

**EFFECT OF DIFFERENT BIOFERTILIZERS ON GROWTH
AND YIELD PARAMETERS OF WHEAT – PEA
INTERCROPPING SYSTEM**

THESIS

Submitted to the

**LOVELY PROFESSIONAL UNIVERSITY,
PHAGWARA, PUNJAB, INDIA**

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

MASTER OF SCIENCE
IN
(AGRONOMY)
BY

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Under the supervision of
Dr. Chandra Mohan Mehta



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PHAGWARA, PUNJAB, INDIA**

2015

CERTIFICATION

This is to certify that the thesis entitled “**Effect of Different Biofertilizers on Growth and Yield Parameters of Wheat – Pea Intercropping System**” submitted in partial fulfilment of the requirements for the degree of Master of Science in Agronomy of the Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, is a record of bonafide research carried out by **BYIRINGIRO Emmanuel**, Registration No. 11313053 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.

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ACKNOWLEDGEMENTS

Though only my names appear on the cover of this thesis, Almighty God and different people contributed to its completion. My thanks go to those people who made it possible and I will cherish their support forever.

I am greatly indebted to my Advisor, Dr. Chandra Mohan Mehta for undertaking the task of supervising this work, his willingness, guidance and assistance in this research. I gained a tremendous amount of knowledge under his supervision.

I thank my co-advisors, Dr. Balkrishna Sopan Bhople (HOD of Agronomy) and Dr. Amit Kesarwani, for their help, encouragements and advices. My special thanks go to all my teachers especially those from Department of Agronomy, School of Agriculture, who passed before me during the whole my master's degree from Lovely Professional University, for their encouragements and unlimited supports.

Furthermore, I am grateful to all my friends and colleagues for their moral support which helped me to stand and focus on my studies. I acknowledge and appreciate their friendship.

I am very much thankful to my family for their love and patience. I am indebted to my parents, uncles, aunts, brothers, and sisters to whom I dedicate this thesis. I extend my thanks to my family members especially my parents for giving birth to me at the fifth place and supporting spiritually throughout my life. I am extremely grateful to Dr. Arun Kumar for his support and comments which helped to explore myself.

Finally, I recognize the financial support from Government of Rwanda through Rwanda Education Board (REB) that sponsored me for the whole programme.

BYIRINGIRO Emmanuel

DEDICATION

To Almighty God, the Creator,

To my past relatives,

To my Mother and my Father,

To my Uncles and Aunts,

To my Brothers and Sisters,

To my Cousins and Nephews,

To all my Friends and Colleagues

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LIST OF ABBREVIATIONS AND ACRONYMS

%	:	Percentage
AMF	:	Arbuscular Mycorrhizal Fungi
CEC	:	Cation Exchange Capacity
DAS	:	Days After Sowing
DMRT:		Duncan Multiple Range Test
dS/m	:	deciSiemens per metre
EC	:	Electrical Conductivity
FYM	:	Farm Yard Manure
IAA	:	Indole-3-Acetic Acid
IPNM	:	Integrated Plant Nutrients Management
K	:	Potassium
LER	:	Land Equivalent Ratio
meq	:	milliequivalents
N	:	Nitrogen
°C	:	degree Celsius
OC	:	Organic Carbon
P	:	Phosphorus
PAU	:	Punjab Agricultural University
PGPR	:	Plant Growth Promoting Rhizobacteria
PGR	:	Plant Growth Regulator
pH	:	Potential of Hydrogen
PSM	:	Phosphate Solubilizing Microorganisms
R	:	<i>Rhizobium</i>
RCBD	:	Randomized Complete Block Design
RH	:	Relative Humidity
SE	:	Standard Error
SPD	:	Split Plot Design
SPSS	:	Statistical Package for Social Sciences
US	:	United States
VAM	:	Vesicular Arbuscular Mycorrhiza

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ABSTRACT

Mineral fertilizers pose a health hazard and affect microbial population in soil by degrading the physical structure of the soil, leading to the lack of oxygen in the plant root zone besides quite expensive and making the cost of production high. The use of biofertilizers in cereal-legume intercropping may induce high production and sustain soil quality while saving environment. It is in this context that a field experiment pertaining to “Effect of Different Biofertilizers on Growth and Yield Parameters of Wheat-Pea Intercropping System” was conducted on farm of Agronomy Department, School of Agriculture, Lovely Professional University (Phagwara-Punjab-India) during *rabi* season 2014-2015 on silt loam soil using split plot layout with RCBD with three replications. Three levels of cropping systems (Pea monoculture, Wheat monoculture and Wheat-pea intercropping) were compared in main plots and five levels of biofertilizers (without biofertilizer, R, PGPR, AMF and R + PGPR + AMF) were applied in sub-plots. Physical (textural class and particles %) and chemical (pH, EC, CEC, OC, N, P and K) properties before and after harvesting were analysed. Observations on growth were taken at the end of flowering and those of yield were recorded at crop maturity. Results of this study indicated that inoculants do not affect plant height and 1000 seeds weight of pea. PGPR inoculation was found significant on pod length and on number of pods per plant. Inoculation with consortia of biofertilizers (R + PGPR + AMF) was having a significant impact on days to maturity, number of branches per plant, number of seeds per pod, pod length and seed yield in pea monoculture as well as in intercropping system. Similar to pea, in wheat cropping system there was no significant impact of biofertilizers on plant height as well as in spike length and days of maturity but inoculation of PGPR showed a significant effect on number of tillers per plant and number of grains per spike in wheat. Addition to this AMF application on wheat showed a significant difference in 1000 grains weight of wheat. The combination of bio-fertilizers (R + PGPR + AMF) was found to be significant in terms of yield and it gave higher yield in wheat. Consortia of biofertilizers (R + PGPR + AMF) had a significant impact over single biofertilizers while comparing land efficiency of intercropping. Overall, the application of combined biofertilizers (R + PGPR + AMF) caused a significant increase in available N, in available K, in available P, in OC and in EC. Therefore, this study supports the statement that application of compatible biofertilizers together in the field can improve the yield and growth of crops in monoculture as well as in intercropping system.

1. INTRODUCTION

All over the world, people tend to live in places with high fertile soils, sufficient rainfall and suitable temperatures. With population pressure, soils nutrients and fertility decrease when farmers are not able to limit losses by adding nutrients to the soil through manures, crop residues, biofertilizers as well as mineral fertilizers. But the mineral fertilizers pose a health hazard and affect microbial population in soil by degrading the physical structure of the soil, leading to the lack of oxygen in the plant root zone besides quite expensive and making the cost of production high.

According to Mokunye *et al.* (1996), soil fertility depletion in nitrogen and phosphorus has been occurred as agriculture is affected by major biophysical constraints. In such situation soil fertility can be restored effectively through bio-fertilizers which are considered as an alternative solution. Biofertilizers can be defined as preparation containing living cells of microorganisms that when inoculated on seed, applied on plant surface or on soil have the capacity to improve the soil fertility and promote growth by converting major nutrients (nitrogen, phosphorus) from unavailable to available form through biological nitrogen fixation and phosphorus solubilising microorganisms (Rokhzadi *et al.*, 2008).

Different sources of biofertilizers such as nitrogen fixers, plant stimulators, phosphorus solubilising microorganisms, plant growth promoting rhizobacteria (PGPR) have been reported by Shekh (2006). Use of bio-fertilizers became very important eco-friendly practice and helped in getting high quality yield (Shevananda, 2008). Microorganisms in biofertilizers usually have specific function; like *Azospirillum* fixes nitrogen and phosphate solubilizing bacteria solubilise phosphorus from the soil and make their easy availability to the plants (Saraswati and Sumarno, 2008). Nitrogen and phosphorus are major nutrients for plant growth, though plants have a limited capacity to take out them from the environment, as consequence they need microorganisms involved in nutrient recycling, to help plants for uptake and absorption of these nutrients at adequate concentrations, while microbes get waste products as food. During this symbiotic relationship, plant rooting systems become stronger and bigger. As the plant root is larger, the more living space and food is available for microorganisms, in a way microorganisms act as biofertilizers (El-khily, 2005).

Improvement of soil fertility by use of biofertilizers like PGPR, mycorrhizal fungi have shown important contribution to nutrient availability in the soil, and also hold together soil particles into the stable aggregates, which bring down soil erosion and ameliorate soil structure (Wu *et al.*, 2005). Different studies have been conducted to evaluate the responses of various plants like field pea (Noor, 2003) and turf grass (Guntoro *et al.*, 2007) to biofertilizer application but findings were still inconsistent. As all plant crops, nitrogen during growth and grain development is important to facilitate protein accumulation in the wheat kernels. Grain legumes and cereals when grown together, legumes can capture and fix to soil the atmospheric nitrogen. Different grain legumes and cereals studies have been conducted to show how cereals are significantly gaining inorganic nitrogen (Jensen, 1996). Pea and barley intercropping systems are commonly practised in temperate but pea and wheat intercropping are rare (Ghaley *et al.*, 2005).

Intercropping is defined as an agronomic practice consists of growing two or more crops on the same land area, which often results in maximisation of productivity, stable yields, adequate use of resources, decrease weeds, plant diseases, and nitrogen losses (Marer *et al.*, 2007). Principle of intercropping is to get high total productivity per unit area and time, besides impartial use of resources and production inputs like labour. Ahmad *et al.* (2001) concluded that field pea and wheat can be intercropped for effective use of land. Shortage of additional production land for crop, decrease in yield per unit area has heightened concerns about sustainable and economically viable cropping systems. Cropping systems like intercropping is an alternative possible manner of increasing productivity.

Intercropping of wheat and legumes has the ability to decrease the quantity of nutrient up taken from the soil as compared to wheat sole. Intercropped legumes compete with wheat for inorganic nitrogen applied on field, in lieu of fixing nitrogen from the air. Though, when nitrogen is not applied, the completion will not occur because the intercropped legumes will fix most of their nitrogen from atmosphere (Adu-Gyamli *et al.*, 2007).

This research was aimed to ascertain **“Effect of Different Biofertilizers on Growth and Yield Parameters of Wheat – Pea Intercropping System”**.

2. REVIEW OF LITERATURE

Plant root system in the soil is surrounded by different soil microorganisms. The narrow zone in the soil that is influenced by root, its secretions and soil microorganisms, is known as Rhizosphere. The Rhizosphere is characterized by a huge amount of carbon (form exudates of root and also the dead root parts) and is sometimes depleted in nutrients (due to the fast nutrient uptake by plants) compared to the bulk soil (Brimecombe *et al.*, 2007).

Soil microorganisms in the soil can affect plants in various ways, either in beneficial or strongly antagonistic. These effects may be mutualists or parasites depending on organisms. The plants and soil microorganisms interaction can be either obligate for both components (where plant or microorganism cannot survive and reproduce solely under the favourable conditions of soil fertility) or facultative (where all components could live and reproduce solely) (Morgan and Whipps, 2001).

To accomplish beneficial interactions to plants, some microorganisms are associated with soil biota, and create a kind of protection to plant. Other organisms act directly and enhance nutrients deliverance to plants. These microorganisms play a crucial role in Nitrogen (N), Phosphorous (P) acquisition, as well as in micronutrients.

2.1 Biofertilizers

Biofertilizer can be defined as an organic preparation containing living microorganisms which can be either applied to plant roots or on soil in the vicinity of root zone (Chen, 2006; Gupta and Sen, 2013). These bioinoculants are expected to improve plant growth and yield. Different sources of biofertilizers such as nitrogen fixers, plant stimulators, phosphorus solubilising microorganisms, plant growth promoting Rhizobacteria have been reported by Shekh (2006). Use of biofertilizers showed a significant important in getting yield of high quality and reduce ecological pollution (Shevananda, 2008). Biofertilizers enhance plant growth by increasing the availability of primary nutrients and stimulate targeted plant growth when inoculated on seed, applied on plant surface or on soil (Muraleedharan *et al.*, 2010).

2.2 The Rhizosphere

The Rhizosphere may be defined as the narrow zone in the soil that includes plants roots and the surrounding soil (Walker *et al.*, 2003), or as the volume of the soil plant roots, association of root hair, and all plant root exudates dwell (Dessaux *et al.*, 2009) while the term 'Rhizobacteria' stands for a group of bacteria that are able to colonize the root environment in the Rhizospheric zone (Kloepper *et al.*, 1991). There are three parts recognized in the Rhizosphere: the Rhizosphere (soil), the Rhizoplane, and the root itself. Among these parts, Rhizosphere is the zone of soil that is dominated plant roots which release plant substrates that have an effect on the microbial activity. On other hand, Rhizoplane is the root surface with strongly adhered soil particles while the root itself is a part of root system (Barea *et al.*, 2005).

2.3 Arbuscular Mycorrhizal Fungi (AMF)

2.3.1 General review

The symbiotic association with AMF in crop plants is widely distributed (Smith and Read, 2008; Smith and Smith, 2011). Great amount of cultivated plants can form arbuscular mycorrhizal. AMF colonisation can improve the growth and development of the plant by providing soil available P. The level of plant growth improvement can be affected by various factors like host plants species, species of fungi, and conditions of the soil (Tawaraya, 2003). AMF amount extends from 5-50% of the soil microbes (Olsson *et al.*, 1999) and hyphae of AMF may attain 54-900 kg/ha (Zhu and Miller, 2003).

The mycorrhizal symbiosis stimulates plant nutrients uptake and promotes environmental stability, and plays a great role in agriculture. AMF are mainly known for the important role in P gaining (Lambers *et al.*, 2008). Through the extended soil volume accessed by the arbuscular mycorrhizal hyphae, plant can gain orthophosphate, inorganic phosphate present in the Rhizosphere solution (Smith and Read, 2008).

AMF can work as filters, holding heavy metal on the hyphae surface known as glomalin, thus keep safe plant from heavy metal concentration in the soil (Audet and Charest, 2007). AMF can also protect plant from stress conditions like (drought, flooding salinity) (Miransari, 2010; Smith *et al.*, 2010; Birhane *et al.*, 2012). The impact of AMF on water-plant relationship and drought has been investigated by Auge (2001).

2.3.2 Effect of agricultural practices on AMF

Applied modern technologies in agriculture affect the mycorrhizal symbiosis. Practices like tillage are physically disturb soil aggregates, and AMF hyphal networks which decrease soil structure, soil fertility, and force more carbon to be allocated to AMF to re-establish these networks. Use of mycorrhizal host crops, continuous cropping systems and no tillage practices, can reduce the application of synthetic inputs, like P, and enhance the strong plant-mycorrhizal symbiotic relationship (Nichols, 2008; Panwar *et al.*, 2008).

2.3.3 AMF function

AMF has the ability to stimulate the plant growth and to increase nutrient uptake such as phosphorus, zinc, copper, boron, molybdenum, iron, and manganese in the soil (Lambert *et al.*, 1979; Shibata and Yano, 2003). AMF associated plants showed to be more tolerant than non-AMF colonized plants to withstand various biotic and abiotic stresses like heavy metals toxic, root pathogens and infection, drought, extreme temperature, saline soils, adverse pH and transplanting (Paraskevopoulou-Paroussi *et al.*, 1997; Ruiz-Lozano *et al.*, 2001; Rabie and Almadini, 2005; Smith and Read, 2008; Turkmen *et al.*, 2008).

Almost plants (80%) can establish an AMF symbiosis association. During this association, AMF colonize roots of plant component and stimulate plant growth (Smith and Read, 2008). Significant changes in root physiology and root exudation for plants associated with AMF have been reported (Posta *et al.*, 1994; Giasson *et al.*, 2008). Nutrient uptake improvement due to AMF colonization showed significant growth of many crop plant species (Jensen, 1982; Barea *et al.*, 1987; Hirata *et al.*, 1988; Weber *et al.*, 1993; Al-Karaki and Clark 1999; Biró *et al.*, 2000; Jia *et al.*, 2004) and trees (Habte and Aziz, 1985; Manjunath and Habte, 1988; Okon *et al.*, 1996). Modified root metabolic functions and selective effect on soil microorganisms occur when AMF catabolise root exudates (Duponnois *et al.*, 2008; Saldajeno *et al.*, 2008).

AMF can improve soil structure by producing a slimy glycoprotein known as glomalin. AMF also takes place in stable soil aggregates formation and create large pores which allow the better growth of the hyphae, which facilitate easier water and air penetration, thus prevent soil erosion (Piotrowski *et al.*, 2004; Nichols, 2008).

2.4 *Rhizobium*

Rhizobium is a nitrogen fixing bacteria (NFB) that can transform inert atmospheric nitrogen to organic compounds (Bakulin *et al.*, 2007). In agronomical point of view *Rhizobium* inoculation ensures nitrogen fixation by legumes instead of nitrogenous fertilizers (Gupta, 2004). The presence of nodules on roots does not mean that the sufficient nitrogen is being fixed for good growth of the host plant (Weaver 1974). Inoculation with effective strains of *Rhizobium* have increased pod yield in ground nut (Sundara-Rao, 1971). The increase in the number of nodules and the development of palm or ginger like nodule proliferations are evident when the plant grew in mixed soil (You *et al.*, 2008).

The nitrogen fixing bacteria include different species such as cyanobacteria, actinomycetes, as well as eubacteria, which are heterotrophic (e.g. *Azotobacter*), autotrophic (*Thiobacillus*), aerobic (*Bacillus*), anaerobic (*Clostridium*) and photosynthetic (*Rhodospirillum*). NFB organisms may live freely (e.g. *Azotobacter*), in symbiotic association with plants or with specialized structures (nodules) given by their host plant (*Rhizobium*) (Graham, 2001).

2.4.1 Rhizobial function

Atmospheric nitrogen stimulates plant growth through root nodule bacteria. The initiation of nodulating bacteria on or around the legume root may affect the infection of some pathogens or reduces the damages they can cause (Akhtar and Siddiqui, 2007). Nitrogen depleted soil give low performance and yield in the absence of inoculation or fertilization. Legumes inoculated with a selected *Rhizobium* strain may augment nodule number per plant, nodule dry weight, plant yield and nitrogen content compared to non-inoculated plants (Beck *et al.*, 1992; El Hadi and Elsheikh, 1999; Kantar *et al.*, 2003). The increase of production for the plant inoculated with rhizobia depends on rhizobial strains and their combination with other microorganisms (Dashti *et al.*, 1997; Rudresh *et al.*, 2005; Wani *et al.*, 2007).

Combination of *Rhizobia* and *Pseudomonas sp.* usually gives a significant increase in dry weight of legumes plants due to nodule induction by *Pseudomonas* (Bolton *et al.*, 1990; Goel *et al.*, 2002; Valverde *et al.*, 2006).

2.4.2 Nutrient uptake

Rhizobia can affect some plant physiological processes like photosynthesis, nodulation and nitrogen fixation in legumes by stimulating plant dry matter and grain yield (Dashti *et al.*, 1997; Kantar *et al.*, 2003). It has been reported that interaction of Rhizotrophic microorganisms can improve nutrients uptake in the soil like P, K, Ca, Mg and N and hence increase yields (Peix *et al.*, 2001; Saini *et al.*, 2004). In Bangladesh *Rhizobium* inoculation showed a surplus up to 80 kg/ha of nitrogen and increase the yield of pea plant over untreated plants (Ahmed *et al.*, 2007).

2.5 Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are bacteria identified in the Rhizosphere and they have the ability to colonize roots and promote the growth of plants. This term PGPR was coined at the first time by Kloepper and Schroth (1978) for the microorganisms that are closely associated with the Rhizospheric region. The PGPR can take place in different ecosystem process, like biological control of plant pathogen, nutrients cycling and/or seedling growth (Persello-Cartieaux *et al.*, 2003).

It was well identified that, in the rhizosphere, only 1-2% of bacteria are able to promote the growth of the plant (Antoun and Kloepper, 2001). Different mechanisms are used by PGPR to promote plant growth like production of diverse compounds (such as phytohormones, organic acids, siderophores), fix atmospheric nitrogen, solubilize phosphate and produce antibiotics that inhibit deleterious rhizobacteria, and production of biological active substances or plants growth regulators (PGRs). Production of these substances is of the important mechanisms used by PGPR to enhance plant growth and plant development (Arshad and Frankenberger, 1998). Therefore the utilization of PGPR to improve plant growth and crop yield has become important in the present as well as in the future agriculture (Pal *et al.*, 2000).

In salt stress areas, PGPR shown significant effect on germination rate, drought tolerance, and weight on shoots and roots, yield and plant growth (Kloepper *et al.*, 2004; Kokalis-Burelle *et al.*, 2006). Another major important of PGPR is the production antibacterial products that control plant pathogen and pest infestations (Dey *et al.*, 2004; Herman *et al.*, 2008; Minorsky, 2008). The systematic resistance in the treated plants were observed when PGPR strains applied to seeds or on seedlings (Yanni *et al.*, 1997; Biswas *et al.*, 2000). The inefficacy of PGPR in the field conditions has often due to their incapability to colonize root of plant (Benizri *et al.*, 2001; Lugtenberg, *et al.*, 2001).

2.6 Interaction of plant associated microorganisms

Managing plant associated microorganisms' population in the Rhizosphere by use of PGPR, AMF, and nitrogen fixing bacteria can give important benefits for ecosystem and environment restoration in depleted lands (Khan, 2002; Khan, 2004).

2.6.1 AMF and *Rhizobium*

Generally maximum plant growth was achieved through mycorrhiza (Azcón-Aguilar, 1983). The interaction of AMF and rhizobial bacteria to enhance plant growth was shown on *Medicago arborea* plant in the Southern of Spain (Herrera *et al.*, 1993a; 1993b); and microbial activities were affected by biogeochemical cycling of important plant nutrients (Jeffries and Barea, 1994). AMF has the ability to increase the availability and uptake of nutrients, especially phosphorus, which has an important role in plant growth. This is due to a strong mycelia present on AMF, which has the ability to expand root surface available for nutrient absorption (especially phosphorus) (Jia *et al.*, 2004; Shockley *et al.*, 2004), and then promote rhizobium infection, enhancing nitrogen-fixation ability and plant growth (Siviero *et al.*, 2008). These findings were in both top growth and total dry weight of lucerne compared to the untreated plants (Pandey *et al.*, 2003). In lucerne (alfalfa) and pea, co-inoculation of *Rhizobium* with AMF enhances greater plant growth than single inoculated plants (Höflich *et al.*, 1994).

2.6.2 AMF and PGPR

Since AMF and PGPR have common habitat (root area), AMF and PGPR when applied together, must interact in their process of root colonization and functioning as they are root-associated microorganisms. Soil microorganisms like PGPR, can affect AMF formation and AMF can influence PGPR populations in the Rhizospheric zone (Barea, 2000). Rhizospheric organisms like AMF and PGPR have the ability to increase availability of essential nutrients, particularly phosphorus, by solubilizing and mineralizing plant nutrients from organic and inorganic sources (Koide and Kabir, 2000; Hodge *et al.*, 2001; Tawarayama *et al.*, 2006; Idris *et al.*, 2009; Richardson *et al.*, 2009). Also AMF and PGPR can improve plant health by inducing plant resistance and by controlling the growth of pathogens (George *et al.*, 1995; Ramamoorthy *et al.*, 2001; Weller *et al.*, 2002).

2.6.3 *Rhizobium* and PGPR

The combination of *Rhizobium* and PGPR can be an alternative solution to improve the nitrogen fixation. The potential of PGPR to ameliorate nodulation have been documented for many legumes species. Generally, increase in nodulation enable higher nitrogenase activity leading in superior dry matter yield. Though, the results change according to the experiment system used. Under field conditions, PGPR strains like *Serratia proteamaculans*, *S.fonticola*, *Pseudomonas fluorescens* and *P. putida* tried out individually or in combination with *R. leguminosarum*, increased emergence, plant vigour, nodulation, nitrogenase activity and root weight of lentil, but did not showed an effect on pea (Chanway *et al.*, 1989).

It has been found by Yadegari *et al.* (2008) that inoculation of *Rhizobium phaseoli* and PGPR strains such as *P. fluorescens* on bean gave promising results on yield and plant growth parameters. In the experiment conducted by Höflich *et al.* (1994), co-inoculation of *Rhizobium sp.* with *P. fluorescens* showed the ability to promote the root development and protect root from pests, also shown the benefit improvement of rhizobia in lucerne, pea and broad bean.

2.6.4 Interactive effects of AMF, *Rhizobium* and PGPR

To attain effective growth, an interaction between microorganisms and host plant is important for optimal use of plant assimilates or microbial metabolites. In other word, functional compatibility must be established between the associated organisms (Höflich *et al.*, 1994). Plant growth and yield can be increased by combining various microbial inoculants with different characteristics. Selected nitrogen fixing bacteria, AMF and PGPR microorganisms are able to produce certain stimulating metabolites.

2.7 Intercropping

In agriculture, different types of cropping systems are applied depending on the local climate, soil and farmer's income. The use of any cropping system is determined by water balance, radiation, temperature and soil conditions (Beek and Bennema, 1972). Therefore the cropping system varies from place to place in the world. In small scale farming, farmers raise their crops in combination to minimize total failure and get different produce to satisfy their family's food, and income generation. To increase productivity per unity area, intercropping is used. Intercropping can simply defined as cultivation of two or more crops simultaneously on the same piece of land has been shown to be beneficial in terms of yield stability, increase in total yield, pest and disease management, weed management, erosion control, and soil fertility amongst others (Willey, 1979a; Innis, 1997; Hauggard-Nielson *et al.*, 2006).

Several factors are usually considered in choosing crop combinations to intercrop. Some of these factors include crop architecture, life cycle and agronomic practices of the crops, environmental conditions, growers demand, local preference, and length of growing seasons (Ofori and Stern, 1987; Fukai and Trenbath, 1993; Connolly *et al.*, 2001). In any case, the selection of component crop that minimize intercrop competition and maximize complementary effects between the component crops in resource use is the ideal (Willey, 1979b; Hauggard-Nielson *et al.*, 2006). Largely, intercropping benefits are usually greater when the growth duration between component crops differs widely (suggesting temporal effects) than when the crops durations are similar (suggesting spatial effects) (Fukai and Trenbath, 1993; Yahuza, 2011).

Cereal-legume based intercropping is one way of growing stable food crops while gaining different benefits from the added crop. This practice has been very popular for smallholder farmers as it was an appropriate cropping system to maintain soil productivity (Ijoyah and Dzer 2012).

2.7.1 Advantages of cereal-legume intercropping over other intercropping systems

The most important intercrops advantages are reached when the combined species differ either morphologically, phonologically or physiologically (Andersen *et al.*, 2007). In intercropping system sowing may be done at different period, and harvest may also differ. However, crops should stay together for a significant period of their growth (Ofori and Stern, 1987). Cereal-legume intercropping system showed positive effects and efficacy of intercrops than the pure cropping. It has been investigated by Kadziulienė *et al.* (2009), that intercropping generates beneficial biological interactions between crops, by increasing grain yield and stability, by efficiency use of available resources and reducing weed pressure.

2.7.2 Points to be considered in cereal-legume intercropping system

Growing together two or more crops, an adequate space is needed for each crop to maximize the cooperation and minimize competition between them. Therefore, the following points such as spatial arrangement (Malezieux *et al.*, 2009); plant density (Andersen *et al.*, 2007; Neumann *et al.*, 2007); maturity period of the crops being grown (Anil *et al.*, 1998), plant architecture (Brisson *et al.*, 2004), should be considered before intercropping application. The choice of the compatible component in intercropping relies on the plant growth habit, land, light water and fertilizer utilization (Brintha and Seran, 2009).

2.7.3 Importance intercropping systems

The very important growth resources needed by crops are water, light and nutrients (Brisson *et al.*, 2004). Above soil surface plant parts compete for light and carbon dioxide and below soil surface compete for water and nutrients (Malezieux *et al.*, 2009).

Water is the most important factor for plant production and it is a vehicle for all soil based resources (Malezieux *et al.*, 2009). The improvement of water use efficiency in intercropping enhances the efficiency use of other resources (Hook and Gascho, 1988). Also, Dolijanovic *et al.* (2007) emphasized that intercropping system utilizes water better than monoculture.

In intercropping leaf canopy may make better special use of light (Waddington and Edward, 1989). Intercropped crops with different plant height, high and low canopy, improve light interception and enhance yields of shorter crops which are planted between with sufficient wider rows of the taller ones (Seran and Brintha, 2010). Light becomes an important factor to determine yield, when morphologically dissimilar crops with different periods of maturity are intercropped (Willey, 1979a). For example, light intercepted was higher in maize-bean intercropping system than in monoculture (Tsubo *et al.*, 2001).

Nutrient uptake in intercropping system can occur spatially and temporally (Anders *et al.*, 1996). Spatial nutrient uptake can be enhanced by the increased root mass, while temporal nutrient uptake occurs when components in intercropping require peak nutrient demands at different times. Different rooting and uptake patterns of the species present in intercropping system improve more efficiency use of available nutrients in the soil (Fujita and Ofosu-Budu, 1996).

It is frequently believed that intercropping system is good for weeds, pests and diseases control in comparison to monocrops. It has been reported by Willey *et al.* (1983), which in intercropping system weed growth is determined by the competitive ability and plant population present in crop community. For instance Steiner (1984) reported weed suppression in maize-groundnut intercropping system.

About pests and diseases, Willey *et al.* (1983) quoted that one component in intercropping can be a barrier to the expansion of pests or disease to the other. For instance, Seran and Brintha (2010) reported that greater infestation of bud worm in sole maize than in intercropped maize with soybean.

It has been reported by Seran and Brintha (2010), that intercropping system can control soil erosion by stopping rain drops from hitting the bare soil. For instance, Reddy and Reddi (2007) reported that taller crops behave as wind barrier for short crops in intercropping system with taller and short components. Moreover, Zougmore (2000) reported that in sorghum-cowpea intercropping, soil loss was reduced up to 50% compared to monocultures.

2.7.4 Wheat-pea based intercropping system

In many different part of the world, wheat and pea are grown as sole crops and there few published reports that investigate the intercropping system of wheat and pea. Both being cool crops, give the impression of extensive research for intercropping.

Intercropping of barley and pea showed the efficiency use of the available growth resources then their corresponding sole crops. It has been reported by Andersen *et al.* (2007) that time and environmental conditions can vary the competitive ability and interactions of different plant species in intercropping system. It has been stated that in intercropping system plant species do not compete with the same resource thus there is an adequate degree of resource complementarity.

Studies done in Europe on barley-pea intercropping, showed that the yield and grain protein concentration were higher than in monocrops (Hauggard-Nielson *et al.*, 2003). Hauggard-Nielson *et al.* (2001) and Jensen (1996) reported that the environmental sources for plant growth, especially nitrogen was more efficient in intercropping compared to sole cropping and nitrogen concentration was increased in intercropped barley grain compared to sole cropped barley (Jensen, 1996). Higher nitrogen proportion provided from nitrogen fixation was observed in intercropped pea plants than in sole cropped pea plants (Izaurrealde *et al.*, 1992; Jensen, 1996 and Jensen; 1998). It has been shown by (Jensen, 1996 and Hauggaard-Nielsen *et al.*, 2001) that greater yield stability and competitive ability towards weeds were observed in barley-pea intercropping system than in sole cropped pea plants.

3. RATIONALE AND SCOPE OF THE STUDY

The soil fertility declines when nutrient content reduced and affects its biological, physical, and chemical components in such a way that they are not able to support and feed plants that lead to limited crop production. The depletion in nutrients is due to continuous cropping and heavy use of chemical fertilizers. Chemical fertilizers have been used intensively to increase crop production all the world. However, they start manifest their negative impact on soil and environment degradation by depositing the harmful compounds. To achieve the sustainable agriculture strategies like use of organic fertilizers, intercropping and biofertilizers should be taken into account. Mainly, cereals and legumes are grown as sole crop. Wheat (as cereal) and pea (as legume) are considered as one of the most important crops in terms of their production and intake. Mostly, wheat and pea are grown as sole crops and fertilized by chemical fertilizers which have negative impact on soil fertility, on environment as well as human health. Few published reports about the intercropping of wheat and pea are available and no published reports where biofertilizers were used. This research was aimed to evaluate the role of different biofertilizers on wheat-pea intercropping system under the following objectives.

OBJECTIVES OF THE STUDY

1. To determine crop response of wheat and pea in intercropping system
2. To find out the efficacy of different biofertilizers on growth and yield of wheat and pea in intercropping system,
3. To find out the effect of different biofertilizers on soil chemical properties.

4. MATERIALS AND METHODS

The field experiment entitled “**Effect of Different Biofertilizers on Growth and Yield Parameters of Wheat – Pea Intercropping System**” was carried out at Lovely Professional University during *rabi* season 2014-2015. The details of materials, procedures adopted, and techniques used during the course of this study are described in this chapter.

4.1 Situation of Experimental Site

The field experiment was conducted in the Research Farm of Department of Agronomy, School of Agriculture, Lovely Professional University, Punjab (India) during Rabi season 2014 -2015. Geographically is situated ($31^{\circ} 15'$ North latitude and $75^{\circ} 42'$ East longitude) at 235 m above mean sea level in Punjab. This experimental site falls in “Central Plain Zone (PB-3) of Punjab.



Figure 4.1 Picture showing the location of study area

4.2 Climatic and weather

The climate of the experimental site is located in Punjab State which experiences by the extreme hot and extreme cold conditions. The annual temperatures in Punjab State range from 1 to 46°C and can reach 49°C during summer and 0°C in winter. Its average rainfall ranges from 960 mm in the sub mountain region and 460 mm in the plains. It is also characterized by heavy rain in the northeast area near the foothills of Himalayas, whereas it receives less rainfall and high temperatures in the area lying in south and west. It experiences also three seasons as follows: Summer season from April and June and it is characterized by the rise in temperatures up to 38°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°C.

4.3 Meteorological data during growing season

Weather and climate are important factors that determining the success or failure of agriculture. Weather influences agricultural operations from sowing to the harvest, the reason why it is important to present the variations of climate during growing season. The mean of weekly meteorological observations were recorded during entire growing season and are represented in Table 4.1. Crops were sown on 26/11/2014. Pea was harvest on 25/3/2015 and wheat was harvested on 22/4/2015. Maximum and minimum temperatures during growing season were 33.49°C and 6.90°C respectively, relative humidity varied between 63 and 85 per cent. There was a total rain of 190 mm during growing period.

Table 4.1 Monthly air temperature, relative humidity and total precipitation from November 2014 to April 2015

Month	Temperature (°C)			RH (%)	Rainfall (mm)
	Maximum	Minimum	Average		
November	26.9	10.9	18.9	63	0
December	17.6	6.9	12.25	80	42.2
January	15.6	7	11.3	85	24.6
February	22.2	10.5	16.35	79	38.6
March	25.5	13.3	19.4	76	84.6
April	33.49	19.17	26.33	62.62	0
Total					190

Source: Department of Meteorology, PAU

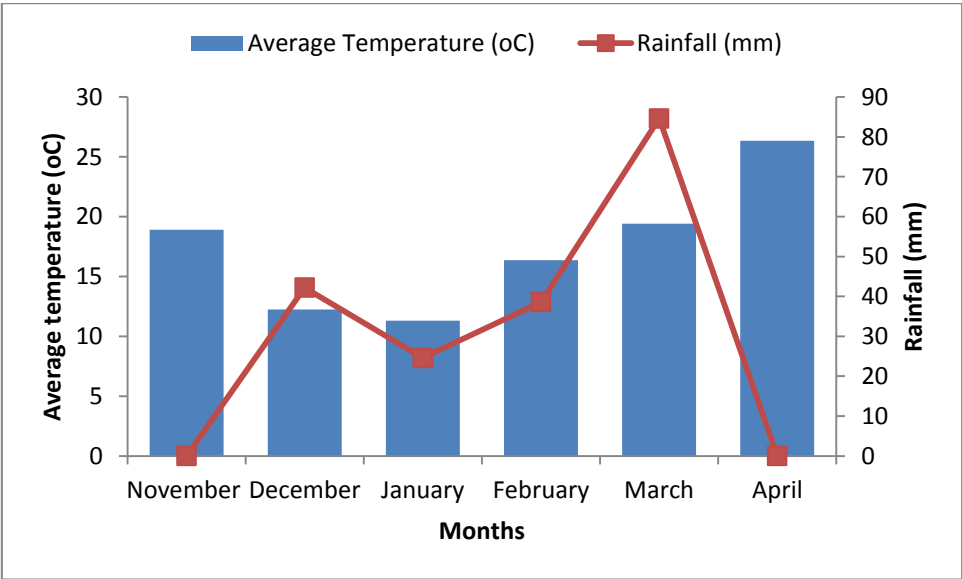


Figure 4.2 Monthly air temperature and total precipitation from November 2014 to April 2015

4.4 Soil Analysis

To find out physical and chemical characteristics of the experimental soil, top soil samples from 0-15 cm depth were collected from each replicates before sowing then after harvesting all crops soil samples were collected from each plot and they were air dried and sieved then a composite sample was obtained by mixing them together for further analysis of both physical and chemical properties. The results of soil analysis before sowing presented in table 4.2 showed that the soil was silt loam, slightly alkaline in reaction, non-saline, low in organic carbon, low in available nitrogen and potassium and medium in available phosphorus status.

Table 4.2: Analytical methods used for soil analysis

No	Parameter	Status/ Value	Method
Physical properties			
1	Textural class	Silt loam	Soil texture triangle Method
2	Particles analysis (%)		International pipette method (Piper, 1950)
	Sand	30	-do-
	Silt	64	-do-
	Clay	6	-do-
Chemical properties			
3	Soil reaction	Alkaline (pH 7.7)	Water suspension (Jackson, 1967)
4	Electrical Conductivity	0.614 dS/m	Water suspension (Jackson, 1967)
5	Organic carbon	0.40%	Rapid titration method (Walkley and Black 1934)
6	Exchangeable Cations	0.347 meq/100 g	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)

4.5 Procedures of soil analysis

4.5.1 Triangle Method for soil textural class

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

4.5.2 Particles distribution (%): International pipette method (Piper, 1950)

For determination of soil texture, 50 g of dried soil were sieved through 2 mm sieve and placed into 500 ml bottle. After that 100 ml of obtained dispersion solution was added into 50 g soil in 500 ml plastic bottle. Sample bottles were shaken at regular intervals for half an hour on shaking machine for preparing homogeneous solution. The obtained solution was transferred into 1000 ml glass measuring cylinder then after water was added to make solution of 1000 ml. As per International approved system, sample solution was shaken for 30 seconds. Depending on the solution temperature and sedimentation chart, first pipetting was done with 50 ml pipette at 10 cm depth. In first pipetting, 50 ml solution were sucked and transferred into 60 ml china dish. The formed sample solution contained mixture of clay and silt particles. Depending on the solution temperature and sedimentation chart, second pipetting was done with 50 ml pipette at 10 cm depth. In second pipetting 50 ml solution were sucked and transferred in 60 ml china dish. This solution contained clay particles in soil sample. Remaining soil solution was transferred in 1 litre. Measuring cylinders and 0.02 mm sieves were washed using jet of water. Sand particles on sieve were collected in china dish. Pipetted solution was transferred in 3 dishes and kept overnight in an oven at temperature of 105°C. Solutions were cooled in desiccators and weight was taken quickly. The weight of fine was determined by deducting the weight of clay, silt and coarse sand particle from 100.

4.5.3 Soil reaction: Water suspension (Jackson, 1967)

About 12.5 g of dried soil were weighed and added into 150 ml beaker, then after 25 ml of distilled water was added and the obtained solution was agitated with glass rod for half an hour and then left for one hour. The electrode was inserted into solution for pH reading. Every time, electrode was washed with distilled water for new record of soil sample.

4.5.4 Electrical Conductivity: Water suspension (Jackson, 1967)

To find out the electrical conductivity of soil, 25 g of dried soil were taken then transferred into 100 ml beaker then after 50 ml of distilled water was added. The suspension was mixed intermittently for half an hour and left it for 30 minutes without any disturbances. Conductivity cell was inserted in solution and EC value was recorded.

4.5.5 Organic carbon: Rapid titration method (Walkley and Black 1934)

To determine organic carbon of soil, 2 g of dried soil samples were weighed and taken into 250 ml conical flask, to which 10 ml of 1 N $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 were added. The content was shaken for a minute and was left for a half an hour to make reaction complete. Then after 200 ml of distilled water, 10 ml of orthophosphoric acid and 4 drops of drops of diphenylamine indicator were added and the violate colour was appeared in the suspension. The obtained solution was titrated with ammonium ferrous sulphate and the point of the titration was marked with the change of colour from violate to bright green. The blank titration was performed in the similar way.

4.5.6 Available Nitrogen: Alkaline Permanganate Method (Subbiah and Asija, 1956)

To determine available nitrogen in the soil, 5 g of dried soil were taken and transferred into the distillation flask of micro-Kjeldhal distillation assembly. About 52 ml of 0.32% $KMnO_4$ solution was added to the distillation unit. From 150 ml conical flask, 10 ml of N/50 H_2SO_4 were pipetted out and mixed with two drops of methyl-red indicator. The conical flask and the delivery tube of the distillation unit were placed in such a way that the delivery tube was well placed into the content of the conical flask. The quantity of 25 ml of 2.5% NaOH solution was added into the distillation flask containing soil and $KMnO_4$ through the set provided in the distillation tube and the inlet was immediately closed with stop-cock. Then after, distillation was started and 30 ml of the distillate was collected. The content of the conical flask was titrated with N/50 NaOH and the end point was indicated with change of colour from pink to yellow.

4.5.7 Cations Exchange Capacity: Ammonium Acetate method (Chapman, 1965)

Quantity of 4 g of dried soil for medium or fine textured soil or 6 g of coarse textured soil were transferred into a 50 ml of centrifuge tube. 33 ml of 1 N CH_3COONa solution of a pH value of 8.2 was added to the soil. Tubes were shaken for 5 minutes in a reciprocating shaker. The tube was centrifuged for 10 minutes at about 2000 rpm. The clear supernatant liquid were decanted as completely as possible and discarded. Then after the sample was treated in the same manner with 33 ml portions of sodium acetate solution, and this treatment was done four times, and each time the supernatant was discarded. The same sample was suspended with 33 ml of 95 % ethanol, and was shaken for 5 minutes in the reciprocating shaker, until the supernatant was clear, then after supernatant was decanted and discarded. The sample was washed three times with 33 ml portions of ethanol. The supernatant liquid from third washing was collect and EC was recorded. The absorbed Na was replaced by shaking the sample with 33 ml portions on neutral normal ammonium acetate for 5 minutes in the reciprocating shaker and centrifuged until the supernatant was clear. Third ammonium acetate extracts was collected into 100 ml volumetric flask and the volume was made then after the sodium concentration in the extract was determined with the help of flame photometer. A series of standard Na was prepared from 10 ppm Na to 100 ppm reading of galvanometer. Concentration of Na of the sample was read after necessary dilution from the standard curve.

4.5.8 Available Phosphorus: 0.5 M NaHCO_3 , pH=8.5 (Olsen *et al.* 1954)

A soil of 1 g of was weighed and transferred into 150 ml conical flask. A pinch of Darco-G 60 and 20 ml of 0.5 NaHCO_3 were added into the conical flask, then after the flask was shaken for half an hour on an electrical shaker and the suspension was filtered through Whatman No.1 filter paper. Similarly a blank solution was prepared. About 5 ml of the extract was transferred into a 25 ml volumetric flask and then after 0.5 ml 5N H_2SO_4 were added and the solution was shaken for a while till CO_2 evolution disappeared. A quantity of 4 ml of ascorbic acid (solution B) was added to it and the volume was made by addition of distilled water then after the flask content was mixed. The intensity of the blue colour developed within a calorimeter was measured at 760 μm wavelength using red filter.

4.5.9 Available Potassium: 1 N Neutral ammonium acetate (Black, 1965)

A quantity of 5 g of dried soil was weighed and was taken into in 150 ml conical flask, then after 52 ml of neutral ammonium acetate solution were added to the flask. The content was shaken for five minutes on mechanical shaker and filtered through Whatman No.1 filter paper. The extract was collected into beaker then after 5 ml of the extract was diluted with distilled water. The diluted extract was atomized flame photometer to note K reading.

4.6. Experimental details

4.6.1. Treatments

The experimental design was comprised of 15 treatments combination with three levels of cropping systems (wheat monoculture, pea monoculture and intercropping system of wheat and pea) and five levels of biofertilizers (Without biofertilizer, Rhizobium, Plant Growth Promoting Rhizobacteria; Mycorrhizal Arbuscular Fungi, and Rhizobium + Plant Growth Promoting Rhizobacteria + Mycorrhizal Arbuscular Fungi). Treatments along with their symbols are presented in Table 4.3.

Table 4.3 Experimental treatments

Treatment code	Description
T1	Pea sole
T2	Pea + R
T3	Pea + PGPR
T4	Pea + AMF
T5	Pea + R + PGPR + AMF
T6	Wheat Sole
T7	Wheat + R
T8	Wheat + PGPR
T9	Wheat + AMF
T10	Wheat + R+PGPR+ AMF
T11	Wheat + Pea Sole
T12	Wheat + Pea + R
T13	Wheat + Pea + PGPR
T14	Wheat + Pea + AMF
T15	Wheat + Pea + R + PGPR + AMF

R: *Rhizobium*; PGPR: Plant Growth Promoting Rhizobacteria; AMF: Mycorrhizal Arbuscular Fungi

4.6.2 Experimental design and layout

The experimental design was laid in split plot design based on Randomized Complete Block Design (RCBD) with three replicates. Treatments were randomly allotted to different plots. The lay out and experiment plan along with treatments are shown here below:

- (i) Total number treatments : $3 \times 5 = 15$
- (ii) Replications : 3
- (iii) Design : SPD (Split Plot Design)
- (iv) Total number of plots : 45
- (v) Gross plot size : $4.0 \text{ m} \times 2.0 \text{ m} = 8.0 \text{ m}^2$
- (vi) Net plot size : $3.6 \text{ m} \times 1.6 \text{ m} = 5.76 \text{ m}^2$
- (vii) Spacing : 30 cm x 10 cm for pea
: 20 cm x 10 cm wheat



Figure 4.3 Picture showing status of experiment at 72 days after sowing

4.7. Biofertilizers and varietal characteristics

4.7.1. *Rhizobium*

Rhizobium culture (*Rhizobium leguminosarum* bv. *viciae*) was collected from Punjab Agricultural University (PAU). Rhizobia act inside the nodules as symbiotic nitrogen fixation, which are symbiotic organs formed on roots of the host plant. The bacteria supply ammonium to plant and the carbon and energy needed for symbiotic nitrogen fixation are from the plant photosynthates. Lodwing *et al.* (2003) found that the metabolic dependence between two symbiotic partners is complex than in single exchange of products of photosynthesis and ammonium.

4.7.2. Plant Growth Promoting Rhizobacteria (PGPR)

Plant Growth Promoting Rhizobacteria (*Pseudomonas fluorescens* sp.) culture was collected from International Biotech, Chak Kala Tibba, Sitto Road Abohar.

4.7.3. Arbuscular Mycorrhizal Fungi

Arbuscular Mycorrhizal Fungi culture (*Glomus mosseae* sp.) was collected from International Biotech, Chak Kala Tibba, Sitto Road Abohar.

4.7.4. Wheat

WH 1105, this wheat variety has been bought from HI-TECH KAMBOJ SEEDS which is located in Indri, Karnal (Haryana) India, and it is a double dwarf variety with an average of height 97 cm. Its ears are medium and tapering in shape white smooth glumes. Its grains are amber, hard, medium bold and lustrous. It is resistant to yellow and brown rust and less susceptible to Karnal bunt and loose smut diseases. It matures in about 157 days. Its average grain yield is 23.1 quintals per acre.

4.7.5. Field Peas

PB-89, this variety has been developed by Punjab Agricultural University (PAU) and it has to be sown between October 15 and November 15, and can give first picking in 85-90 days. It is medium dwarf, vigorous, having more number of well filled pods. Average green pod is 60 quintal per acre.

4.8. Field operations

The field preparations were done by applying the primary and secondary tillage, using mould board plough and harrow respectively which were mounted on a tractor. It was followed by planking of the field using planker. Once the field was levelled uniformly, the layout was carried out manually. The treatments beds of 4 m x 2 m were, paths and water channels were prepared according to the experimental layout. The sequence of all operations is presented in table 4.4.

Table 4.4 The schedule of various agronomical operations done during growing period

Sr. /No.	Operation	Date
1	Ploughing and planking of the field	November 14, 2014
2	Pre sowing irrigation	November 15, 2014
3	Lay out of the of the experiment	November 24, 2014
4	Seed Inoculation with <i>Rhizobium</i>	November 26, 2014
5	Sowing with AMF and PGPR soil application	November 26, 2014
6	Thinning of pea	January 12, 2015
7	Irrigation	March 10, 2015
8	Rain	
		January 9, 2015
		February 3, 2015
		February 15, 2015
		March 1, 2015
		March 2, 2015
		March 8, 2015
9	First weeding	January 2, 2015
	Second weeding	February 7, 2015
10	Harvesting	
	Pea	March 26, 2015
	Wheat	April 24, 2015

4.8.1. Biofertilizers application

- (i) **Rhizobium:** The *Rhizobium* culture used in the experiment was applied by seed treatment method just before sowing. Before sowing, the seeds were first treated with *Rhizobium* culture and then left to dry under shade for about 30 minutes followed by sowing.
- (ii) **PGPR:** was applied by soil application to the respective plots at the rate of 2 kg/ha manually during sowing time.
- (iii) **AMF:** was applied by soil application to the respective plots at the rate of 2 kg/ha manually during sowing time.

4.8.2. Crop raising

4.8.2.1. Seed and sowing of pea

Seeds were inoculated by *Rhizobium* culture. Seeds were sown at the seed rate of 75 kg/ha and sown on 26th November 2014.

4.8.2.2. Seed and sowing of wheat

Seeds were sown at the rate of 100 kg/ha on 26th November 2014.

4.8.2.3. Weeding and thinning

Two hand weeding were done at 38 days after sowing and at 74 days after sowing. Thinning was done at 48 days after sowing to obtain uniform plant stand.

4.8.2.4. Irrigation

Crops were raised under irrigated conditions but the irrigation was provided if needed because the rain was too much during growing season.

4.8.2.5. Treatment evaluation

In order to determine the effect of different biofertilizers on growth and yield parameters of wheat and pea intercropping system, needed observations were recorded and are given here below:

4.9. Growth and Yield parameters

4.9.1. Growth parameters for Pea

4.9.1.1. Plant height (cm)

Six plants were selected randomly from each plot and tagged permanently then used for measurement of plant height. Height of each tagged plant was measured at the end of flowering stage using meter scale from the ground to the top and average of six plants was calculated as mean plant height.

4.9.1.2. Number of branches per plant

Number of branches was computed from six plants selected randomly from each plot and tagged permanently. The average of six plants leaves was calculated as mean branches.

4.9.1.3. Days to maturity

Number of days to maturity was computed from date of sowing.

4.9.2. Yield parameters for Pea

4.9.2.1. Pod length

Six pods were selected randomly from tagged plants then used for determination of number of pods length. Lengths of six pods were computed and average was computed as mean pod length.

4.9.2.2. Number of pods per plant

Six plants were selected randomly from each plot and tagged permanently then used for determination of number of pods per plant. Pods from each tagged plant were computed and average of pods from six plants was computed as mean number of pod per plant.

4.9.2.3. Number of seeds per pod

At the harvest time, six pods were randomly selected from each plot and the total seed were counted to work out the average of number of seeds per pod.

4.9.2.4. 1000 seeds weight (g)

1000 seeds from six tagged plants selected randomly from each plot were used to determine 1000 seeds weight.

4.9.2.5. Seed yield (kg/ha)

At crop maturity stage, all pea plots were harvested manually then pods were threshed to collect the seeds and seed weight was recorded. Then after plot yield was converted into kg/ha by the following formula:

$$\text{Seed yield (kg/ha)} = \frac{\text{Seed yield plot (kg)}}{\text{Plot size (m}^2\text{)}} \times 10000$$

4.9.3. Growth parameters for Wheat

4.9.3.1. Plant height (cm)

Six plants were selected randomly from each plot and tagged permanently then used for measurement of plant height. Height of each tagged plant was measured as the distance in cm from the base to the end of spike and average of six plants was calculated as mean plant height.

4.9.3.2. Days to maturity

Number of days to maturity was computed from date of sowing.

4.9.3.3. Number of tillers per plant

Total number of tillers was computed from six plants tagged and selected randomly from each plot. The average of six plants tillers was calculated as mean number of tillers.

4.9.3.4. Spike length

From six randomly selected plants, six spikes were also selected randomly and used to determine their length. The average of these six spikes length was work out as mean spike length.

4.9.4. Yield parameters for Wheat

4.9.4.1. Number of grains per spike

At the harvest time, six spikes were randomly selected from each plot and the total seed were counted to work out the average of number of seeds per spike.

4.9.4.2. 1000 grains weight

1000 seeds from six tagged plants selected randomly from each plot were used to determine 1000 seeds weight.

4.9.4.3. Grain yield (kg/ha)

At crop maturity stage, all wheat plots were harvested manually then were threshed to collect the seeds and yield weight was weighed. On the basis of grain yield of each plot, grain yield (kg. ha⁻¹) was calculated by the following formula:

$$\text{Grain yield (kg/ha)} = \frac{\text{Grain yield plot (kg)}}{\text{Plot size (m}^2\text{)}} \times 10000$$

4.10. Data analysis procedure

All data were statistically analysed 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. The land equivalent ratio (LER) was determined as described by Willey (1985) using the formula:

$$\text{LER} = \frac{\text{Intercrop Field Pea}}{\text{Pea Monoculture}} + \frac{\text{Intercrop Wheat}}{\text{Wheat Monoculture}}$$

5. RESULTS AND DISCUSSIONS

This chapter includes results and discussions of the experiment entitled ‘**Effect of Different Biofertilizers on Growth and Yield Parameters of Wheat – Pea Intercropping System**’ conducted during *rabi* season of 2014-2015 in the Research Farm of Department of Agriculture, Lovely Professional University; Punjab (India). The observations on growth and yield parameters of pea and wheat were recorded during the course of investigation and statistically analysed and the significant of results were verified. Results with main effects and significant interaction have been described in details in succeeding paragraphs. To provide better understanding, some of the characters have been represented graphically.

5.1. Biometric parameters of pea**5.1.1. Effect of different biofertilizers on plant height and number of days to maturity of pea**

The data pertaining to plant height are presented in Table 5.1 and Figure 5.1. The comparison of treatment mean with different biofertilizers indicated that there was no significant difference in pea plant height ($p < 0.05$). The maximum average plant height of 50 cm was recorded in T4 (Pea + AMF) while minimum average plant height of 42.88 cm was recorded under T1 (Pea sole) with no biofertilizers application. Even though application of biofertilizers was not significant in terms of plant height, the highest plant height was observed in T4 (Pea + AMF). Results from this investigation showed that mycorrhizal plants were taller than non-mycorrhizal plants. These findings agree with Diederish and Manske (1990) who reported the maximum growth in plant inoculated with mycorrhizal fungi. Bahadur *et al.* (2006) in pea, Biswas and Patra (2007) in green gram, Djebali *et al.* (2010) in common bean and Ramana *et al.* (2010) in French bean, also supported increased plant height and other growth parameters due to inoculation of VAM over no inoculated plants. This might be due to the fact that AMF has the ability to colonize plant root and enhance various effects on plant growth, biomass allocation and photosynthesis (Fidelibus *et al.*, 2000). AMF increases root surface area and facilitates uptake of soil water and nutrient especially uptake of PO_4^{3-} , NH_4^+ , K^+ and NO_3^- (Marschner and Dell, 1994; Hayman, 1983). Also, Tarafdar and Marschner (1994) reported that mycorrhizal plants performed better than non-mycorrhizal in terms of plant height.

On other hand, a significant difference ($p < 0.05$) in days to maturity was observed between treated and untreated plants (Table 5.1 and Figure 5.1). The maximum average number of days to maturity of 122 days was observed under T15 (Wheat + Pea + R + PGPR + AMF) which was at par with T5 (Pea + R + PGPR + AMF) while the minimum average number of days to maturity was found in T1 (Pea sole) and T13 (Wheat + Pea + PGPR) with statistical similar effect with 119.33 days. The extended period to maturity in plant inoculated with combined biofertilizers might be caused by the proper conditions provided by biofertilizers which produced plant growth promoting hormones thus increased root's absorbency and improved plant growth status then finally prolonged crop maturity. Kenndy (2001) confirmed this in his experiment. Also Haque *et al.* (2006) reported that increase in nitrogen might the factor to delay phenological stages including crop maturity as nitrogen enhance vegetative growth. Results from this study are in accordance with Javahey and Rokhzadi (2011) who reported prolonged phenological stages due to biofertilizers on sunflower.

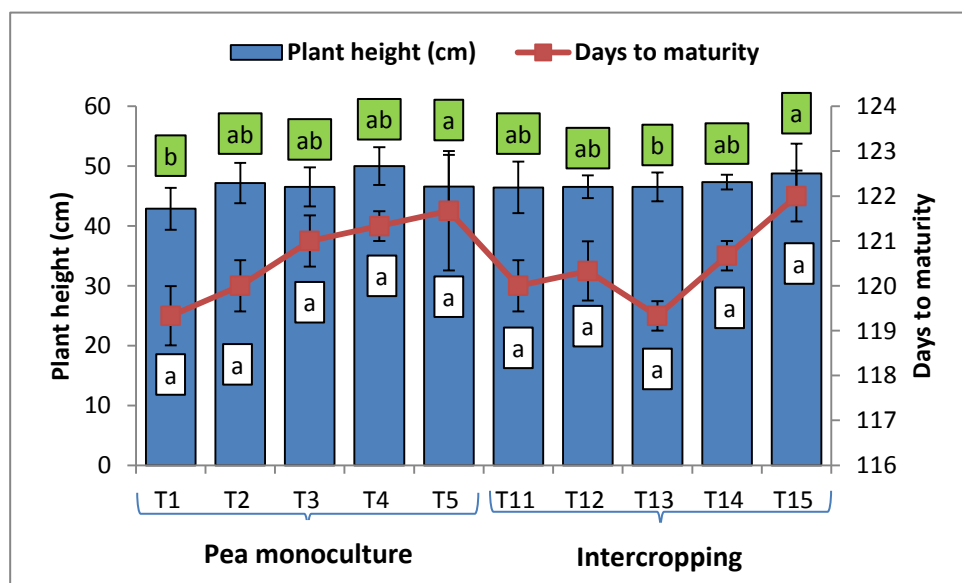


Figure 5.1 Effect of biofertilizers on plant height and days to maturity of pea

5.1.2. Effect of different biofertilizers on number of branches per plant and pod length (cm) of pea

It is apparent from data (Table 5.1 and Figure 5.2) that there was a significant difference ($p < 0.05$) in number of branches of pea plant. Similar statistical maximum number of branches was recorded in T15 (Wheat + Pea + R + PGPR + AMF) and T4 (Pea + AMF) with 3.90 and 3.05 means branches per plant respectively while the minimum number of branches per plant was 2.39 noticed in T1 (Pea sole). The maximum number of branches in these treatments might be attributed to the fact that the component biofertilizers such *Rhizobium* fixed atmospheric nitrogen through nodules hence increases plant height, branches per plant, and number of nodules (Zahran, 1999 and Rudresh *et al.*, 2005). PGPR also has the ability to produce phytohormones, organic acids, siderophores, fix atmospheric nitrogen, solubilize phosphate (Arshad and Frankenberger, 1998). AMF enhance phosphate nutrition in legumes, which results in plant growth and nitrogen fixation (Cluett and Boucher, 1983). The results obtained in this investigation are in line with El-Mansi *et al.* (2000) who reported the increase in branches by application of biofertilizers.

Results pertaining to pod length (cm) are presented in Table 5.1 and Figure 5.2 indicated a significant difference ($p < 0.05$). The longest pod was observed in T3 (Pea + PGPR) and T13 (Wheat + Pea + PGPR) which was statistically similar with 8.05 and 7.94 cm respectively. The shortest pod was found in case of T11 (Wheat + Pea Sole) with 5.94 cm. The significant effect of biofertilizers application on pod length in pea might attributed to the PGPR which enhanced plant growth by synthesizing plant growth promoting hormones (Dobbelaere *et al.*, 2003), facilitating nutrients uptake from soil (Çakmakçı *et al.*, 2006) or preventing plant diseases (Selvakumar *et al.*, 2009). The results obtained are in line with that found by Rather *et al.* (2010) who reported significant increase in pod length, number of pods per plant, number of seeds per pod, 100 grain weight of pea increased by co-inoculation of *Rhizobium*, *Azotobacter* and PSB. Negi *et al.* (2006) reported that pod length was significantly increased under the influence of biofertilizers.

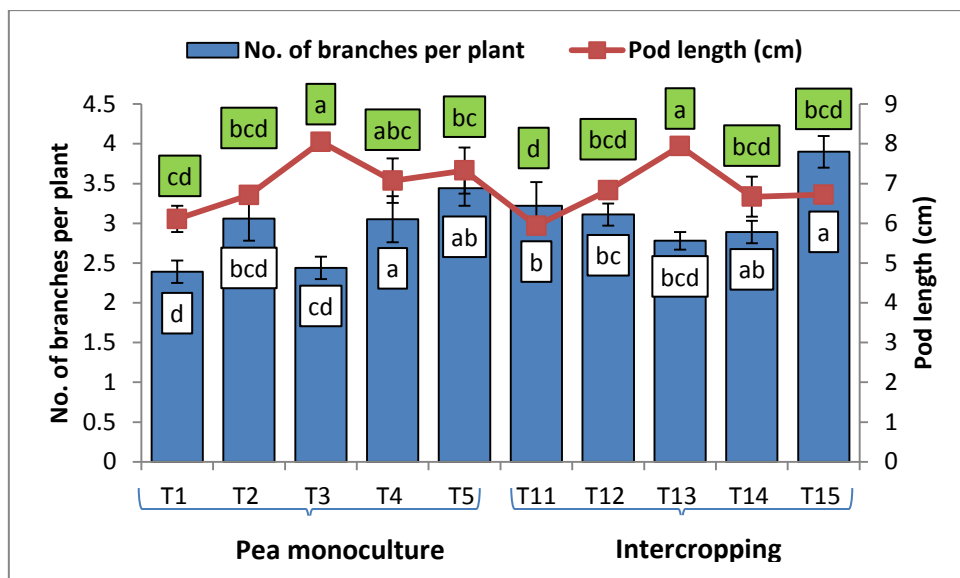


Figure 5.2 Effect of biofertilizers on number of branches per plant and pod length (cm) of pea

5.1.3. Effect of different biofertilizers on number of pods per plant and number of seeds per pod of pea

The application of biofertilizers on pea was significant ($p < 0.05$) on number of pods per plant. The data presented in Table 5.1 and Figure 5.3 showed that the maximum average number of pods per plant was recorded in T5 (Pea + R + PGPR + AMF) which was statistically similar to T3 (Pea + PGPR) with 5.60 and 5.44 pods per plant respectively. The minimum number of pods per plant of 2.67 pods was found in case of T11 (Wheat + Pea). The significant effect of biofertilizers on number of pod per plant might be due to the fact that *Rhizobium* is nitrogen fixing bacteria (NFB) that can transform inert atmospheric nitrogen to organic compounds (Bakulin *et al.*, 2007). Also increment in number of pod per plant might be due to the improvement of phosphate uptake and growth in leguminous by AMF (Ezawa *et al.*, 1995). Glick (1995) reported maximum number of pods per plant due to PGPR which stimulates growth by fixing atmospheric nitrogen, production of siderophores which chelate iron and make it available for the plant root, solubilizing phosphorus and secretion of phytohormones. The above factors and results are in accordance with Pramanik and Bera (2012) who reported that *Rhizobium*, PSB and VAM significantly increased number of pod per plant, weight of pod per plant, number of seeds per plant, test weight, seed yield, stalk yield and harvest index in chickpea. Also the obtained findings are in line with Zhang *et al.* (2002) who reported an increment of number of pods per plant, number of seeds per pod in two soybean cultivars. Moreover, Kazemi *et al.* (2005) found that the inoculated seeds of

soybean by biofertilizers induced significant number of pods per plant, number of seeds per plant, thousand grain weights and grain yield in soybean.

It is evident from the data (Table 5.1 and Figure 5.3) that different biofertilizers showed a significant difference ($p < 0.05$) in number of seeds per pod. The maximum number of seeds per pod was noticed in T5 (Pea + R + PGPR + AMF) with 6.76 seeds while the minimum number of seeds per pod was found in case of T1 (Pea sole) with 5.33 seeds per pod. The significant effect of biofertilizers on number of seeds per pod in treated plant might be attributed to provision of needed nitrogen and phosphorus. Later is known as the essential cell division, root and seed formation (Gizawy and Mehasen, 2009). Elshanshoury (1995) reported increased nutrient uptake like NO_3^- , NH_4^+ , PO_4^{3-} , K^+ and Fe^{2+} in inoculated plants. The results found corroborate the ones of Srivastava and Ahlawat (1995) and Yadav (2009) in pea. Biofertilizers induced significant number of pods per plant, number of seeds per plant, thousand grain weights and grain yield in soybean.

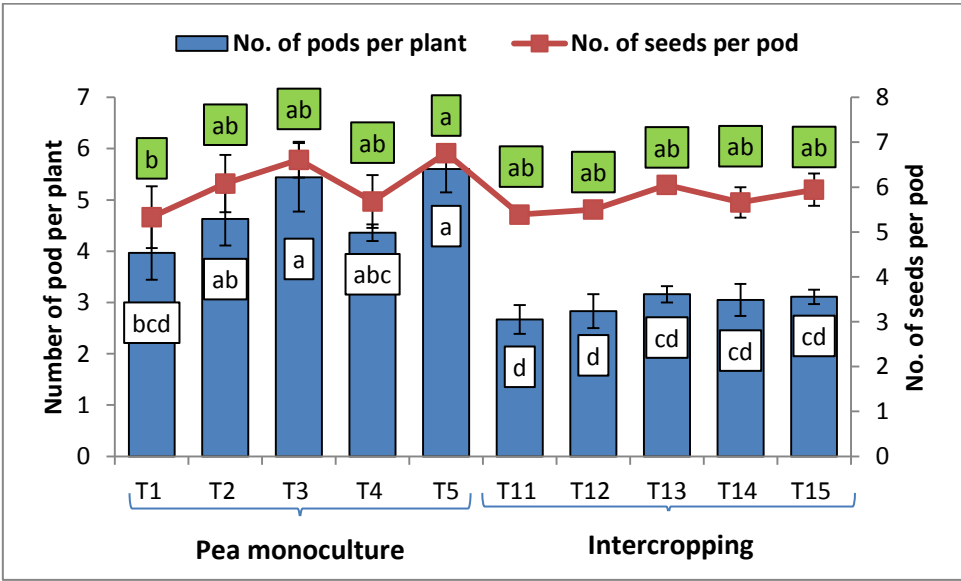


Figure 5.3 Effect of biofertilizers on number of pods per plant and number of seeds per pod of pea

5.1.4. Effect of different biofertilizers on 1000 seeds weight (g) and seed yield (kg/ha) of pea

Data regarding 1000 seeds weight (g), presented in Table 5.1 and Figure 5.4 revealed that application of biofertilizers on pea had no significant difference ($p < 0.05$). The average maximum 1000 seeds weight of 146.67 g was obtained in T14 (Wheat + Pea + AMF) while the minimum 1000 seeds weight was 119.67 g recorded in T11 (Wheat + Pea Sole). Although the inoculated plants showed highest records compared to uninoculated. This better performance of inoculated plants over non-inoculated plants might be attributed to the fact that biofertilizers have the ability to solubilize, to enhance plant growth by increasing biological fixation, promoting availability of micro elements and releasing phytohormones. The same results were observed by Kazemi *et al.* (2005) who reported the increase in 1000 seeds weight due to biofertilizers inoculation and Zhang *et al.* (2002) who reported increase in 100 seeds weight of two soybean cultivars.

Data pertaining to seed yield (kg/ha) is given in Table 5.1 and Figure 5.4 indicated that the maximum average seed yield (kg/ha) in pea was found in case of T5 (Pea + R + PGPR + AMF) while the minimum average seed yield (kg/ha) was recorded in T11 (Wheat + Pea Sole) with 605.09 kg/ha and 176.03 kg/ha respectively. Significant effect of combined biofertilizers showed higher seed yield compared to other treatments. This might be due to the fact that component biofertilizers can promote and induce nutrient uptake and their availability in the soil. These results are in agreement with Negi *et al.* (2006). Also the yield attributes improvement including seed yield due to biofertilizer inoculation might attributed to the increased and balanced nutrients availability (N and P). The results from this present study are in agreement with Sharma and Namdeo (1999). Variation in pea yield across this investigation might be attributed to weather conditions such as temperatures, intense and uneven rainfall distribution which in some cases caused water logging (Table 4.1 and Figure 4.2).

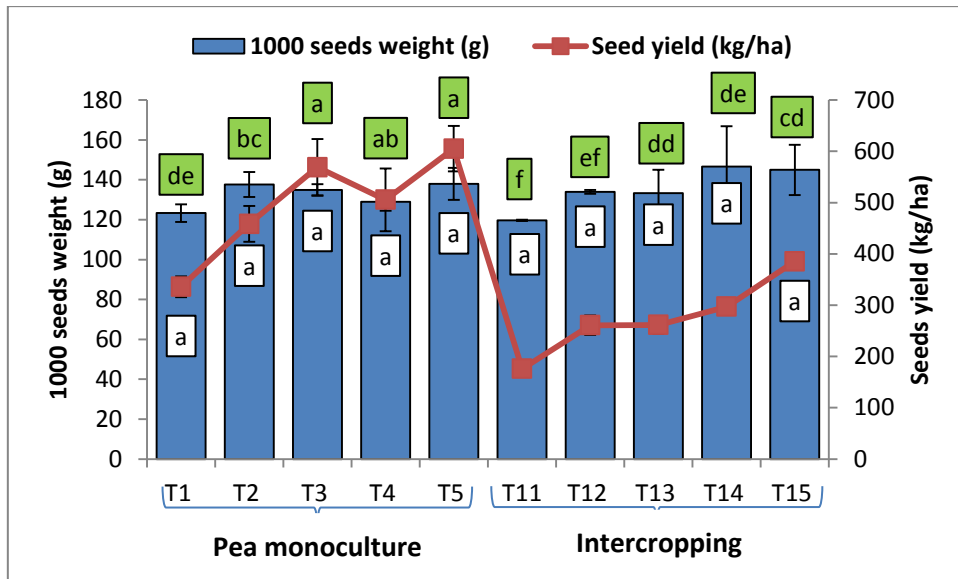


Figure 5.4 Effect of biofertilizers on 1000 seeds weight (g) and seed yield (kg/ha) of pea

Table 5.1 Effect of different biofertilizers on growth and yield parameters of pea (*Pisum sativum* L.) variety PB 89 in monoculture and in intercropping

PEA MONOCULTURE								
Treatments	Plant height (cm)	Days to maturity	No. of branches per plant	Pod length (cm)	No. of pods per plant	No. of seeds per pod	1000 seeds weight (g)	Seed yield (kg/ha)
T1 = Pea sole	42.88 ^a ±3.51	119.33 ^b ± 0.66	2.39 ^d ±0.14	6.11 ^{cd} ±0.33	3.97 ^{bcd} ±0.53	5.33 ^b ±0.69	123.33 ^a ±4.40	335.77 ^{de} ±20.25
T2 = Pea + R	47.16 ^a ±3.38	120.00 ^{ab} ± 0.57	3.06 ^{bcd} ±0.28	6.71 ^{bcd} ±0.20	4.63 ^{ab} ±0.52	6.08 ^{ab} ±0.64	137.67 ^a ±6.22	458.35 ^{bc} ±34.96
T3 = Pea + PGPR	46.52 ^a ±3.28	121.00 ^{ab} ± 0.57	2.44 ^{cd} ±0.14	8.05 ^a ±0.05	5.44 ^a ±0.67	6.61 ^{ab} ±0.40	135.00 ^a ±2.88	568.81 ^a ±55.23
T4 = Pea + AMF	50.00 ^a ±3.16	121.33 ^{ab} ± 0.33	3.05 ^a ±0.29	7.07 ^{abc} ±0.56	4.36 ^{abc} ±0.16	5.68 ^{ab} ±0.59	129.00 ^a ±4.58	505.39 ^{ab} ±61.38
T5 = Pea + R + PGPR + AMF	46.59 ^a ±5.31	121.67 ^a ± 1.33	3.44 ^{ab} ±0.22	7.33 ^{bc} ±0.58	5.60 ^a ±0.45	6.76 ^a ±0.08	138.00 ^a ±8.00	605.09 ^a ±44.20
PEA INTERCROPPING								
T11 = Wheat + Pea sole	46.44 ^a ±4.29	120.00 ^{ab} ±0.57	3.22 ^b ±0.30	5.94 ^d ±0.05	2.67 ^d ±0.28	5.39 ^{ab} ±0.14	119.67 ^a ±0.33	176.03 ^f ±5.37
T12 = Wheat + Pea + R	46.55 ^a ±1.92	120.33 ^{ab} ±0.66	3.11 ^{bc} ±0.14	6.83 ^{bcd} ±0.16	2.83 ^d ±0.33	5.50 ^{ab} ±0.00	134.00 ^a ±1.00	260.85 ^{ef} ±19.19
T13 = Wheat + Pea + PGPR	46.52 ^a ±2.39	119.33 ^b ±0.33	2.78 ^{bcd} ±0.11	7.94 ^a ±0.11	3.16 ^{cd} ±0.16	6.05 ^{ab} ±0.14	133.33 ^a ±11.66	285.4 ^{de} ±17.16
T14 = Wheat + Pea + AMF	47.33 ^a ±1.24	120.67 ^{ab} ±0.33	2.89 ^{ab} ±0.14	6.67 ^{bcd} ±0.50	3.05 ^{cd} ±0.31	5.66 ^{ab} ±0.34	146.67 ^a ±20.27	297.07 ^{de} ±12.94
T15 = Wheat + Pea + R + PGPR + AMF	48.77 ^a ±4.97	122.00 ^a ±0.57	3.90 ^a ±0.20	6.72 ^{bcd} ±0.20	3.11 ^{cd} ±0.14	5.94 ^{ab} ±0.36	145.00 ^a ±12.58	382.37 ^{cd} ±8.51

R: *Rhizobium*, PGPR: Plant Growth Promoting Rhizobacteria, AMF: Arbuscular Mycorrhizal Fungi and T: Treatment. Values are means ± SE, n=3, the mean followed by similar letter(s) are not significantly different at p<0.05, according to DMRT (Duncan's Multiple Range Test) for separation of mean.

5.2. Biometric parameters of wheat

5.2.1. Effect of different biofertilizers on plant height (cm) and days to maturity of wheat

Results presented in Table 5.2 and Figure 5.5 revealed that, plant height of wheat plant was not significantly ($p < 0.05$) affected by application of biofertilizers. The highest average plant height was observed in case of T15 (Wheat + Pea + R + PGPR + AMF) with 67.52 cm while the lowest plant height was 62.66 cm and was recorded in T13 (Wheat + Pea + PGPR). The application of biofertilizers in wheat had no significant effect on plant height. However, the inoculated plant showed highest records compared to uninoculated plants. This behaviour of inoculated plant might be attributed to N-fixing activity and secretion of growth promoting substances such as IAA (Indole-3-Acetic Acid), gibberellins and cytokinin (El-Shanshoury, 1995.) and mineralization of certain nutrients (EL-Demerdash *et al.*, 1992). The results from this investigation are in accordance with Rashid *et al.* (1998) and Ahemed *et al.* (1998) who reported higher plant height for wheat in IPNM (Integrated Plant Nutrient Management). Selvakumar *et al.* (2009) and Gomaa *et al.* (2002) had reported the increase in plant height, leaf number and leaf area with biofertilizer and / or organic fertilizer result in yield increase.

A perusal of data from Table 5.2 and Figure 5.5 indicated that the application of biofertilizers gave no significant effect on number of days to maturity in wheat plant. The maximum number of days to maturity was found in case of T12 (Wheat + Pea + R) with 148 days while the minimum number of days was recorded in T6 (Wheat sole) with 146 days. The effect on days to maturity by application of biofertilizers not was significant. Though, inoculated plants gave highest values over uninoculated plants. This might be attributed to the growing substances produced by biofertilizers which induced phytohormones and plant nutrients availability in the soil. This result is in line with Mardalipour *et al.* (2014) who reported that nano biofertilizers increased growing period length in wheat.

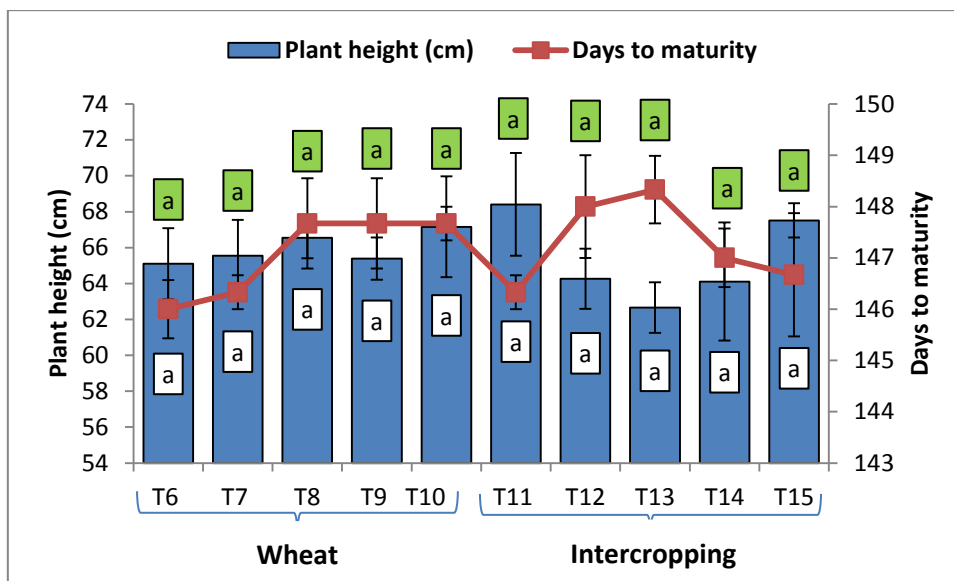


Figure 5.5 Effect of biofertilizers on plant height (cm) and days to maturity of wheat

5.2.2. Effect of different biofertilizers on productive tillers per plant and spike length (cm) of wheat

The effectiveness of all treatments on number of productive tillers per plant was presented in Table 5.2 and Figure 5.6. Comparison of different treatment means indicated that number of productive tillers per plant were significantly affected by application of biofertilizers at $p < 0.05$. The maximum number of productive tillers per plant was obtained in T8 (Wheat + PGPR) with 6.5 tillers per plant with the minimum number of tillers was 4.38 tillers per plant and was recorded in T11 (Wheat + Pea sole). The maximum number of productive tillers in treatment inoculated by PGPR may be explained by the fact that PGPR increased nitrogen uptake, solubilized phosphorus, produced siderophores and secreted phytohormones needed to chelate iron and make it available to plant (Gyaneshwar *et al.*, 1998). Results from this investigation are in line of Sial *et al.* (2003) who observed maximum number of effective tillers in IPNM (Integrated Plant Nutrients Management). Also the results are in agreement with Idrees *et al.* (2002) who reported increase in number of tillers in wheat IPNM.

Results on spike length (cm) of wheat indicated that there was no significant effect ($p < 0.05$) of biofertilizers application on spike length (Table 5.2 and Figure 5.6). The longest spike was produced in T14 (Wheat + Pea + AMF) with 10.65 cm while the shortest spike of 10.01 cm was recorded in T11 (Wheat + Pea sole).

The improved performance in spike length in T14 inoculated with AMF might be attributed to the fact that AMF has the ability to increase absorption and translocate mineral nutrients from soil to host plant (George *et al.*, 1995), to induce tolerance towards biotic (Singh *et al.*, 2000) and abiotic stresses (Gaur and Adholeya, 2004). The results from this study are in line with Rashid *et al.* (1998) and Ahmed *et al.* (1998) who found that in wheat, IPNM enhanced higher yield attributes in rainfed areas. Also Chatha *et al.* (2005) observed higher spike length when mineral and organic fertilizers were combined. Findings from this study are not in agreement with Ahmed (1972) and Agawal and Singh (1976).

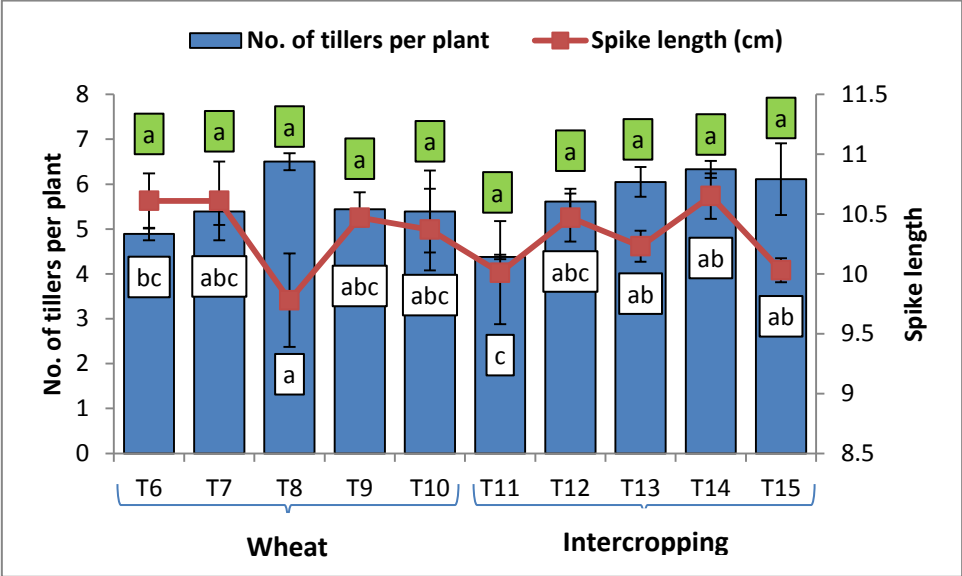


Figure 5.6 Effect of biofertilizers on productive tillers per plant and spike length (cm) of wheat

5.2.3. Effect of different biofertilizers on number of grains per spike and 1000 grains weight (g) of wheat

Data pertaining to the number of grain per spike of wheat is presented in 5.2 and Figure 5.7. The comparison of different treatment means indicated that the number of grain per spike of wheat monoculture was significantly ($p < 0.05$) influenced by application of different biofertilizers. The maximum number of grains per spike was observed in T14 (Wheat + Pea + AMF) with 42.72 grains per spike while the minimum average number of grains per spike was recorded in T11 (Wheat + Pea sole) with 35.05 grains per spike. Maximum number of grains per spike was recorded in T14 in which wheat and pea were inoculated with AMF.

This might be resulted from AMF which increased phosphorus availability by colonizing roots and through extended hyphae of AMF that enlarge the effective surface outside of the roots into rhizosphere (Manske, 1990, Manske *et al.*, 1995). Results from this study corroborate with Bahrani *et al.* (2010) who reported positive effect of biofertilizers on grains per spike. Idrees *et al.* (2002) also confirmed improvement in number of grains per spike when mineral and organic fertilizers were integrated.

Weight of 1000 grain has a great importance on final wheat yield. Data pertaining to 1000 grain weight (g) of wheat intercropped with pea presented in 5.2 and Figure 5.7. Comparison of different treatment means indicated that application of different biofertilizers was significant ($p < 0.05$) on 1000 grain weight. As regard of results maximum mean 1000 grain weight (42.72 g) was observed in T9 (Wheat + AMF) while the minimum 1000 grain weight of 38.70 g was found in case of T11 (Wheat + Pea sole). Positive effect of AMF may be due to the ability of biofertilizers to promote the availability of phosphorus and others plant nutrients (Kucey *et al.*, 1989, Tiwari *et al.*, 1989). The results from this study are in line with Afzal *et al.* (2005) who observed significant effect on 1000 weight when phosphorus was in combination with PSM.

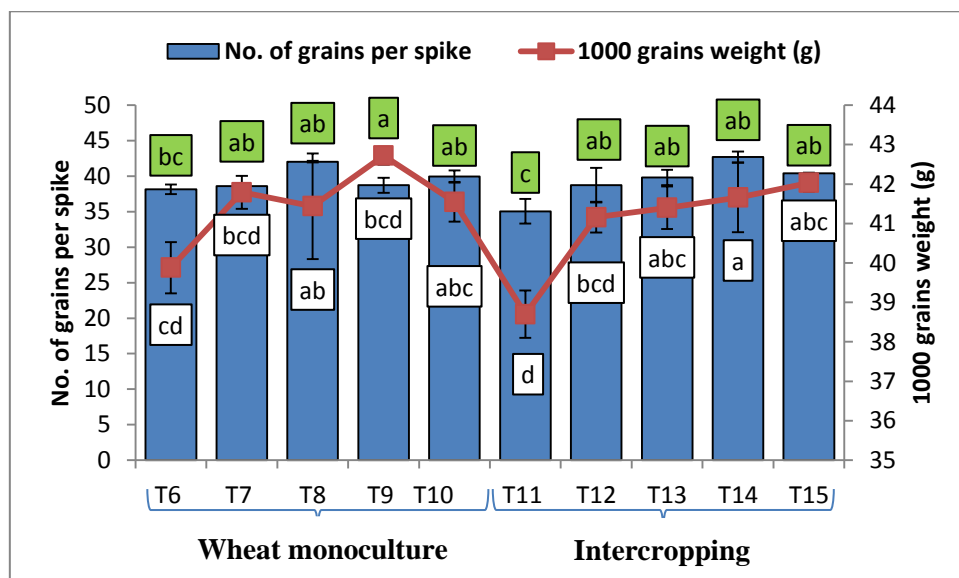


Figure 5.7 Effect of biofertilizers on number of grains per spike and 1000 grains weight (g) of wheat

5.2.3. Effect of different biofertilizers on grain yield (kg/ha) of wheat

A number of various morphological and physiological mechanisms interacting during vegetative stages of wheat and result in grain yield. The data regarding grain yield (Table 5.2 and Figure 5.8) of wheat showed that application of biofertilizers was significant ($p < 0.05$). The maximum average grain yield of 7469.20 kg/ha was recorded in T10 (Wheat + R + PGPR+ AMF) and the minimum grain yield of 2309.70 kg/ha was noticed in T11 (Wheat + Pea sole). The significant effect of biofertilizers on grain yield was observed in crop with combined biofertilizers. This might be due to better root development that results in more nutrient uptake along with production of plant growth promoting substances. This also could be due to the fact that wheat was treated with *Rhizobium*, AMF and PGPR and might be explained by the fact that PGPR can directly improve plant growth by secreting phytohormones and by rising nutrient uptake (Lippmann *et al.*, 1995) or by enhancing plant resistance to pathogens (Liu *et al.*, 1995a,b). It has been observed by Wiehe and Höflich (1995) that *Rhizobium leguminosarum* bv.trifolii, multiplied and survived in non-legumes (corn, rape *Brassica napus* L. and wheat). Apart from N-fixation by *Rhizobium* in non-legumes, it can also produce phytohormones like IAA (Wang *et al.*, 1982), siderophores (Guerinot, 1991) and phosphorus solubilisation (Chabot *et al.*, 1996). AMF are able to promote mineral nutrients by deliver them to host plant through their extended hyphal network (George *et al.*, 1995). Moreover, organic fertilizers released nutrients slowly and prevent the losses by leaching (Arshad *et al.*, 2004; Anup Das *et al.*, 2010). The results obtained are in accordance with Sharma and Singh (2008) and Khaliq and Sanderz (2000). Different researchers, Zorita and Canigia (2009) reported that application of biofertilizers enhance grain yield in wheat. Similarly, Kizilkaya (2008); Sary *et al.* (2009) and Daneshmand *et al.* (2012). Enhancement in yield and its components might be due to secretion of plant growth substances which increase the availability of nutrients (Vessy, 2003 and Piccinin *et al.*, 2013).

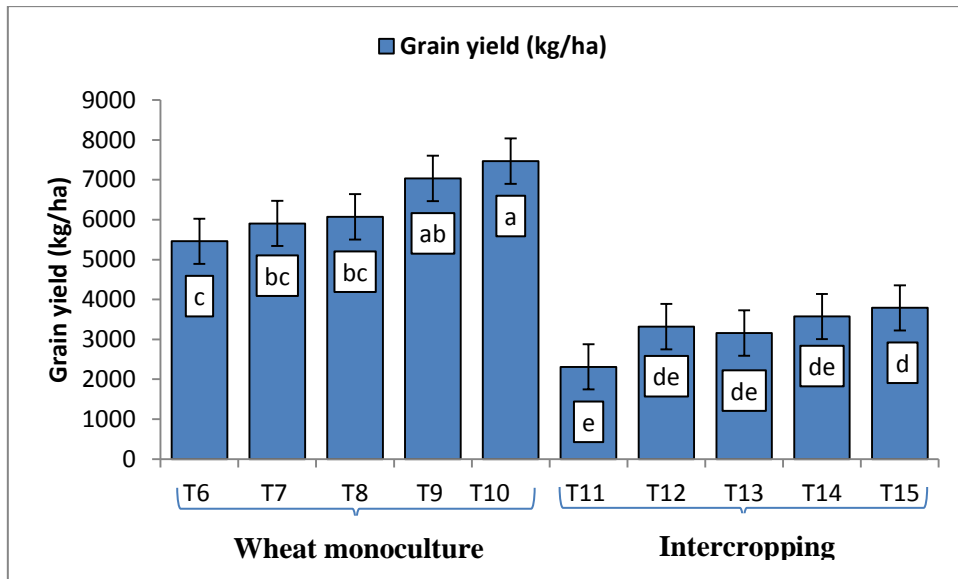


Figure 5.8 Effect of biofertilizers on grain yield (kg/ha) of wheat

Table 5.2 Effect of different biofertilizers on growth and yield parameters of wheat (*Triticum aestivum* L.) variety WH1150 in monoculture and in intercropping

WHEAT MONOCULTURE							
Treatments	Plant height (cm)	Days to maturity	No. of tillers per plant	Spike length (cm)	No. of grains per spike	1000 grains weight (g)	Grain yield (kg/ha)
T6 = Wheat sole	65.11 ^a ±1.98	146.00 ^a ±0.57	4.89 ^{bc} ±0.14	10.61 ^a ±0.23	38.16 ^{cd} ±0.67	39.88 ^{bc} ±0.65	5460.20 ^c ±243.44
T7 = Wheat + R	65.55 ^a ±2.00	146.33 ^a ±0.33	5.39 ^{abc} ±0.30	10.61 ^a ±0.33	38.61 ^{bcd} ±0.14	41.79 ^{ab} ±0.42	5906.60 ^{bc} ±386.66
T8 = Wheat + PGPR	66.55 ^a ±1.14	147.67 ^a ±0.88	6.50 ^a ±0.19	9.78 ^a ±0.39	42.05 ^{ab} ±0.11	41.44 ^{ab} ±1.34	6073.20 ^{bc} ±11.97
T9 = Wheat + AMF	65.39 ^a ±1.18	147.67 ^a ±0.88	5.44 ^{abc} ±0.38	10.47 ^a ±0.02	38.72 ^{bcd} ±1.07	42.72 ^a ±0.23	7033.70 ^{ab} ±890.67
T10 = Wheat + R + PGPR+ AMF	67.16 ^a ±2.81	147.67 ^a ±0.33	5.39 ^{abc} ±0.91	10.37 ^a ±0.34	39.94 ^{abc} ±0.86	41.55 ^{ab} ±0.50	7469.20 ^a ±738.21
WHEAT INTERCROPPING							
T11 = Wheat + Pea sole	68.41 ^a ±2.86	146.33 ^a ±0.33	4.38 ^c ±0.05	10.01 ^a ±0.43	35.05 ^d ±1.73	38.706 ^c ±0.60	2309.70 ^e ±115.43
T12= Wheat + Pea + R	64.27 ^a ±1.68	148.00 ^a ±1.00	5.61 ^{abc} ±0.29	10.47 ^a ±0.20	38.72 ^{bcd} ±2.43	41.166 ^{ab} ±0.39	3318.80 ^{de} ±76.07
T13= Wheat + Pea + PGPR	62.66 ^a ±1.41	148.33 ^a ±0.66	6.05 ^{ab} ±0.33	10.23 ^a ±0.13	39.83 ^{abc} ±1.08	41.403 ^{ab} ±0.54	3158.80 ^{de} ±95.56
T14= Wheat + Pea + AMF	64.11 ^a ±3.29	147.00 ^a ±0.57	6.33 ^{ab} ±0.19	10.65 ^a ±0.19	42.72 ^a ±0.77	41.663 ^{ab} ±0.88	3573.30 ^{de} ±159.31
T15= Wheat + Pea + R + PGPR+ AMF	67.52 ^a ±0.96	146.67 ^a ±1.20	6.11 ^{ab} ±0.80	10.03 ^a ±0.10	40.39 ^{abc} ±0.11	42.030 ^{ab} ±0.22	3790.60 ^d ±118.71

R: Rhizobium, PGPR: Plant Growth Promoting Rhizobacteria, AMF: Arbuscular Mycorrhizal Fungi and T: Treatment. Values are means ± SE, n=3, the mean followed by similar letter(s) are not significantly different at p<0.05, according to DMRT (Duncan's Multiple Range Test) for separation of mean.

5.3 Effect of different biofertilizers on land equivalent ratio at pea and wheat monoculture crop and in intercropping

The yield obtained at wheat-pea intercropping (Table 5.3) showed that, in intercropping, the productivity was different from the monoculture. Thus, in pea monoculture the maximum average yield was recorded in T5 (Pea + R + PGPR + AMF) with 605.09 kg/ha which was 269.32 kg/ha more than pea without biofertilizer and when pea was in intercropping with the same biofertilizers the yield was reduced to 385.37 kg/ha. Similarly, Mutungamiri *et al.* (2001) reported a decrease in bean yield in maize-bean intercropping system.

In wheat monoculture, maximum average yield of 7469.2 kg/ha was produced by T10 (Wheat + R + PGPR+ AMF) which is 2009 kg/ha more than wheat without biofertilizers and when wheat is in intercropping the yield reduced to 3790.60 kg/ha. The reduction in cereal yield in this study might be attributed to the interspecific completion of resources (Nnoko and Doto, 1980; Francis *et al.* 1982; Caballero *et al.* 1995 and Assefa and Ledin, 2001).

Regarding the land equivalent ratio presented in Table 4.3, showed the highest LER value was 1.139 followed by 1.131, 1.096 and 1.022 respectively. These results showed that in sole crop, there would be 13.9%, 13.1%, 9.6% and 2.2% respectively more land areas to produce the same yield as in intercropping. The lowest land equivalent ratio was 0.947.

The data in Table 5.3 showed that the inoculation of biofertilizers in wheat-pea intercropping system increased the LER compared to non-inoculated plants. Dhima *et al.* (2007) reported that when LER value is greater than 1, intercropping has advantages over monoculture in terms of use of resources for crop growth. On other hand, when LER is less than 1, means that use of resources in monoculture is more efficient than in intercropping and it will better to grow both crops separately (Francis and Sanders, 1978). The superiority of inoculated plant over non-inoculated in terms of LER during this investigation might due to the fact that the light, water, carbon dioxide and nutrients competition between components. Advantage from cereal-legume intercropping systems has been reported by Banik, (1996) in wheat-legume; Chen *et al.* (2004) in pea and barley; Li *et al.* (1999) in maize faba bean.

Table 5.3 Yield and the land equivalent ratio (LER) of pea and wheat in monoculture crop and intercropping

Treatments	Pea	Wheat	LER		Total LER
			Pea	Wheat	
T1 = Pea sole	335.77	-	-	-	-
T2 = Pea + R	458.35	-	-	-	-
T3 = Pea + PGPR	568.81	-	-	-	-
T4 = Pea + AMF	505.39	-	-	-	-
T5 = Pea + R + PGPR + AMF	605.09	-	-	-	-
T6 = Wheat sole	-	5460.2	-	-	-
T7 = Wheat + R	-	5906.6	-	-	-
T8 = Wheat + PGPR	-	6073.2	-	-	-
T9 = Wheat + AMF	-	7033.7	-	-	-
T10 = Wheat + R + PGPR+ AMF	-	7469.2	-	-	-
T11 = Wheat + Pea sole	176.03	2309.7	0.524	0.423	0.947
T12= Wheat + Pea + R	260.85	3318.8	0.569	0.562	1.131
T13 = Wheat + Pea + PGPR	285.4	3158.8	0.502	0.520	1.022
T14= Wheat + Pea + AMF	297.07	3573.3	0.588	0.508	1.096
T15= Wheat + Pea + R + PGPR+ AMF	382.37	3790.6	0.632	0.507	1.139

5.4 Effect of different biofertilizers on soil chemical properties of post harvested soil.

5.4.1 Effect of different biofertilizers on available nitrogen and available potassium status of post harvested soil.

Nitrogen is an essential nutrient in vegetative growth of the crop. Comparison of various treatments means of different biofertilizers in Table 5.4 and Figure 5.9 showed a significant increase in soil available nitrogen as compared to the treatments without biofertilizers. The maximum average available N of 266.55 kg/ha was observed in T10 (Wheat + R + PGPR+ AMF) while the lowest value of available nitrogen was 168.17 kg/ha recorded in T11 (Wheat + Pea sole) where no biofertilizer was applied.

This significant increase of available N recorded in T10 might be attributed to the fact that component biofertilizers contributed to the soil microorganisms which enhance the availability of nutrients in the soil and plant were not able to uptake all nutrients. The results obtained in this investigation are in line with findings of Sharma *et al.* (2009). Wu *et al.* (2005) also reported an increase in availability of nitrogen in his experiment on effect of

biofertilizers on soil properties and the growth of *Zea mays*. Mahajan *et al.* (1996) also observed increment in soil N with IPNM after harvest.

A significant increase in soil available K at $p < 0.05$ was observed in treatments T15 (Wheat + Pea + R + PGPR+ AMF) with highest value of 312.92 kg/ha of available potassium followed T5 (Pea + R + PGPR + AMF), T10 (Wheat + R + PGPR+ AMF), T4 (Pea + AMF) and T9 (Wheat + AMF) with 310.78, 284.23, 233.18 and 225.77 kg/ha respectively. The lowest record of 149.26 kg/ha was observed in case of T1 (Pea sole) where no biofertilizer was applied. The significant increase in potassium might be attributed to the fact that when microorganisms' cultures are applied to the soil, they enhance organic residues decomposition hence releasing inorganic nutrients which become available for plant uptake (Javaid and Mahmood, 2010). The present results agree with Kaihura *et al.* (1999) and Blaise *et al.* (2005) who reported that FYM increased soil K. Stephen and Nybe (2003) reported increased soil N, P, K and Ca.

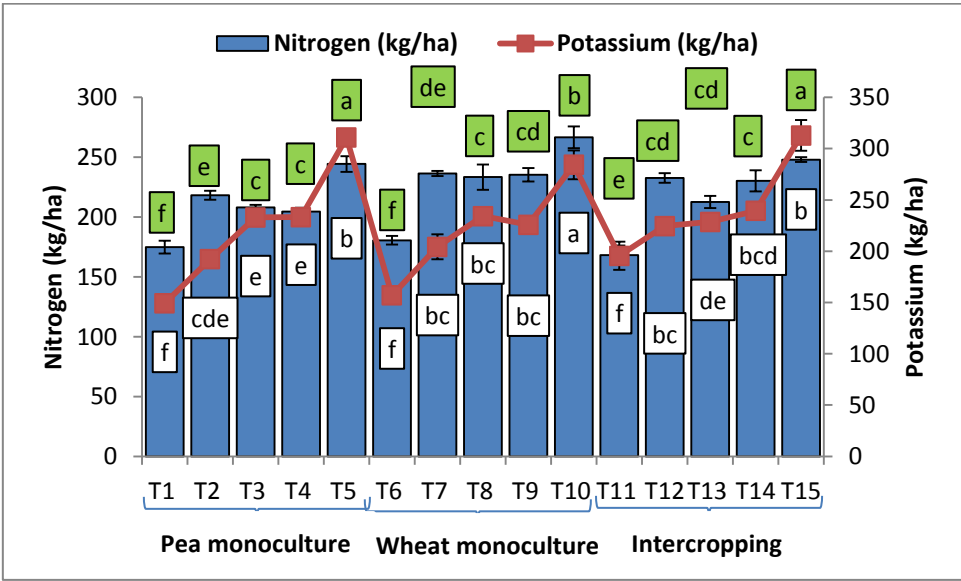


Figure 5.9 Effect of biofertilizers on nitrogen and potassium status of post harvested soil.

5.4.2 Effect of different biofertilizers on phosphorus and pH status of post harvested soil.

Data pertaining to available phosphorus in soil after harvesting is given in Table 5.4 and Figure 5.10. Comparison of means of different biofertilizers caused a significant increase in soil available phosphorus and the highest value was recorded in T15 (Wheat + Pea + R + PGPR+ AMF) and T5 (Pea + R + PGPR + AMF) which were statistically similar to each other with 28.30 kg/ha and 28.20 kg/ha respectively. The lowest value was noticed in T6 (Wheat sole = without biofertilizers) with 19.03 kg/ha. The effect of biofertilizers on available P was significant. This significant increase might probably due to the availability of nitrogen coupled with phosphorus from biofertilizers which may enhance the use of other nutrients. The significant increase also might be attributed to the fact that organic materials have the ability to cover sesquioxides then reduce P-solubilization hence increased P availability in soil solution (Bhardwaj and Omanwar, 1992). Similar results were reported by (Yadav, 2001) in cowpea. Morari *et al.* (2008) reported increase of available P due to organic fertilizers use. Also, Ipinmoroti *et al.* (2008) reported that application of organic manure results in higher build-up of N, P, K, Ca, Mg and organic carbon. Also, findings from this study are in line with Subramaniam and Kumaraswamy (1989) who found available P content of soil.

A critical examination of data in Table 5.4 and Figure 5.10 revealed that soil pH was significantly increased at ($p < 0.05$). Statistical similar effect on soil pH value ranged from 8.00 to 8.13 for all of treatments except T1. The portable reason behind the increased pH of soil was irrigation water used because at 60 DAS irrigation water had pH of 8.5 and at 90 DAS, pH was 9. Results from this study was disagree with Dhonde and Bhakare (2008) and Chang *et al.* (1991) who reported that FYM, wheat straw and *glycidia* leaves with NPK fertilizers significantly increase OC, whereas soil pH and EC were not affected significantly. Also, the obtained results differed from Chang *et al.* (1991) who reported that organic fertilizers have to lower soil pH.

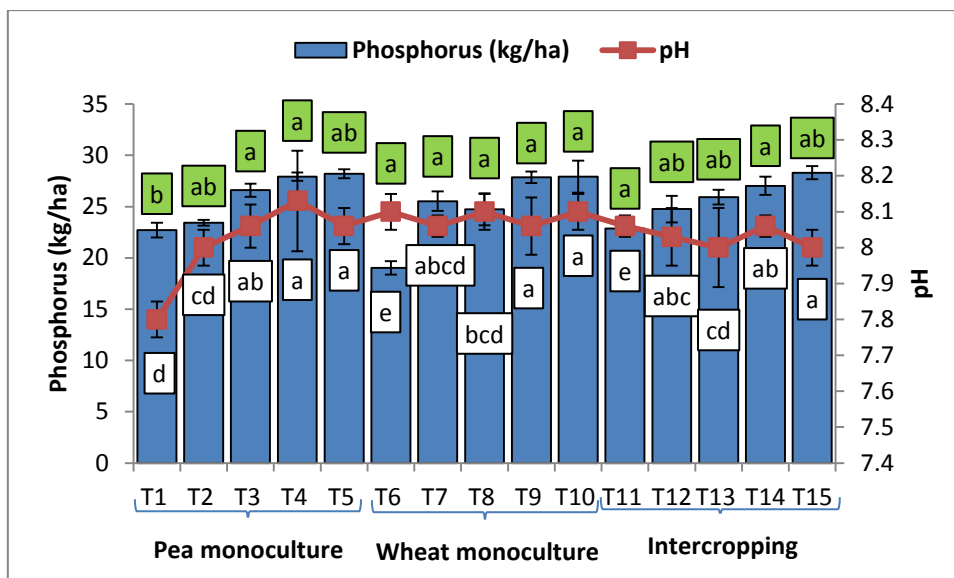


Figure 5.10 Effect of biofertilizers on phosphorus and pH status of post harvested soil.

5.4.3 Effect of different biofertilizers on OC and CEC status of post harvested soil.

Data pertaining to organic carbon (%) in soil after harvesting is given in Table 5.4 and Figure 5.11. Comparisons of biofertilizers treatments caused a significant increase in soil organic carbon ($p < 0.05$). The maximum organic carbon of 0.56 % was observed in T15 (Wheat + Pea + R + PGPR + AMF) while the minimum organic carbon of 0.43 % recorded in T1 (Pea sole). The increase in organic carbon might be due to microbial inoculants that are able to release bound nutrients in most organic matter at right time. Results from this study are in line with Sharma (2014) who reported significant increase in organic matter due to biofertilizers. Also results from this investigation agree with Rajendra (2005) who reported that the organic manures improve the organic matter content and in turn support soil microorganisms. This is in line with the results of Yadav *et al.* (2009) who documented that FYM application gave a significant increment in organic carbon in soil.

It is apparent from data in Table 5.4 and Figure 5.11 that application of biofertilizers caused a significant increase in CEC (meq/100g) in post-harvest soil. The maximum CEC value of 0.51 meq/100g was observed in T13 (Wheat + Pea + PGPR) while the minimum value of 0.32 meq/100g was recorded in T10 (Wheat + R + PGPR+ AMF).

The significant increase in CEC can be directly correlated with increased in soil organic matter. This results agree with Dadhich *et al.* (2011) who reported that application of FYM increased OC, CEC and available of NPK in the soil. Also Nandwa (1995) reported that animal manure and compost increase water holding capacity and CEC.

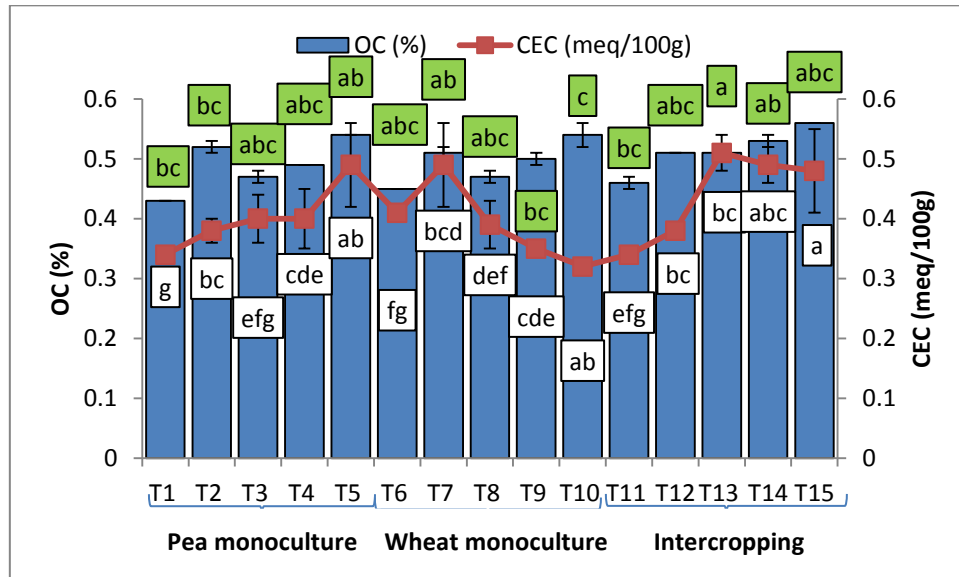


Figure 5.11 Effect of biofertilizers on OC and CEC status of post harvested soil.

5.4.4 Effect of different biofertilizers on EC status of post harvested soil.

Data from Table 5.4 and Figure 5.12 pertaining to EC (mmhos/cm) showed a significant difference ($p < 0.05$) due to application of biofertilizers. The maximum EC of 0.21 mmhos/cm was recorded in T4 (Pea + AMF), T7 (Wheat + R), T13 (Wheat + Pea + PGPR) and T15 (Wheat + Pea + R + PGPR+ AMF) which were statistical at par to each other. The T10 (Wheat + R + PGPR+ AMF) showed EC of 0.15 mmhos/cm. Results of before sowing soil analysis Table 3.2 showed that EC was 0.614 mmhos/cm. Post-harvest soil analysis showed a decrease in EC might be due to organic acids produced by PGPR (Das and Singh, 2014).

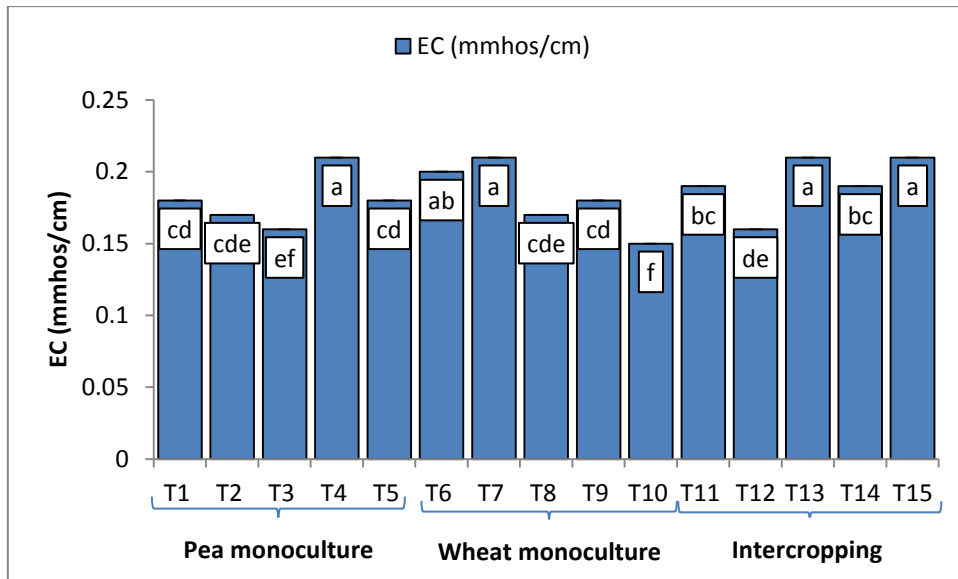


Figure 5.12 Effect of biofertilizers on EC of post harvested soil

Table 5.4 Effect of different biofertilizers on nutrients status of post harvested soil.

PEA MONOCULTURE							
Treatments	Nitrogen (kg/ha)	Potassium (kg/ha)	Phosphorus (kg/ha)	pH	OC (%)	CEC (meq/100g)	EC (mmhos/cm)
T1 = Pea sole	174.83 ^f ±5.26	149.26 ^f ±2.15	22.70 ^d ±0.72	7.80 ^b ±0.05	0.43 ^g ±0.00	0.34 ^{bc} ±0.00	0.18 ^{cd} ±0.00
T2 = Pea + R	218.23 ^{cde} ±3.66	192.17 ^e ±5.76	23.43 ^{cd} ±0.29	8.00 ^{ab} ±0.05	0.52 ^{bc} ±0.01	0.38 ^{bc} ±0.02	0.17 ^{cde} ±0.00
T3 = Pea + PGPR	207.90 ^e ±2.20	232.94 ^c ±7.65	26.60 ^{ab} ±0.64	8.06 ^a ±0.06	0.47 ^{efg} ±0.01	0.40 ^{abc} ±0.04	0.16 ^{ef} ±0.00
T4 = Pea + AMF	204.63 ^e ±3.20	233.18 ^c ±4.05	27.93 ^a ±0.40	8.13 ^a ±0.14	0.49 ^{cde} ±0.00	0.40 ^{abc} ±0.05	0.21 ^a ±0.00
T5 = Pea + R + PGPR + AMF	244.30 ^b ±6.51	310.78 ^a ±8.27	28.20 ^a ±0.45	8.06 ^{ab} ±0.05	0.54 ^{ab} ±0.00	0.49 ^{ab} ±0.07	0.18 ^{cd} ±0.00
WHEAT MONOCULTURE							
T6 = Wheat sole	180.53 ^f ±3.67	156.91 ^f ±5.68	19.03 ^e ±0.66	8.10 ^a ±0.05	0.45 ^{fg} ±0.00	0.41 ^{abc} ±0.0	0.20 ^{ab} ±0.00
T7 = Wheat + R	236.40 ^{bc} ±2.13	204.23 ^{de} ±12.18	25.53 ^{abcd} ±0.95	8.06 ^a ±0.03	0.51 ^{bcd} ±0.01	0.49 ^{ab} ±0.07	0.21 ^a ±0.00
T8 = Wheat + PGPR	233.40 ^{bc} ±10.60	234.26 ^c ±1.50	24.73 ^{bcd} ±1.56	8.10 ^a ±0.05	0.47 ^{def} ±0.01	0.39 ^{abc} ±0.04	0.17 ^{cde} ±0.00
T9 = Wheat + AMF	235.37 ^{bc} ±5.67	225.77 ^{cd} ±1.68	27.86 ^a ±0.56	8.06 ^a ±0.08	0.50 ^{cde} ±0.01	0.35 ^{bc} ±0.00	0.18 ^{cd} ±0.00
T10 = Wheat + R + PGPR+ AMF	266.55 ^a ±9.05	284.23 ^b ±14.02	27.93 ^a ±1.56	8.10 ^a ±0.05	0.54 ^{ab} ±0.02	0.32 ^c ±0.00	0.15 ^f ±0.00
WHEAT-PEA INTERCROPPING							
T11 = Wheat + Pea sole	168.17 ^f ±8.12	195.47 ^e ±13.75	22.86 ^e ±0.23	8.06 ^a ±0.03	0.46 ^{efg} ±0.01	0.34 ^{bc} ±0.00	0.19 ^{bc} ±0.00
T12= Wheat + Pea + R	232.57 ^{bc} ±4.02	224.46 ^{cd} ±2.04	24.76 ^{abc} ±1.29	8.03 ^{ab} ±0.08	0.51 ^{bc} ±0.00	0.38 ^{abc} ±0.01	0.16 ^{de} ±0.00
T13 = Wheat + Pea + PGPR	212.67 ^{de} ±5.05	228.57 ^{cd} ±5.19	25.93 ^{cd} ±0.71	8.00 ^{ab} ±0.11	0.51 ^{bc} ±0.01	0.51 ^a ±0.03	0.21 ^a ±0.00
T14= Wheat + Pea + AMF	230.27 ^{bcd} ±8.74	239.29 ^c ±3.63	27.03 ^{ab} ±0.88	8.06 ^a ±0.03	0.53 ^{abc} ±0.01	0.49 ^{ab} ±0.03	0.19 ^{bc} ±0.00
T15= Wheat + Pea + R + PGPR+ AMF	248.00 ^b ±1.91	312.92 ^a ±15.13	28.30 ^a ±0.64	8.00 ^{ab} ±0.05	0.56 ^a ±0.00	0.48 ^{abc} ±0.07	0.21 ^a ±0.00

R: Rhizobium, PGPR: Plant Growth Promoting Rhizobacteria, AMF: Arbuscular Mycorrhizal Fungi and T: Treatment. Values are means ± SE, n=3, the mean followed by similar letter(s) are not significantly different at p<0.05, according to DMRT (Duncan's Multiple Range Test) for separation of mean.

6. SUMMARY AND CONCLUSION

The present study was conducted to investigate the 'Effect of Different Biofertilizers on Growth and Yield Parameters of Wheat-Pea Intercropping System'. Perusal of the data from this study yielded in the following salient findings:

1. Application of biofertilizers showed no significant influence on plant height and 1000 seeds weight in pea monoculture.
2. PGPR inoculation was found significant on pod length in pea monoculture and in pea intercropped with wheat. Also it showed significant influence on number of pods per plant in pea monoculture.
3. Inoculation with combined biofertilizers (R + PGPR + AMF) was found to be significant on days to maturity, number of branches per plant, number of seeds per pod and seed yield in pea monoculture and it showed a significant influence on days to maturity, number of branches per plant and on pod length in pea intercropped with wheat. It gave the highest yield in pea monoculture.
4. Application of biofertilizers showed no significant effect on plant height, number of days to maturity and spike length in wheat.
5. Inoculation of PGPR showed a significant effect on number of tillers per plant and number of grains per spike in wheat monoculture and in wheat intercropped with pea respectively.
6. Inoculation of AMF found to be significant on 1000 seed weight of wheat monoculture.
7. The combination of biofertilizers (R + PGPR + AMF) was found to be significant in terms of yield and it gave higher yield in wheat monoculture.
8. Combination of biofertilizers (R + PGPR + AMF) showed superior land efficiency of intercropping in comparison with the single biofertilizer or without biofertilizer and its record was 1.139. That means a 13.9% area advantage of intercrops over monoculture.
9. Application of combined biofertilizers (R + PGPR + AMF) caused a significant increase in available N in wheat monoculture (T10), in available K in pea monoculture (T5), in wheat monoculture (T10) and in wheat-pea intercropping (T15). Also it showed a significant increase in available P in wheat-pea intercropping and pea monoculture respectively. OC also was significantly increase due to application of

combined biofertilizer application and finally increased EC in wheat-pea intercropping.

10. Application of AMF showed a significant increase in EC for wheat monoculture (T4) and wheat-pea intercropping (T13). Rhizobium showed to increase EC in wheat monoculture (T7).
11. Application of PGPR showed a significant increase in wheat-pea intercropping (T13).

On the basis of this experiment results it can be concluded that intercropping altered yield of component plants. Yield of monoculture was higher than their intercrop yields. The seed yield of pea was more affected by intercropping and being reduced with 54.04 % when inoculated with PGPR and was less reduced with 36.31 % when inoculated with combined biofertilizers (*Rhizobium* + PGPR + AMF). Yield of wheat was more affected by intercropping in plots without biofertilizer application and was less affected when treated with *Rhizobium*. All inoculated plants showed better land efficiency in intercropping as compared to their respective monocultures and the combination of *Rhizobium* + PGPR + AMF showed higher values of 1.139. That means, 13.9% more land area to produce the same yield as in intercropping. Also, intercropping affected nutrient availability in different treatments of pea monoculture as compared to its respective intercrop. In general, combination of biofertilizers (*Rhizobium* + PGPR + AMF) was found to work perfectly in monoculture as well as in intercropping in terms of soil nutrient availability of post-harvest soil. Therefore, it can be concluded that combined biofertilizer application use (R + PGPR + AMF) was the most effective in terms of yield in all cropping patterns (pea monoculture, wheat monoculture and wheat-pea intercropping) and can be used to reduce the use of chemical fertilizers for sustainable crop production in terms of yield and soil fertility as well as environmental safety. However, more and intense systematic studies are required to provide better understanding of biofertilizer use in making crop production more profitable income generating.

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APPENDIX



Picture showing status of experiment at 20 days after sowing



Picture showing status of experiment at 30 days after sowing after 1st hand weeding



Picture showing status of experiment at 50 days after sowing after 2nd hand weeding



Picture showing status of experiment at 65 days after sowing



Picture showing status of experiment at 72 days after sowing



Picture showing status of experiment at 112 days after sowing



Picture showing status of pea at 119 days after sowing



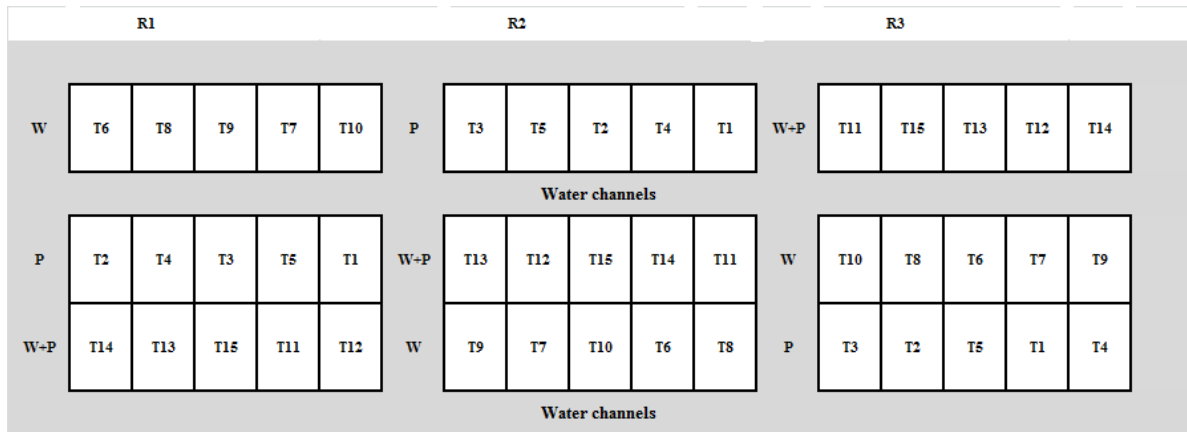
Picture showing status of wheat at 140 days after sowing



Picture showing yield from some plots for pea and wheat respectively



Seed yield and grain yield of pea and wheat respectively



T1	Pea sole	T6	Wheat Sole	T11	Wheat + Pea Sole
T2	Pea + R	T7	Wheat + R	T12	Wheat + Pea + R
T3	Pea + PGPR	T8	Wheat + PGPR	T13	Wheat + Pea + PGPR
T4	Pea + AMF	T9	Wheat + AMF	T14	Wheat + Pea + AMF
T5	Pea + R + PGPR + AMF	T10	Wheat + R+PGPR+ AMF	T15	Wheat + Pea + R + PGPR + AMF
W=	Wheat	P=	Pea	T=	Treatment
				Plot size =	4m x 2m

Figure showing experimental design and layout