soil quality*. Antone van Leeuwenhock, 81, pp.343-351.

Barea, J., azcon-aguilar, C. And azcon, R. (1987). Vesicular-arbuscular mycorrhiza improve both symbiotic n2 fixation and n uptake from soil as assessed with a 15n technique under field conditions. *New phytologist*, 106(4), pp.717-725.

Barea, J. (1997) Mycorrhiza-bacteria interactions on plant growth promotion. In Plant Growth Promoting Rhizobacte ria. Ogoshi, A., Kobayashi, K., Homma, Y., Kodama, F., Kondo, N., and Akino, S. (eds). Paris, France: OECD Press, pp. 150–158.

Barea, J. (2002) Rhizosphere and mycorrhiza of field crops. In Biological Resource Management: Connecting Science and Policy (OECD). Toutant, J.P., Balazs, E., Galante, E., Lynch, J.M., Schepers, J.S., Werner, D., and Werry, P.A. (eds). Berlin: INRA, Editions and Springer, pp. 110–125.

Barea,J., Azcon, R. and Azcon-Aguilar C. 1992. arbiscular mycorrhizal fungi. In: nitrogen-fixing systems. Methods Microbiol. pp.391-416.

Batut, J., Andersson, S. and O'Callaghan, D. (2004). The evolution of chronic infection strategies in the alpha-proteobacteria. *Nat Rev Micro, 2(12), pp.933-945*.

Bhat,M., Rashid, A., Faisul,R., Mahdi,S., Haq,S. and Raies A. 2010. Effect of *Rhizobium* and Vesicular arbuscular mycorrhizae fungi on green Gram (*Vigna radiata L.Wilczek*) under temperate conditions. *Research Journal of Agricultural Sciences.*, 1(2), pp.113-118.

Biró, B., Köves-Péchy, K., Vörös,I., Takács, T., Eggenberger, P. and Strasser, R. (2000) Interrelations between Azospirillum and Rhizobium nitrogen fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile AMF- free or normal conditions. *Appl Soil Ecol* 15, pp.159–168

Biswas, A. and Patra, A.(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.

Black C. 1965. Methods of soil analysis Part I Am. Soc. Agron. Inc. Publi. Madison Wisconsin USA.

Boardman A., Greenberg D., Vining A. and Weimer D. (1996). *Cost Benefit Analysis: Concepts and Practice*. Englewood Cliffs, NJ: Prentice Hall.

Bolan, N., Robson, A. and Barrow, N. (1987). Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. *Plant Soil*, 99(2-3), pp.401-410.

Borch, K., Brown, K. and Lynch, J. 1998. Improvement of bedding plant quality and stress resistance with low phosphorus. *Hort Technology* 8, pp.575–579.

Brockwell, J., Bottomley, P. and Thies, J. (1995). Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. *Plant Soil*, 174(1-2), pp.143-180.

Bucher, M., Rausch, C. and Daram, P. (2001). Molecular and biochemical mechanisms of phosphorus uptake into plants. *Journal of Plant Nutrition and Soil Science*, 164(2), pp.209-217.

Burchi, F., Fanzo, J. and Frison, E. (2011). The Role of Food and Nutrition System Approaches in Tackling Hidden Hunger. *IJERPH*, 8(12), pp.358-373.

Chakrabarty J., Chatterjee N. and Dutta S. (2007). Interactive Effect of VAM and *Rhizobium* on Nutrient Uptake and Growth of *Vigna mungo*. *Journal of Mycopathology Re-search*, 45 (2), pp. 289-291.

Chapman, H. (1965). Cation-exchange capacity. In: Black CA (ed.). Methods of soilanalysis - Chemical and microbiological properties. *Agronomy* 9, pp.891-901.

Chaurasia,B. and Khare,P. (2005). *Hordeum vulgare*: A suitable host for mass production of arbuscular mycorrhizal fungi from naturalsoil. *J. Applied ecology and environmental research*.4(1), pp.45-53.

Clark, A. (ed.) 2007. Managing cover crops profitably. 3rd ed. Sustainable agriculture research and education program handbook series, bk 9. Sustainable Agriculture Research and Education, College Park, MD

Clark, R. and Zeto, S. (2000). Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition*, 23(7), pp.867-902.

Clugston, G. and Smith, T. (2002). Global nutrition problems and novel foods. *Asia Pac J Clin Nutr*, 11(s6), pp.S100-S111.

DÃ©nariÃ©, J., DebellÃ©, F. and PromÃ©, J. (1996). Rhizobium Lipo-Chitooligosaccharide Nodulation Factors: Signaling Molecules Mediating Recognition and Morphogenesis. *Annu. Rev. Biochem.*, 65(1), pp.503-535.

Daniels, B. and Trappe, J. (1980). Factors Affecting Spore Germination of the Vesicular-Arbuscular Mycorrhizal Fungus, Glomus epigaeus. *Mycologia*, 72(3), p.457.

Demir S. and Akkopru A. (2007). Using of Arbuscular Mycorrhizal Fungi (AMF) for biocontrol of soil-borne fungal plant pathogens Chincholkar, Mukerji (Eds.), Biological control of plant diseases, Haworth Press, USA ,pp. 17–37

Denarie, J., Debelle, F. and Prome, J. (1996). *Rhizobium* lipochitooligosaccharide nodulation factors - signaling molecules mediating recognition and morphogenesis. *Annual Review of Biochemistry*. 65, pp.503-535.

DjÃ©bali, N., Turki, S., Zid, M. and Hajlaoui, M. (2010). Growth and development responses of some legume species inoculated with a mycorrhiza-based biofertilizer. *Agriculture and Biology Journal of North America*, 1(5), pp.748-754.

Elzebroek, T., and Wind K. (2008). Guide to cultivated plants. CAB International, Oxfordshire, UK.

Fageria, N., Baliger V and Jones C. 1997. *Growth and mineral nutrition of field crops*. 2nd Ed. Marcel Dekker, Inc. NY, USA.

Food and Agriculture Organization. (2012). [Online] Available at: http://faostat.fao.org (Accessed 24 April 2015). FAO, Rome

Fujiyoshi,M., Nakatsubo, T., Ogura, S. and Horikoshi, T. (2000). Estimation of mycelial biomass of arbuscular mycorrhizal fungi associated with the annual legume *Kummerowia striata* by ergosterol analysis. *Ecol.Res.*,15, pp. 121-131.

Gage, D. (2004). Infection and Invasion of Roots by Symbiotic, Nitrogen-Fixing Rhizobia during Nodulation of Temperate Legumes. *Microbiology and Molecular Biology Reviews*, 68(2), pp.280-300.

Gamalero, E., Martinotti, M., Trotta, A., Lemanceau, P. and Berta, G. (2002). Morphogenetic modifications induced by Pseudomonas fluorescens A6RI and Glomus mosseae BEG12 in the root system of tomato differ according to plant growth conditions. *New Phytologist*, 155(2), pp.293-300.

Garbaye, J. (1994). Tansley Review No. 76 Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytologist*, 128(2), pp.197-210.

Geneva, M., Zehirov, G., Djonova, E., Kaloyanova, N., Georgiev, G. and Stancheva I. (2006). The effect of inoculation of pea plants with mycorrhizal fungi and rhizobium on nitrogen and phosphorus assimilation. Plant and Soil Environment, 52(10), pp.435-440.

Gill, N. andVear. 1980. Agricultural botany. 3rd ed. (K.C. Vear and D.J. Barnard eds.) Gerald Duckworth and Co., Ltd, London.

Gryndler, M. (2000) Interactions of arbuscular mycorrhizal fungi with other soil organisms. In Arbuscular Mycorrhizas: Physiology and Function. Kapulnik, Y., and Douds, D.D.J. (eds). Dordrecht, the Netherlands: Kluwer Academic Pub- lishers, pp. 239–26

Hakoomat A., Muhammad A. And Shakeel R. (2004). Interactive effect of seed inoculation and phosphorus application on growth and yield of Chick pea(*Cicer arietinum L.*). *International journal of Agricultural Biology*. 6, pp.95-110.

Hanlon EA, McNeal BL, Kidder G. 1993. Electrical Conductivity Interpretations. Fla. Coop. Extn. Ser., IFAS, Univ. of Fla., Gainesville, FL.

Hartnett, D. and Wilson, G. (1999). Mycorrhizae Influence Plant Community Structure and Diversity in Tallgrass Prairie. *Ecology*, 80(4), p.1187.

Hay, R. (1998). Growth and Mineral Nutrition of Field Crops. By N. K. FAGERIA, V. C. BALIGER and C. A. JONES. 23x15 cm. Pp. xi+624 with 173 text-figures. New York: Marcel Dekker Inc.: 2nd Edition, 1997. Price h/b: $195.00, ISBN 0 8247 0089 9. *New Phytologist*, 138(4), pp.743-750.

Hegde, D., Dwivedi, B. and Sudhakara S. (1999). Biofertilizers for Cereal Production in India Review. *The Indian Journal of Agricultural Sciences* **69,** pp.73-83.

Hodge, A., Campbell, C. and Fitter, A. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, 413(6853), pp.297-299.

Ide Franzini, V., AzcÃ³n, R., Latanze Mendes, F. and Aroca, R. (2010). Interactions between Glomus species and Rhizobium strains affect the nutritional physiology of drought-stressed legume hosts. *Journal of Plant Physiology*, 167(8), pp.614-619

Isobe, K. and Tsuboki, Y. (1998). The Relationship between Growth Promotion by Arbuscular Mycorrhizal Fungi and Root Morphology and Phosphorus Absorption in Gramineous and Leguminous Crops. *Japanese journal of crop science*, 67(3), pp.347-352.

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

Jackson M. L. 1967. *Soil Chemical Analysis*. Prentice Hall of India, Limited, New Delhi. P 219 221

JIA, Y. (2004). The Influence of Rhizobium and Arbuscular Mycorrhizal Fungi on Nitrogen and Phosphorus Accumulation by Vicia faba. *Annals of Botany*, 94(2), pp.251-258.

Johnson, N., Copeland, P., Crookston, R. and Pfleger, F. (1992). Mycorrhizae: Possible Explanation for Yield Decline with Continuous Corn and Soybean. *Agronomy Journal*, 84(3), p.387.

Kanaujia, S., Rastogi, K., Sharma, S. and Raj, N. (1999). Response of phosphorus, potassium and *rhizobium* inoculation on pod yield and economics of pea *(Pisum sativum* L.) cv. Lincon. *Haryana Journal of Horticulture Sciences*, 28, pp. 117-118.

Karim M., Islam F., Akkas Ali M. and Haque F. (2001) "On- Farm Trail with *Rhizobium* Inoculants on Lentil," *Bang-ladesh Journal of Agricultural Research*, 26, pp. 93-94

Kemery, R. and Dana,M. (1995). Prairie remnant soil as a source of mycorrhizal inoculum. *Hort science* 30(5), pp.1015-1016.

Knee, E., Gong, F., Gao, M., Teplitski, M., Jones, A. , Foxworthy, A., Mort, A. and Bauer, W. (2001). Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. *Molecular Plant- Microbe Interactions.* 14, pp.775-784.

Koske, R. (1981). A preliminary study of interactions between species of vesicular-arbuscular fungi in a sand dune. *Transactions of the British Mycological Society*, 76(3), pp.411-416.

Kucey, r. And bonetti, r. (1988). Effect of vesicular-arbuscular mycorrhizal fungi and captan on growth and N2 fixation by rhizobium -inoculated field beans. *Can. J. Soil. Sci.*, 68(1), pp.143-149.

Kumar, B., Ram, M. and Mallaiah K. (2010). Studies on nodulation, biochemical analysis and protein profiles of Rhizobium isolated from Indigofera species. *Malaysian Journal of Microbiology*, 6(2), pp.133-139

Kumar, V. and Sharma,R. (2006). Path coefficient analysis for pod yield and its attributes in garden pea (Pisum sativum L. sub sp. hortense Asch. and Garebn.). *Annals Agric. Res*. 27(1), pp.32-36

Kytoviita, M. (2000). Do symbiotic fungi refresh themselves by incorporating their own or closely related spores into existing mycelium?. *Oikos*, 90(3), pp.606-608.

Linderman, R.(2000) Effects of mycorrhizas on plant tol- erance to diseases. In Arbuscular Mycorrhizas: Physiology and Function. Kapulnik, Y., and Douds, D.D.J. (eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, pp. 345–365.

Lipman J. and Conybeare A. (1936) “Preliminary Note on the Inventory and Balance Sheet of Plant Nutrients in the United States,” *New Jersey Agricultural Experiment Station Bulletin*, p. 607.

Lugo, M. and Cabello, M. (2002). Native Arbuscular Mycorrhizal Fungi (AMF) from Mountain Grassland (Cordoba, Argentina) I. Seasonal Variation of Fungal Spore Diversity. *Mycologia*, 94(4), p.579.

Makoi, J. and Ndakidemi, P. (2009). The agronomic potential of vesicular-arbuscular mycorrhiza (VAM) in cereals– legume mixtures in Africa. *African* Journal of *Microbiology* Research, 3, pp. 664-675.

Mandimba, R. (1995). Contribution of nodulated legumes on the growth of *Zea mays* L. under various cropping systems. *Symbiosis*.19, pp.213–222

Manke, N., Potdukhe, S., Bramhankar,S. and Padghan, P.(2008). Effect of Rhizobium and VAM on growth parameters and yield of mungbean. *Journal of Plant Disease Sciences*. 3(2): pp.145-159

Mathimaran, N., Ruh, R., Vullioud, P., Frossard, E. and Jansa, J. (2005). Glomus intraradices dominates arbuscular mycorrhizal communities in a heavy textured agricultural soil. *Mycorrhiza*, 16(1), pp.61-66.

McGee, R. (2012).Seed yield of Austrian winter field peas intercropped with winter cereals. *Agron. J.* 77, pp. 913-91

McGONIGLE, T., MILLER, M., EVANS, D., FAIRCHILD, G. and SWAN, J. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, 115(3), pp.495-501.

Meyer, J. and Linderman, R. (1986). Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, Pseudomonas putida. *Soil Biology and Biochemistry,* 18(2), pp.185-190.

Mia M. and Shamsuddin, Z. (2010). Rhizobium as a crop enhancer and biofertilizer for increased cereal production. Afr J Biotechnol 9: 6001–6009

Miller, R., Jastrow, J. and Reinhardt, D. (1995). External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia*, 103(1), pp.17-23.

Mohd, S. and Siddiqui, Z. (2009).Effects of phosphate solubilizing micro organisms and *Rhizobium sps.* On the growth, nodulation, yield and root-rot disease complex of Chick pea under field condition. *African Journal of Biotechnology*. 8(15), pp.3489-3496.

Moll, R., Kamprath, E. and Jackson, W. (1982). Analysis and Interpretation of Factors Which Contribute to Efficiency of Nitrogen Utilization1. *Agronomy Journal*, 74(3), p.562.

Moradi, R., Rezvani Moghaddam, P., Nasiri Mahallati, M. and Nezhadali, A. (2011). Effects of organic and biological fertilizers on fruit yield and essential oil of sweet fennel (Foeniculum vulgare var. dulce). *Spanish Journal of Agricultural Research*, 9(2), p.546.

Mortimer, P., PÃ©rez-FernÃ¡ndez, M. and Valentine, A. (2008). The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated Phaseolus vulgaris. *Soil Biology and Biochemistry,* 40(5), pp.1019-1027.

Morton, J. B. and G. L. Benny(1990). Revised classification of arbuscular mycorrhizal fungi (zy gomycetes) - a new order, glomales, 2 new suborders, glomineae and gigasporineae, and 2 new families, acaulosporaceae and gigasporaceae, with an emendation of glomaceae. *Mycotaxon* 37, pp. 471-491.

Mukherjee, P. and Rai, R. (2000). Effect of arbuscular mycorrhizae and phosphate solubilizing bacteria on growth, yield and phosphorus uptake in wheat (*Triticum aestivam*). *Indian. J. Agron.* 45(3), pp. 602-607

Murray, G. and Swensen, J. (1985). Seed Yield of Austrian Winter Field Peas Intercropped with Winter Cereals1. *Agronomy Journal*, 77(6), p.913.

Nautiyal, C., Chauhan, P., DasGupta, S., Seem, K., Varma, A. and Staddon, W. (2010). Tripartite interactions among Paenibacillus lentimorbus NRRL B-30488, Piriformospora indica DSM 11827, and Cicer arietinum L. *World J Microbiol Biotechnol*, 26(8), pp.1393-1399.

Nehl, D., Allen, S. and Brown, J. (1997). Deleterious rhizosphere bacteria: an integrating perspective. *Applied Soil Ecology*, 5(1), pp.1-20.

Newton, W. (2000). Nitrogen fixation in perspective. In *Nitrogen fixation: from molecules tocrop productivity*., *F. O. Pedrosa, M. Hungria, M. G. Yates & W. E. Newton(Eds)Kluwer Publishers*. *Dortrecht, Netherlands* pp. 3-8

Oliveira, A. and Oliveira, L. (2005). Seasonal dynamics of arbuscular mycorrhizal fungi in plants of Theobroma grandiflorum Schum and Paullinia cupana Mart. of aN agroforestry system in Central Amazonia, Amazonas State, Brazil. *Brazilian Journal of Microbiology*, 36(3), pp.262-270.

Olsen, S., Cole, C., Watanabe, F. and Dean L. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dept. Agric. 939:1-19

Pandey, D., Singh, J., Kashyap, N. and Dwivedi, G.(2003). Response of vesicular arbuscular mycorrhyzae (VAM), *rhizobium* and phosphrous sources on nodulation, growth, yield of pea cv. VL-7. *Crop Research,* 25 (2), pp. 333-336.

Pinton, R., Varanini, Z. and Nannipieri, P. (2001). The rhizosphere as a site of biochemical interactions among soil components, plants and microorganisms. *In Rhizosphere : Biochemistry and Organic Substances at the Soil-plant Interface.Pinton, R, Vananini, Z & Nannipieri (Eds) Marcel Dekker Publishers, New York* p1-18.

Piper C. (1950). Soil and plant analysis. Academic press. New York. Fide: Majumdar, SP, Singh RA. 2000. In: Analysis of soil physical properties. Agrobios (India), Jodhpur, V. pp. 105-151.

Rajendran, K. and Jayasree, R. (2007). Effect of Biofertilizers on quality seedling production of *Acacia nilotica*. *Journal of Non-timber products*, 14, pp. 5-11.

Ramana, V., Ramakrishna, M., Purushotham, K. and Reddy, K. (2010). Effect of biofertilizers on growth, yield attributes and yield of French bean (*Phaseolus vulgaris* L.). *Legume Research*, 33 (3).

Rambelli, A. (1973) The rhizosphere of mycorrhizae. In Ectomycorrhizae. Marks, G.L., and Koslowski, T.T. (eds). New York, USA: Academic Press, pp. 299–343.

Rathje, (1959). Jackson, M. L.:Soil chemical analysis. Verlag: Prentice Hall, Inc., Englewood Cliffs, NJ. 1958, 498 S. DM 39.40. *Z. Pflanzenernaehr. Dueng. Bodenk.*, 85(3), pp.251-252.

RICHARDS, L. (1954). Diagnosis and Improvement of Saline and Alkali Soils. *Soil Science*, 78(2), p.154.

Rodino, A., Metral, R., Guglielmi, S. and Drevon, J. (2009). Variation among common-bean accessions (Phaseolus vulgaris L.) from the Iberian Peninsula for N2-dependent growth and phosphorus requirement. *Symbiosis*, 47(3), pp.161-174.

Sankaranarayan, C. and Sundarababu, R. (2001). Influence of moisture and pH on the efficieny of vesicular mycorrhiza, Glomus mosseae against Meloidogyne incognita on black gram(vagna mungo L.). *J.Biol. Control*. 15(1), pp. 69-72.

Schubler, A., Schwarzott, D.and Walker, C. (2001). A new fungal phylum, the Glomeromycota:phylogeny and evolution. *Mycol.Res*. 105, pp.1413-1421.

Sharma, D., Yadav, A., Sharma, R., Yadav, A. and Sharma, R. (1997). Effect of dates of sowing and phosphorus fertilization on growth, pod yield and disease in pea (*Pisum sativum* L.). *Annals of Agricultural Research*, 18 (4), pp. 564-566.

Shockley, F., McGraw, R. and Garrett, H. (2004). Growth and nutrient concentration of two native forage legumes inoculated with Rhizobium and Mycorrhiza in Missouri, USA. *Agroforestry Systems*, 60(2), pp.137-142.

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

Siviero, M., Motta, A., Lima, D., Birolli, R., Huh, S., Santinoni, I., Murate, L., Castro, C., Miya-uchi, M., Zangaro, W., Nogueira, M. and Andrade, G. (2008) Interaction among N-fixing bacteria and AM fungi in Amazonian legume tree (*Schizolobium amazonicum*) in field conditions. *Applied Soil Ecology*, **39**, pp. 144–152.

Smith, S. and Read, D. (1997). Mycorrhizal Symbiosis. Second Edition. Academic Press, Toronto, Canada.

Smith, S. E., D.J Read. 1997. Mycorrhizal symbiosis. San Diego :, Academic Press.

Snell, P.D. and Snell, G.T. 1949. *Calorimetric methods of analysis*, 3rd End. Vol. II D. Van. Nostrand Co., Inc., New York.

Staddon, P. Heinermeyer and Fitter, A.(2002). Mycorrhizae and global environmental change. *Research at different scales. Plant and soil*. 244, pp.253-261

Subba Roa NS. 2001. An appraisal of biofertilizers in India. The biotechnology of biofertilizers, ed. S.Kannaiyan, *Narosa Pub. House, New*.

Subbiah, B. Asija G. (1956). A rapid procedure for the estimation of nitrogen in soils. Curr.Sci. 25, pp.259-260.

Sylvia,D. and WilliamsS.(1992). Vesicular arbuscular mycorrhizae and environment stress,.In :Mycorrhizae in sustainable agriculture, Eds., Bethlenfelvay, G.J and R.G. Linderman publication-54, American society of Agron, Madison, pp:101-124

Tajini, F., Suriyakup, P., Vailhe, H., Jansa, J. and Drevon, J. (2009). Assess suitability of hydroaeroponic culture to establish tripartite symbiosis between different AMF species, beans, and rhizobia. *BMC Plant Biology*, 9(1), p.73.

Talaat N. and Abdallah A. (2008). Response of Faba Bean (*Vicia fava* L.) to Dual Inoculation with *Rhizobium* and VA Mycorrhiza under Different Levels of N and P Fertilization. *Journal of Applied Sciences Research*, 4,(9), pp. 1092-1102.

Tang, C., Hinsinger, P., Jaillard, B., Rengel, Z. and Drevon, J. (2001). Effect of phosphorus deficiency on the growth, symbiotic N 2 fixation and proton release by two bean (Phaseolus vulgaris) genotypes. *Agronomie*, 21(6-7), pp.683-689.

Tate R. (2003) . Soil microbiology (symbiotic nitrogen fixation) New York, N.Y: John Wiley & Sons, Inc., pp. 307–333.

Tavasolee A., Aliasgharzad N., Salehijouzani G., Mardi M. and Asgharzadeh A. (2011). “Interactive Effects of *Arbuscular mycorrhizal* Fungi and *rhizobial* Strains on Chickpea Growth and Nutrient Content in Plant,” *African Journal of Microbialogy*, 10, pp. 7585-7591.

Tomar, A., Kumar, N., Pareek, R. and Chaube, A. (2001). Synergism among VAM, phosphate solubilizing bacteria and *rhizobium* for symbiosis with black gram (*Vigna mungo* L.) under field conditions. *Pedosphere*, 11 (4), pp. 327-332.

Toro, M., azcon, R. And barea, J. (1998). The use of isotopic dilution techniques to evaluate the interactive effects of Rhizobium genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by Medicago sativa. *New Phytologist*, 138(2), pp.265-273.

van der Heijden, M., Klironomos, J., Ursic M., Moutoglis P., Streitwolf- Engel R. , Boller T., Wiemken, A. and Sanders. I. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. nature 396(6706), pp. 69-72

VAVILOV, N. (1951). The Origin, Variation, Immunity and Breeding of Cultivated Plants. *Soil Science*, 72(6), p.482.

Viera-Vargas, M., Souto, C., Urquiaga, S. and Boddey, R. (1995). Quantification of the contribution of N2 fixation to tropical forage legumes and transfer to associated grass. *Soil Biology and Biochemistry*, 27(9), pp.1193-1200.

Vivas, A., AzcÃ³n, R., BirÃ³, B., Barea, J. and Ruiz-Lozano, J. (2003). Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of Trifolium pratense L. under lead toxicity. *Can. J. Microbiol.*, 49(10), pp.577-588.

Walker, T., Bais, H., Grotewold, E. and Vivanco, J. (2003). Root exudation and rhizosphere biology. *Plant Physiology*. 132, pp.44-51.

Walkley, A. and Black. I. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci*., 37,pp. 29-37.

Wang, X., Pan, Q., Chen, F., Yan, X. and Liao, H. (2010). Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. *Mycorrhiza*, 21(3), pp.173-181.

Wani, S. and Lee K. (2002). Population dynamics of nitrogen fixing bacteria associated with pearl millet (*P. americanum* L.). In: biotechnology of nitrogen fixation in the tropics, University of Pertanian, Malaysia, 21-30

Warner, A. and Mosse, B. (1980). Independent spread of vesicular-arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society*, 74(2), pp.407-410.

World Bank 1996. Natural resource degradation in Sub-Saharan Africa: Restoration of soil fertility: A Concept Paper and Action Plan.Washington, DC. Mimeo.

Xavier, L. (2002). Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy. *Soil Biology and Biochemistry*, 34(2), pp.181-188.

Yadav, O. (2001). Effect of nitrogen sources and biofertilizer on growth and quality of cowpea. M.Sc. (Ag.) Thesis, Rajasthan Agricultural University, Bikaner

Yawalkar, K., Agarwal, J. and Bokde, S. (1996). Manures and fertilizers. Edi. VIII, Agri Horticultural Publishing House 52, Nagpur, 297-299.

Zuccarni, P.(2007).Mycorrhizal Infection Ameliorates Chloro- phyll Content and Nutrient Uptake of Lettuce Exposed to Saline Irrigation. *Plant Soil Environment*, 53, pp.283-289.

Appendix

Picture showing Crop view after germination

Plant stand in field 40 DAS

Weeding operations

Crop view at flowering time

Plant analysis for nutrients (phosphorous) estimation in laboratory

Plant at its full pod filling stage

Biomass harvested into the polyethylene Bags