MOLECULAR MARKER FOR CELLULOSE DEGRADING MICROBIAL COMMUNITY ECOLOGY TOWARDS SUSTAINABLE AGRICULTURE

A thesis submitted in fulfillment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY

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CERTIFICATE

Certified that the thesis **"Molecular Markers For Cellulose Degrading Microbial Community Ecology Towards Sustainable Agriculture"** which is submitted by Mr. Machiavelli, in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, is a record of the candidate's own independent and original research work carried out by him under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.

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DECLARATION

I hereby declare that the work which is being presented in this thesis **"Molecular Marker For Cellulose Degrading Microbial Community Ecology Towards Sustainable Agriculture"** submitted by me for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, is true and original record of my own independent and original research work carried out under the supervision of Prof. Sunil Khanna, Area Director, Bioinformatics & Biotechnology, NIIT University, Neemrana, Alwar, Rajasthan, India and Dr. N. Tejo Prakash, Associate Professor, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, Punjab, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

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TABLE OF CONTENTS

ABBREVIATIONS

%	Percentage
($x10^6$ cfu / g dry wt. of soil)	$x10^6$ colony forming unit per gram dry weight of soil
°C	Degree centigrade
bp	Base pair
cfu /g dry wt. of soil	Colony forming units per gram dry weight of soil
cm	Centimeter
DNA	Deoxyribonucleic acid
DNSA	3,5-Dinitrosalicylic acid
dNTP	2'-deoxynucleoside-5'-triphosphate
EDTA	Ethylenediamine-tetra acetic acid
g	Gram
$g\ l^{-1}$	Grams per litre
hr	Hour
hrs	Hours
kb	Kilo base
L	Litre
m	meter
mg	Milligram
mg/kg (ppm)	Milligrams per kilogram (parts per million)
mg/mL	Milligram per millilitre
mL	Milliliter
mM	Millimoles
N	Nitrogen

OC	Organic carbon
P	Phosphorous
PCR	Polymerase chain reaction
Q	Quintal
Q/ acre	Quintal per acre
rDNA	Ribosomal deoxyribonucleic acid
RDP	Ribosomal Database Project
RNA	Ribonucleic acid
₹	The Indian rupee (INR) is the official currency of India.
₹/ quintal	Rupee per quintal
rpm	Revolution per minute
sec	Second
Tris	Tris-(hydroxymethyl-) aminomethane
V	Volume of the solution
W	weight in grams
µg	Microgram
µg L^{-1}	Microgram per litre
µg ml^{-1}	Micrograms per millilitre
µl	Microlitre
µM glucose/ g dry wt. soil/ hr	Micromoles of glucose released per gram dry weight of soil per hour
µM PNP/ g dry wt. soil/ hr	Micromoles of p-nitro phenol released per gram dry weight of soil per hour

Chapter 1

Introduction

INTRODUCTION

The global necessity to increase agricultural production from a steadily decreasing and degrading land resource base has placed considerable strain on the fragile agro-ecosystems. Current strategies to maintain and improve agricultural productivity via high input practices places considerable emphasis on 'failsafe' techniques for each component of the production sequence with little consideration to the integration of these components in a holistic, systems approach. While the use of mineral fertilizers is considered the quickest and surest way of boosting crop production, their cost and other constraints deter farmers from using them in recommended quantities. In recent years, concepts of integrated plant nutrient management have been developed, which emphasize maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality. This is done in an integrated manner appropriate to each cropping system and farming situation. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological processes and soil biodiversity. An understanding of microbial diversity perspectives in agricultural context, is important and useful to arrive at measures that can act as indicators of soil quality and plant productivity (Tilak *et al*., 2005).

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem (Corstanje *et al*., 2007). It is a critical resource not only for agricultural production and food security but also towards maintenance of most life processes. The functions of soil biota are central to decomposition processes and nutrient cycling (Vikram *et al.*, 2007). In this context, the long-lasting challenges in soil microbiology are development of effective methods to know the types of microorganisms present in soils, and to determine functions

which the microbes perform *in-situ*. Microbial community ecology in soils plays an important role in agricultural practices. They have major roles in nutrient cycling, and in pathogenic and beneficial interactions with crop plants. Plant growth promoting bacteria promote growth directly, e.g. by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators (Kloepper, 1997; Abbas *et al.,* 2002). Cellulose is the most abundant organic compound in the biosphere, comprising almost 50% of the biomass synthesised by photosynthetic fixation of CO_2 (Eriksson *et al*., 1990). Growth and survival of micro-organisms important in most agricultural soils is influenced by carbon availability in the soils. Cellulases play an important role in carbon availability and so can be used to give preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies (Makoi and Ndakidemi, 2008). In soil ecosystems, phosphatases play a critical role in plant growth by enhancing the availability of phosphorus due to enhanced solubilisation and remobilisation of phosphate (Speir and Ross, 1978). Understanding the dynamics of enzyme activities in these systems is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth in biologically-managed systems because of a higher quantity of organic C found in those systems (Aon and Colaneri, 2001).

In recent decades, Intensification of agriculture and increases in population pressure has reduced the structural stability of soils. To cope with low productivity, inorganic fertilizers have been intensively used for the past two decades. This escalated the soil problems including structural degradation, reduction of organic matter and cellulose. Agricultural residues are a rich source of cellulose (Hameeda *et al.,* 2006;

Mahadevaswamy, *et al.,* 2007). Research efforts now focused on discovering new enzymes from microbial diversity in the soil, the most appropriate practices that may positively influence their activities for improved plant growth as well as improving the biological environments in order to sustain other life types (Makoi and Ndakidemi, 2008). For successful functioning of introduced microbial bioinoculants and their influence on soil health, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behaviour in soil habitats (Hill, 2000).

In Indian state of Punjab, the rice–wheat cropping system has dominated other cropping systems because of the introduction of high yielding varieties of rice and wheat crops, availability of favourable soil and climatic factors, subsidized resource availability backed by the assured procurement system. With the large scale adoption of rice–wheat cropping system even on coarse textured soils, Punjab state has led to overexploitation of water resources coupled with increased energy used for pumping water and has caused a decline in the water table in central districts. Presently, many of shallow wells, which made the cultivation of rice–wheat successful in the state, have dried up. It has become impossible for the marginal and small farmers, with an average holding of less than 2 ha, to replace shallow wells with costlier deep wells. Farmers and planners are seeking alternate suitable crops with low water and energy requirements for diversification and sustaining agricultural productivity and hydrology in the state. Shrinking water resources in northwest India calls for diversification from a rice–wheat cropping system to low-water-requiring crops and development of water-efficient technologies in Punjab state (Jalota *et al*., 2006).

Microbial community ecology in soils plays an important role in agricultural practices. Switching away from chemical inputs towards inputs such as compost, manures, and green manures, sometimes leads to a decrease in yields. This may be because the right populations of microbes for breaking down cellulose and other roles that are necessary to release the nutrients into the soil are not present in sufficient numbers. Thus there is a need to differences between conventional and sustainable agricultural practices, concentrating on soil bacterial and fungal population and diversity.

Very little is known about the contribution made by microbial diversity to the maintenance of soil fertility, and mediation between pest/pathogen incidence and crop quality. Soil health is closely linked to microbial diversity, and that relatively minor change in environmental conditions can dramatically alter population structures. Therefore there is the need to understand the role of biodiversity in maintenance of soil fertility, be able to recognize and measure its key components, develop reliable monitoring methods and also understand the benefits, both economic and nutrition status of the soil.

In the present study we have assessed the response of rice–chickpea cropping as alternative to rice-wheat cropping rotation and demonstrated viability of the alternative system so as to convince the farmers. The attempt has been made to study the role microbes improving soil organic matter such as cellulose degraders and phosphate solubilizers under diversified cropping pattern. Efforts were made to isolate and screen the most efficient cellulose degrading bacteria and study the population dynamics of the cellulose degrading bacteria to understand their role in maintaining the organic fertility of soil.

Chapter 2

Review of Literature

REVIEW OF LITERATURE

2.1 Sustainability in agriculture

Sustainable agriculture has become an important pre-requisite to provide food and nutrition to millions of impoverished people across the world (Reddy *et al.*, 2003; Schojonning *et al*., 2004). Sustainable agricultural practices are expected to facilitate sufficient food and fibre security; environmental stewardship; economic viability and finally social stability and equality (Kirchmann and Thorvaldsson, 2000). The present environmental concerns such as degradation of soil vis-a-vis loss in fertility, caused due to intensive conventional agricultural practices, are also envisaged to be reduced through sustainable agricultural systems (Douglass, 1985; Reeve, 1990; Lal, 1994; Mina *et al*., 2008). Conventional agriculture often rely on the use of synthetic fertilizers; pesticides; fossil fuel based energy; monocultures; excess tillage and irrigation; and narrow or absence of crop rotations (Mader *et al*., 1999; Wells *et al*., 2000). Whereas sustainable agricultural practices is dominantly associated with organic, biodynamic and agroforesty systems requiring low environmentally friendly inputs (Reeve, 1990; Cook and Lee, 1995; Kristensen *et al*., 1995; Stockdale *et al*., 2001; Robson *et al*., 2002; Hole *et al*., 2005)

Stark (2005) comprehensively reviewed prominent organic farming practices that are associated with sustainable crop management. In addition, extensive reviews by Unwin *et al.* (1995); Condron *et al.* (2000); Stolze *et al.* (2000); Tinker (2000); Hole *et al.* (2005) also outlined the salient features of organic farming practices over the conventional systems.

It is argued that land and water resources would tend to be over-utilized in a manner that is unsustainable in circumstances where the returns to these resources are very high

(Chadha *et al.,* 2004). The case of Punjab agriculture, where in recent years, a substantial proportion of agricultural area has come under wheat–rice cropping pattern is a case in point. The dependence of Punjab on groundwater through tube-well irrigation is increasing over the years (Sidhu, 1999). There is a general agreement among scholars that firstly, wheat–rice cropping pattern is largely responsible for declining ground water table in Punjab (Johl, 1986; Prihar, 1990; Singh, 1992) and secondly, that the wheat–rice cropping system is becoming unsustainable over time as the yield levels of these two major crops are stagnating (Kumar, 1998; Sidhu and Singh 2003). However, the existing evidence does not throw adequate insight into the stage of groundwater depletion during which the wheat–rice cycle becomes unsustainable. The calls for diversification from a wheat–rice cropping system to more efficient and sustainable cropping cycle or rotation and a moving a step towards crop diversification in Punjab state is the need of the hour.

2.2 Soil quality

Mausbach and Seybold (1998) defined soil quality as capacity of a soil to fulfil certain functions such as production of biomass; buffering, filtering and transforming capacity; providing habitat to diverse variety of biota. The soil quality is significantly dependent of the specific use it is being attributed and is an indicator of economic viability; agricultural and environmental sustainability; food quality; and ultimately the health of human and livestock. Therefore, the sustainable agricultural practice necessarily involves proper management of soils to preserve and improve its quality (Doran and Jones, 1996; Schjonning *et al*., 2004).

2.3 Soil nutrients

2.3.1 Soil Nitrogen (N)

The overall productivity of organic cropping systems is limited by N supply due to a combination of the prohibition of the use of soluble N fertilisers and the unavailability of alternative organic N fertiliser materials, such as farmyard manure for fertilisation. Legumes are an important source of nitrogenous nutrients for most organic systems and crops under organic management are almost exclusively dependent on soil biological processes to provide sufficient amounts of N from soil organic matter by mineralisation. While mineralisation of N from soil organic matter plays an important role in conventional cropping systems, the availability of soluble mineral fertilisers reduces the dependence on these soil processes. Further the use of green manures is considered good management practice for both conventional and organic systems due to their many positive effects on soil fertility and quality (Greenland, 2000; Watson *et al.*, 2002). The dinitrogen (N_2) fixing pulse legume, chickpea (*Cicer arietinum*), has been widely grown throughout South and West Asia and the Mediterranean region for centuries, usually in sequence with either summer or winter cereals. It is only recently, however, that N_2-fixation inputs and N balances, i.e. the difference between N_2 fixation inputs and N in harvested products (outputs), as well as rotational benefits of chickpea have been quantified. The rotational benefits of chickpea, i.e. increased soil N (nitrate and total N) and cereal crop yields following chickpea, appear to be more consistent as reported by Ahlawat *et al.*, (1981) in a study in north-west India. In the northern grains belt of India, Dalal *et al.*, (1998) reported higher soil nitrate levels following growth of chickpea than followed by wheat (average of +35 kg N ha^{-1}) resulting in higher yields of grain (+0.83 t

ha^{-1}, equivalent to 40% increase) and higher grain proteins (+14%). They concluded that the beneficial effects of chickpea were mediated through enhanced N supply to the wheat from the chickpea, rather than from disease break effects. Legume-based cropping systems will not only reduce nitrogen losses, but they may also increase the proportion of crop residue carbon that is sequestered in stable soil organic matter (Drinkwater *et al.*, 1998).

2.3.2 Soil Organic Carbon (SOC)

Low input farming systems in the tropics have adverse impact on soil organic carbon (SOC) largely through depletion of nutrients, non-return of plant residues to soil and low productivity. Legume-based systems along with appropriate soil management and nutrient management options enhance SOC contents in the tropics even without any external organic inputs (Wani *et al.*, 2003). The soil carbon pool composed of soil organic and inorganic C play an important role in the carbon cycle. Soil organic C equilibrium is governed by a number of interacting factors such as temperature, moisture, texture, quantity and quality of organic matter, methods of organic matter application, soil tillage and cropping system. C sequestration can be augmented by increasing the quantity of organic matter returned or added to the soil, or by reducing the SOC loss by oxidation or erosion or by a combination of both. The changes in soil organic C contents are also directly associated with changes in microbial biomass carbon and biological activity in the soil. Besides living plant roots and organisms, soil microbial biomass is a living portion of soil organic matter. Maintenance of biological activity through residue management is a means for retaining organic matter and improving nutrient availability in the rainfed farming system. The response to changes in inputs of organic material is quicker in soil microbial biomass than in soil organic matter (Powlson and Jenkinson,

1981). Microbial biomass contains labile fraction of organic C and N, which are mineralized rapidly after the death of microbial cells. Organic carbon levels increased with continuous cropping, particularly when legumes were included in the improved systems (Wani *et al.*, 1994; Chander *et al.*, 1997). It was observed that over a period of 5 years the change in SOC was negative under cereal–cereal sequences, whereas the SOC increased in cropping sequences with legume component (Singh *et al.*, 1996)

2.3.3 Soil Phosphorus (P)

In agricultural ecosystems, phosphorus constraints are much more critical because phosphorus in the harvested crops is removed from the system, with only limited quantities being returned through crop residues and animal manures. As a result, extreme phosphorus deficiencies are quite common where no supplementary sources of this element are applied to soils. (Brady and Weil, 2002). The phosphorus cycle in soil is a system which involves soils, land and microorganisms. Major processes include the uptake of soil phosphorus by plants, recycling (the return of plant and animal residues), biological turnover (mineralization and immobilization), fixation to clay, solubilization. Phosphorus is not supplied through biochemical fixation but, must come from other sources to meet plant requirement. These sources include commercial fertilizers, animal manures, plant residues, wastes and native compounds of phosphorus, both inorganic and organic already present in the soil (Stevenson, 1986). Soil microorganisms are integral components of the soil - plant system and of particular importance is their influence on plant nutrients such as phosphorus. Microorganisms can affect the P supply to higher plants through the decomposition of organic P compounds, through the immobilization of available phosphates and by promoting the solubilization of fixed or insoluble mineral

forms (chelating agents). The microbial biomass itself contains a large pool of nutrients that are potentially available to plants.

2.4 Role of Enzymes in Soil Biochemical Reactions

Biochemical actions in soil are the basis of the functioning of the terrestrial ecosystem. They are affected by chemical and physical structure of soil together with season and crop. Although other trophic levels also contribute to the cycling of nutrients, the microbial community is responsible for the primary decomposition of organic macromolecules into forms suitable, e.g., for plant uptake (Vepsalainen *et al.*, 2004). Biochemical reactions are dependent on or related to the presence of enzymes. Many reactions involving soil organic matter transformations may be catalysed by enzymes existing outside the microorganisms and plant root system.

From an ecological point of view, extracellular enzymes are of particular interest because they catalyse the rate-limiting steps of decomposition of macromolecules. These enzymes are constantly being synthesised, accumulated, inactivated and/or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrients cycling (Tabatabai, 1994; Dick, 1997). The activities of these enzymes in soils undergo complex biochemical processes consisting of integrated and ecologically-connected synthetic processes, and in the immobilisation and enzyme stability (Khaziyev and Gulke, 1991).The activities of soil enzymes are largely regulated by soil moisture, temperature, pH and substrate (nutrient) availability (Nannipieri *et al.*, 1983), as also the organic matter content, composition and activity of its living organisms and intensity of the biological processes (Stevenson, 1986). In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable sub-strates that serve as energy sources for micro-organisms (Kiss *et al.*, 1978). Makoi and Ndakidemi

(2008) reviewed extensively on potential role of soil enzymes in regulating soil functions. The authors indicated that these enzymes include amylase, arylsulphatases, β-glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease and urease released from plants (Miwa *et al.,* 1937), animals (Kanfer *et al.,* 1974), organic compounds and micro-organisms (Dick and Tabatabai, 1984; Richmond, 1991) and soils (Gareshamurthy *et al.,* 1995). Therefore, to facilitate integrated biological assessment of soils it is important to understand the role of these enzymes in influencing soil biological activities (Makoi and Ndakidemi, 2008).

2.4.1 Soil Cellulase

Cellulose is the most abundant organic compound in the biosphere and primarily bioavailable through litter. It comprises about 50% of the photosynthetically fixed CO_2 (Eriksson *et al.,* 1990; Hameeda, 2006). Deng and Tabatabai (1994) reported that the growth and survival of the microbiota is dominantly dependent on the soil cellulose as a carbon source. The environmental factors that influence the bioaccessibility of cellulose include water availability, oxygen availability, redox potential, temperature variability, nutrient status, and most importantly interactions among cellulolytic and noncellulolytic microbes. The microorganisms hydrolyze and metabolize insoluble cellulose with the help of extracellular cellulases. The biochemistry of cellulose systems aerobic and anaerobic bacteria as well as fungi has been extensively reviewed by Lynd et al. (2002). The classification of cellulase system was based on their mode of catalytic action and thereby termed as (a) Endoglucanases or 1,4-β-D-glucan-4-glucanohydrolases (EC 3.2.1.4); (b) Exoglucanases: (i) 1,4-β-D-glucan glucanohydrolases (also known as cellodextrinases) (EC 3.2.1.74); (ii) 1,4-β-Dglucan cellobiohydrolases

(cellobiohydrolases) (EC 3.2.1.91); and (iii) β-glucosidases or β-glucoside glucohydrolases (EC 3.2.1.21).

Cellulose utilization is largely an aerobic process, and the primary cellulolytic bacterial isolates were *Cytophaga, Bacillus* and *Cellulomonas* strains. While in composting cattle manure, filamentous bacteria from the genera *Micromonospora*, *Cytophaga*, and *Sporocytophaga* were the most numerically abundant isolates and fungi were found to be much less abundant (Godden and Penninckx, 1984). The activity of cellulases in soil is significantly influenced by factors (Makoi and Ndakidemi, 2008) such as temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon (Rubidge, 1977; Gomah, 1980; Tabatabai, 1982; Klein, 1989; Deng and Tabatabai, 1994; Alf and Nannipieri, 1995), quality of organic matter/plant debris and soil mineral elements (Burns, 1978; Hope and Burns, 1987; Klein, 1989; Sinsabaugh and Linkins, 1989; Deng and Tabatabai, 1994) and the trace elements from fungicides (Arinze and Yubedee 2000; Atlas *et al.,* 1978; Vicent and Sisler, 1968). Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme better so that it may be used more regularly as a predictive tool in our soil fertility programmes (Makoi and Ndakidemi, 2008).

2.4.2 Soil Phosphatases

The microorganisms are the most important sources of soil phosphatases in their environment (Ladd, 1985; Haas *et al.,* 1992). These phosphatases are released into the soil by active exudation, leakage or cell lyses (Tadano *et al.*, 1993). The microorganisms possessing a phosphate-solubilizing ability can also convert the insoluble phosphatic compounds into soluble forms in soil and make them available to the crops (Kang *et al.*,

2002; Pradhan and Sukla, 2005). The role of rhizospheric organisms in mineral phosphate solubilization have been extensive studied in naturally abundant rhizospheric microorganisms genera including *Bacillus* and *Pseudomonas* (Illmer and Schinner, 1992), while *Aspergillus* and *Penicillium* (Motsara *et al.,* 1995; Khan *et al.,* 2007)

Soil phosphatases play a major role in the mineralization processes (dephosphorylation) of organic P substrates. Appiah and Thompson (1974) proposed that the mineralization of organic phosphorus is principally a microbial phenomenon and becomes important after the initial breakdown of soil organic matter. The initial breakdown could be the rate – limiting step of organic phosphorus mineralization. The organic phosphorus mineralization in soil also correlates with that of nitrogen and carbon mineralization (Thompson *et al.,* 1954).

The hydrolysis of esters and anhydrides of phosphoric acid are catalyzed by phosphatase group of enzymes (Schmidt and Lawoski 1961). Along with cellulases, phosphatases can also be used as good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Eivazi and Tabatabai, 1988; Dick and Tabatai, 1992; Dick *et al.,* 2000; Makoi and Ndakidemi, 2008). The phosphatase activity in soils is prominently due to the mechanisms induced in and by plants as adaptation to facilitate phosphate availability. These include enhanced transcription activity of acid phophatases and their release from plant roots (Muchhal *et al.,* 1996; Daram *et al.,* 1999; Kai *et al.,* 2002; Karthikeyan *et al.,* 2002; Versaw and Harrison, 2002; Hayes *et al.,* 1999; Li and Tadano, 1997). The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties, (Ndakidemi, 2006; Izaguirre-Mayoral and Carballo, 2002) as well as crop management practices (Ndakidemi, 2006; Patra *et al.,* 1990; Staddon *et al.,* 1998; Wright and Reddy, 2001). For example, Li *et al.* (2004)

reported that chickpea roots were also able to secrete greater amounts of acid phosphatase than maize. There have been few studies examining the influence of management options in the ecosystem on phosphatases activity in soil where most crops are grown (Makoi and Ndakidemi, 2008).

2.5 Soil Microbial Environment

Soil fertility and productivity are significantly dependent upon soil biota such as macrofauna, mesofauna, microfauna and microflora that constantly interact through ecological phenomena such as predation, competition, etc. (Metting 1993; Coleman *et al*., 2004). These interactions ultimately facilitate in nutrient cycling, availability and retention, decomposition of organic materials, soil organic matter build-up and stabilisation of soil aggregates (e.g. Ritz *et al.,* 1994; Insam and Rangger 1997; Liesack *et al.,* 1997; Maier *et al.,* 2000; Coleman *et al.,* 2004).

Amongst various soil communities, microorganisms such as bacteria and fungi play important role in biogeochemical cycling of nutrients (Jenkinson and Ladd, 1981; McGill and Cole 1981). This important role is facilitated by diverse variety of enzymes such as dehydrogenases, proteases, phosphatases, etc. The enzyme activity in turn influences the soil pH, organic matter content, nutrient content and buildup of soil fertility.

2.5.1 Influence of diversified cropping pattern on soil microbial activities

The agricultural practices and the cropping pattern reportedly influence the microbial community and diversity appreciably. These factors include different fertilisation regimes (Hatch *et al.,* 2000; O'Donnell *et al.,* 2001; Sarathchandra *et al.,* 2001; Belay *et al.,* 2002), herbicide and pesticide application (Johnsen *et al.,* 2001; Seghers *et al.,* 2001; Dungan *et al.,* 2003), crop rotations (Campbell *et al.,* 1991;

Campbell *et al.,* 1992; Campbell *et al.,* 1999), manure applications (Girvan *et al.,* 2003), heavy metal contamination (Brohon *et al.,* 2001; Turpeinen *et al.,* 2004), wet/ dry cycles (Lundquist *et al.,* 1999b) and different tillage systems (Lupwayi *et al.,* 1998; Ibekwe *et al.,* 2002; Zaitlin *et al.,* 2004). In turn, organic and sustainable agricultural practices are observed to provide ecologically beneficial environment that stimulates soil microbial community and its functions with little or no negative effect on the soil productivity (Fraser *et al.,* 1988; Fauci and Dick 1994; Yeates *et al.,* 1997; Lundquist *et al.,* 1999a; Ryan 1999; Belay *et al.,* 2002; Bending *et al.,* 2004; Hole *et al.,* 2005.

Therefore, it is necessary to understand the role of structure and function of soil microbial communities and how they are affected by farming practices so as to improve the sustainability of agroecosystems (Thomas and Kevan 1993; Kennedy and Smith 1995; Beare 1997).

2.5.2 Molecular marker for tracking the fate of cellulose degrading microbes by DNA fingerprinting

Molecular tracking refers to the following up of biological phenomena at molecular level by using molecular, i.e. DNA/RNA-based, techniques without any genetic manipulation, such as genetic fingerprinting of marker genes. The use of molecular techniques to identify microorganisms is currently a key tool to study rhizosphere ecology (Barea *et al.,* 2005). Soil microbial communities can be genetically fingerprinted and the community members identified by comparison with fragment sizes or sequences in databases. The PCR-based community fingerprinting techniques have several advantages (Wintzingerode *et al.,* 1997). They are 1) rapid, and allow parallel analyses of multiple samples, 2) reliable and highly reproducible, 3) provide both qualitative and quantitative information on populations within a community, and 4) allow

the assessment of phylogenetic affiliation of community members by comparison with fragment sizes or sequences in databases. Differences in genomic fingerprints of chromosome structure among isolated bacterial stains can be obtained with highly repeated sequence characterization (Tiedje *et al.,* 1999; Kirk *et al.,* 2004).Genetic diversity among microorganisms has been widely studied by PCR-based techniques, which have opened avenues for the development of new and inventive molecular typing methods. Genotypic relationships among microorganisms have been determined by analyzing the genomic DNA with PCR-based methods (Pooler *et al.,* 1996). Repetitive sequence-based PCR (Rep-PCR) has been used successfully to generate DNA fingerprints to distinguish between genetically unrelated isolates and closely related bacterial strains. It involves the use of primers based on the short repetitive elements derived from highly conserved palindromic inverted repeat regions dispersed throughout the prokaryotic kingdom (Jersek *et al.,* 1999; Laguerre *et al.,* 1996). Amplification of the regions between adjacent repetitive extragenic elements gives strain-specific DNA fingerprints. Primer sets based on the repetitive elements present in the bacterial genome, ERIC (enterobacterial repetitive intergenic consensus), REP (repetitive extragenic palindromes), and BOX, yield genomic fingerprints specific to pathovars and strains of gram negative bacteria (Giselle and Lindstorm, 1994; Smith *et al.,* 1995). The inter-REP and inter-ERIC profiles are specific for bacterial strains within a species (Versalovic *et al.,* 1991). rRNA genes are ubiquitous in living organism and therefore, the rRNA operon forms an attractive locus for molecular typing by PCR-based ribotyping. rRNA operons are present in multiple copies, and amplimers generated by PCR ribotyping indicate the intraspecies genetic diversity in the number and structure of ribosomal operons (Vaughan-Smith *et al.,* 1995). The sequences of multicopy rRNA genes are nearly

identical and homogeneous (Hillis *et al.*, 1991). They are separated by spacer regions which exhibit a large variation in sequence and length at the genus and species levels (Jensen *et al.*, 1993). The aim of the present study was to evaluate intraspecies genetic diversity among cellulose degrading bacterial strains isolated from agricultural soils under different cropping patterns. The sampling sites were located in different areas of Patiala-Punjab regions, and the duration of sampling at the sites also varied with seasons.

2.6 Management practices and impact on soil microbial activities in agroecosystems

In agricultural systems, biodiversity performs ecosystem services beyond production of food, fibre, fuel, and income (Altieri and Nicholls, 2004). Examples include recycling of nutrients, control of local microclimate, regulation of local hydrological processes, regulation of the abundance of undesirable organisms, and detoxification of noxious chemicals (Altieri, 1999). These renewal processes and ecosystem services are largely biological, therefore their persistence depends upon maintenance of biological diversity (Altieri and Nicholls, 2004). A number of management techniques are known to sustain soil biodiversity, increasing, in turn, soil quality. Fig. 1 shows that there are many agricultural practices and designs that have the potential to enhance functional biodiversity, and others that negatively affect it (Altieri, 1995).

The soil quality degraded by intensive use of synthetic chemicals for increasing crop production and therefore, use of bio-agents as efficient microbes, plant growth promoting bacteria (PGPR), biofertilizers or biopesticides is an integral part of sustainable farming (Srivastava *et al.*, 2007). Agricultural practices that maintain adequate soil organic matter content favour the proliferation of soil biota (Reid, 1985).

The practice of adding straw mulch on the soil surface increased soil organic matter and the number of living organisms as much as threefold. Similarly, the application of organic matter enhanced earthworm and microorganism biomass as much as fivefold (Ricou, 1979). Because increased biomass generally is correlated with increased biodiversity (Sugden and Rands, 1990), it is logical to assume that the increase in biomass of microbes represents an increase in biodiversity (Pimentel *et al.*, 1992).

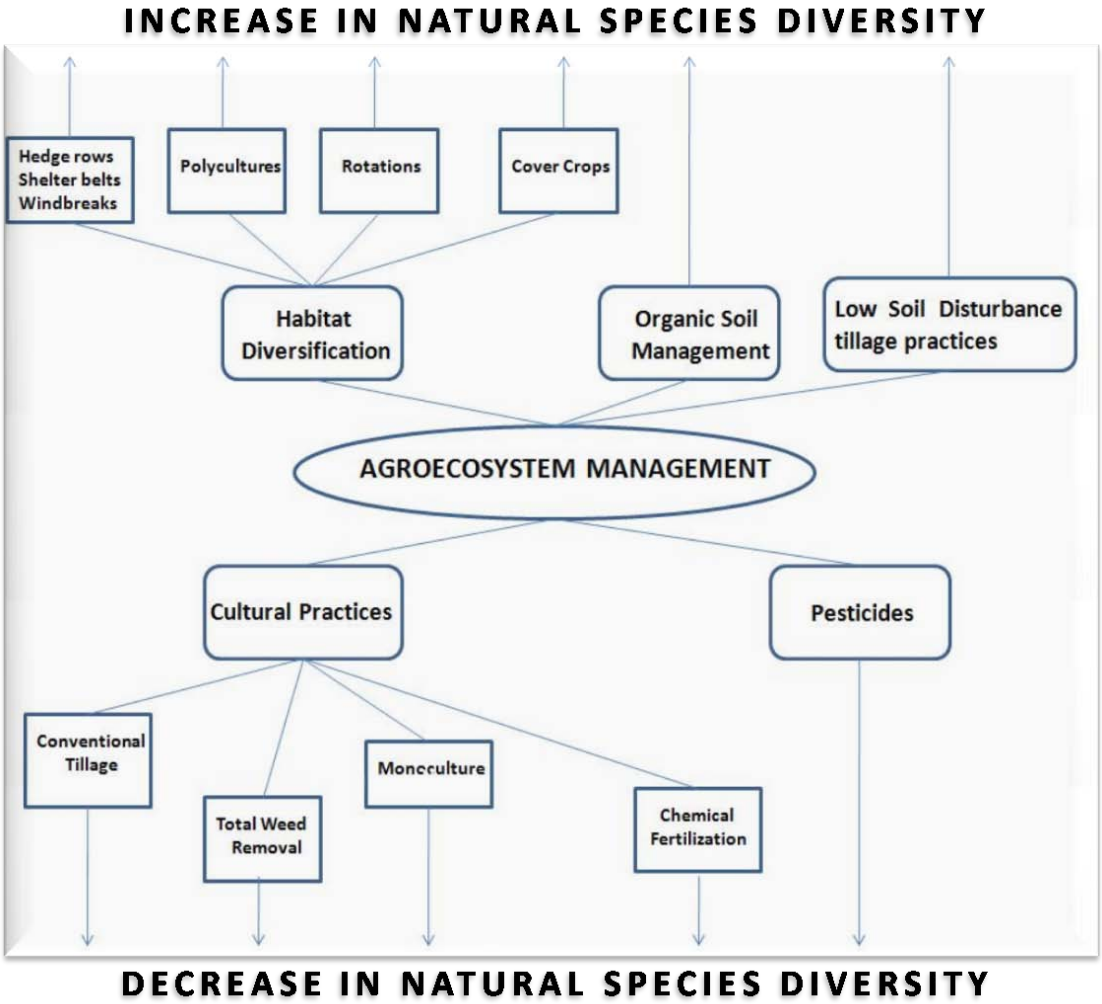


Fig. 1: The effects of agroecosystem management and associated cultural practices on the natural biodiversity adapted from Altieri (1995).

Physical disturbance of the soil caused by tillage and residue management is a crucial factor in determining soil biotic activity and species diversity in agroecosystems.

Soil biodiversity reductions are negative because the recycling of nutrients and proper balance between organic matter, soil organisms and plant diversity are necessary components of a productive and ecologically balanced soil environment (Hendrix *et al.*, 1990). A crop-rotation system with suitable plant associations included may well be in the position to make the best use out of the soil by mobilizing and, at the same time, renewing continuously its biotic potential. For instance, microbial diversity was significantly higher under wheat preceded by red clover green manure or field peas than under wheat following wheat (continuous wheat) or summer fallow. The reports indicate that legume-based crop rotations support diversity of soil microbial communities and may affect the sustainability of agricultural ecosystems (Lupwayi *et al.*, 1998). Changes in community structure and Biolog potential occurred in some soils in response to winter cover crops, although effects were not observed until cover crop incorporation; greater amounts of fungal and protozoan FAME markers were detected in some cover cropped soils compared to winter fallow soils (Schutter *et al.*, 2004).

Chapter 3

Materials and methods

MATERIALS AND METHODS

3.1 Field trials

The field experiments for present study were established during 2002-2004 at four blocks of Patiala district (Punjab, INDIA) namely Patiala (PT), Rajpura (RJ), Derabassi (DB) and Samana (SM). Every block has two adjacent plots of one acre each. Either of the plots was sown with wheat (*Triticum* spp.) as control and chickpea (*Cicer arietinum L.*) as a test during rabi season. These plots were sown with rice (*Oryza sativa*) during kharif season to investigate the wheat-rice-wheat and chickpea-rice-chickpea cropping patterns. However, at Rajpura (RJ), one field was sown with sunflower (*Helianthus annuus*) during rabi season followed by rice (*Oryza sativa*) to study the sunflower-rice rotation. The quality seeds of chickpea kabuli variety (K-1088) were procured from Sikanderpur Agricultural Farm, Sirsa. The wheat (PBW-343), sunflower (Pioneer-3342) and Taraori basmati rice (HBC-19, Karnal Local) varieties were used in the present study. The Punjab Agricultural University prescribed package of practices for rabi (PAU, 2002a) and kharif seasons (PAU, 2002b) were followed for the above experiments and continuously monitored (See photographs in ANNEXURE II). The recommended basal dose of fertilizers (NPK), seed treatment and practices (PAU, 2002a; 2002b) were used for the all studied cropping patterns. Weeds were controlled during crop growth by hand weeding and integrated pest management practices were followed to control pest infestation. Soil samples were collected at different intervals (sowing time, midtime/flowering stage and harvesting of crop) for physicochemical and microbiological analysis as described in section 3.3.

3.2 Plot studies

The plot studies were performed to track the population dynamics and activities of mass inoculated efficient cellulose degrading bacterial strains. Chickpea and sunflower crops were sown in 2 x 2m plots according to randomised block design. The recommendations for the *rabi* and *kharif* crop cultivation were adopted as describes in section 3.1 during studies and continuously monitored. The mass inoculation of bacterial strains used in the present study were grown in 10 litre shake flasks containing LB medium and incubated at (37°C, 120 rpm). After 36 h, the cells were harvested and washed with 10mM phosphate buffer (pH 6.8). Pellets were resuspended in buffer and inoculated to achieve 2 x 10^8 cfu/g soil in three treatment plots and one plot was kept as control. Control plot received only cell-free phosphate buffer. The mass of soil was calculated upto 10 cm. After regular intervals of time during different stages of crop growth, soil samples were collected as described in section 3.3 and was taken to evaluate the population dynamics of microbes and soil parameters (microbial load, enzymatic activity, organic carbon, total nitrogen, available phosphorus and microelements).

3.3 Sample collection

Soil samples used in the present study were collected from agricultural fields of wheat (*Triticum spp*.), chickpea (*Cicer arietinum L*.), sunflower (*Helianthus annuus*) during rabi season and followed by rice (*Oryza sativa*) during kharif season in agricultural plots sown in 25 acres of the farmer's field in representative villages of four different blocks: Patiala, Derabassi, Rajpura and Samana. Experiments were conducted during the both summer-winter seasons of three consecutive years (2002–2004). Soil samples were collected randomly at from 0-30 cm of depth by alcohol sterilized implements and sterile

containers at nine different sites in one acre field. The 3 composites samples were made from 9 collected samples. Soils were processed the same day for microbial and enzymatic activities using standard protocols and stored at 4^{o}C for soil analysis. Air-dried and pulverized soil samples were analyzed for organic carbon, nitrogen, available phosphorus, potassium, moisture level, and pH by standard method (Jackson, 1967).

3.4 Isolation and cultivation of bacterial species

For isolation and enumeration of cultivable bacteria, soil samples were diluted in saline (0.85%) and plated on soil extract agar (Appendix I) for total microbial count, pikovskaya agar (Appendix I) for phosphate solubilisers count and Bushnell Hass Agar (BHA) with 1% carboxy methyl cellulose as sole source of carbon (Appendix I) for cellulose degraders count and incubated for 24-48 hrs at 37°C. The bacterial colonies from the plate were counted. All the samples were plated in triplicates in three different dilutions and the colonies counted were expressed as colony forming units per gram dry weight of soil (cfu/ g dry wt. of soil).

3.5 Morphological and Biochemical studies of soil isolates

3.5.1 Gram staining

a) Bacterial smear from actively growing cells were spread on a glass slide and heat fixed.
b) Filtered crystal violet was flooded for 10 sec.
c) Briefly, washed in water to remove excess crystal violet.
d) Gram's iodine was flooded for 10 sec and washed briefly in water.

e) Further, it was decolourised with acetone until the moving dye front has passed the lower edge of the section and washed immediately in tap water.

f) Safranine was used to counter stain for 15 sec and washed with water to remove the excessive stain. Visualized under microscope at different magnifications.

3.5.2 Catalase test

a) Small amount of bacterial cells were placed onto a clean microscope slide.

b) A few drops of H_2O_2 (3%) was added onto the smear.

c) A positive result was the rapid evolution of O_2 as evidenced by bubbling.

d) A negative result was no bubbles or only a few scattered bubbles.

3.5.3 Oxidase test

a) Small amount of organism from an agar slant or plate was obtained with a sterile swab.

b) One drop of reagent (N,N,N',N'-tetramethyl phenylenediamine dihydrochloride) was placed onto the culture on the swab.

c) Positive reactions turned the bacteria violet to purple immediately or within 10 to 30 seconds. Delayed reactions were ignored.

3.5.4 Nitrate reduction test

Nitrate media (Appendix I) was used to determine the ability of an organism to reduce nitrate (NO_3) to nitrite (NO_2) using the enzyme nitrate reductase. The isolates were grown on nitrate broth containing potassium nitrate as a source of nitrate. After incubating the nitrate broth, 2-3 drops of sulfanilic acid and α-naphthylamine were added. The formation of red color indicated a positive result for nitrate reduction.

3.6 Antibiotic profiling of bacterial isolate

Bacterial isolates were grown in Luria broth (LB) until the absorbance (OD 550) reached to 1.0. The grown bacterial cells were spread on Luria agar and precoated twenty antibiotic discs (Himedia Lab., India) were kept on it. These plates were incubated at 37 ^{o}C and the inhibition zone was noted. The antibiotics used were: Norfloxacin (10 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Cefuroxime (30 µg), Ciprofloxacin (5 µg), Cefaperazone (75 µg), Ceftazidime (30 µg), Roxithromycin (30 µg), Calaritromycin (15 µg), Co-Trimoxazole (25 µg), Netillin (30 µg), Cefaclor (30 µg), Cephotaxime (30 µg), Cephadroxil (30 µg), Azithromycin (15 µg), Ampicillin/Cloxacillin (10/10 µg), Penicillin (10 units), Amikacin (30 µg), Sparfloxacin (5 µg) and Ampicillin/sublactam (10/10 µg). The antibiotic sensitivity of the soil isolates were further tested on different concentration (10 – 150 µg) of Ampicillin.

3.7 Molecular methods

3.7.1 Extraction of DNA

Chromosomal DNA was extracted by a modified ROSE (rapid one step extraction) method (Steiner *et al.,* 1995). Total DNA was extracted from bacterial cultures in Luria broth. After 24h, 50 mL of culture was removed and centrifuged at 3000 X *g* for 5 min, after which the cells were washed in 0.85% NaCl solution, re-centrifuged and resuspended in 2 mL of TEN buffer (100 mM EDTA; 150 mM NaCl; 100 mM Tris-HCl, pH=8.0) containing 4 mg/mL lysozyme. The suspension was incubated at 37°C for 45 min and 0.5 mL of 8.5% SDS was added, followed by incubation at 75°C for 30 min before the addition of 1.5 mL of potassium acetate (5 M, pH=5.2) and incubation for 20 min at 4°C. The DNA was extracted with phenol - chloroform -isoamylalcohol,

precipitated with isopropanol, washed with 70% ethanol, briefly dried and re-suspended in 200 µL of TE buffer.

3.7.2 Electrophoresis of DNA on agarose gels

DNA was loaded on agarose gels prepared in 0.5x TBE, pH 8.0 (Appendix I) using a 6x loading dye (Appendix I). For genomic DNA 0.7 % (w/v), for amplified PCR product 1.2% (w/v), and for RFLP patterns 1.5% (w/v) of agarose concentrations were used in gel. The nucleic acids were then electrophoresed at 50 volts for 45-60 min, stained with Ethidium bromide (0.5 µg/mL) and visualized on a U.V. transilluminator.

3.7.3 ERIC PCR based DNA fingerprinting

Enterobacteriaceae Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to obtain DNA fingerprints of the cellulose degraders. PCR primer sequences (Versalovic, *et al.,* 1991), ERIC – IR (5' – 3') – ATG TAA GCT CCT GGG GAA TCA C- and ERIC – 2 (5' – 3') - AAG TAA GTG ACT GGG GTG AGC G- were obtained from Life Technologies, USA. Single isolated colonies were picked at random from the LA plates, suspended in 50 µl water, and lysed by heating for 10 min at 95°C. Cell lysate was centrifuged (9000X*g*, 3 min) at 4°C, and 2 µl of the supernatant was used in the reaction mixture. The reaction mixture (25 µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin (wt/vol), 0.25 mM each of dNTPs, 0.375 µM each of primers ERIC-1R and ERIC-2, and 2.0 units of Taq polymerase (Life Technologies, USA). The amplification was done on Genamp PCR system (Applied Biosystem, USA). The PCR conditions are as follows: initial denaturation at 94°C for 4 min followed by 41 cycles at 94°C for 1 min, at 50°C for 1 min and at 72°C for 2 min. The final extension was done at 72°C for 5 min. The reaction was terminated by using a loading dye (1 µl) containing 15% Ficoll, 0.25% bromophenol

blue, and 0.25% xylene cyanol. Each PCR product was resolved by electrophoresis on a 1.5% agarose gel.

3.7.4 Amplification of 16S rDNA and Purification of PCR products

For amplification of 1.5 kb gene of 16S rRNA, the primers used were: Forward primer 5' –AGAGTTTGATCCTGGCTCAG – 3' and reverse primer 5' – ACGGGCGGTGTGTTC – 3' (Weisberg *et al.*, 1991). DNA amplification was performed with Genamp PCR system (Applied Biosystem, USA). Reaction mixture for the PCR contained 1X PCR buffer (Invitrogen, USA), each deoxynucleotide triphosphate at a concentation of 200µm, 1.5 mM $MgCl_2$, each primer at a concentration of 0.1 µm and 2.5U of Taq DNA polymerase (Invitrogen, USA) in a final volume of 100 µl. PCR conditions were as follows: Preheating at 92^oC for 2min, 36 cycles of 92^oC for 1min, 48^oC 30sec and 72^oC for 2min and final extension 72^oC for 6min. Amplified DNA was verified by electrophoresis of aliquots of PCR product (5µl) in 1.0% agarose gel in 1X TAE buffer. PCR product was cloned into the pDrive-cloning vector and transformed into *E. Coli* DH5α (Hanahan, 1983). 16S rDNA sequence of the bacterial isolates and similar sequences were aligned using the Clustal W and dendrograms were generated by the distance neighbour-joining method using MEGA4 software (Tamura *et al.*, 2007). Numbers are percent gap values after 1000 bootstrap replications. Only values above 75% were considered.

3.7.5 Restriction analysis of DNA samples by agarose gel electrophoresis

Sterile water was added in a sterile microfuge tube containing amplified DNA solution and made up a volume of 17 µl (500 ng). The appropriate 10x restriction enzyme assay buffer was added and mixed thoroughly by tapping the tube. 1 µl (2-5 units) of the restriction enzyme was added, mixed by tapping the tube. The mixture was incubated at

the appropriate temperature for 1-2 hr. To stop the reaction, 4-5 µl gel-loading buffer was added, mixed by vortexing briefly (as the DNA samples need to be analyzed directly on agarose gel).

3.7.6 Sequencing

The sequence was generated by chain termination method using an Applied Biosystems automatic sequencer (Bangalore Genie, India).

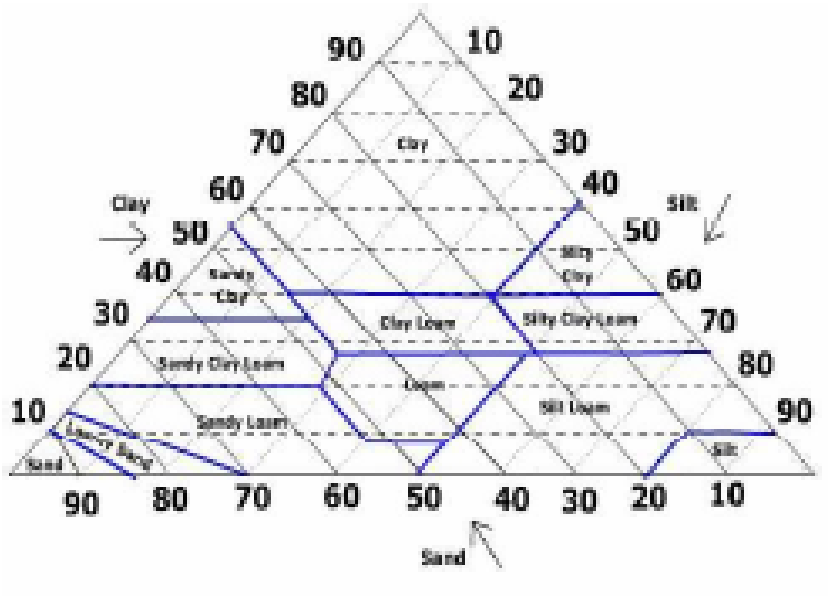
3.7.7 Analysis of sequence data

Partial 16S rDNA sequences similarities were calculated for only unambiguously determined nucleotide positions. The 16S rDNA gene sequences of soil isolates were compared with those available in GenBank database using BLAST program (Altschul *et al.,* 1997) and SEQMATCH of RDP-II (Cole *et al*., 2003). The sequences of closely related bacteria were retrieved from RDP-II and aligned using multiple alignments CLUSTALW program (Thompson *et al.,* 1997). The evolutionary distance was calculated by Kimura 2 parameter and phylogenetic dendograms were constructed by neighbor-joining using MEGA4 software (Tamura *et al.,* 2007). For analysis, 1000 bootstrap replicates were performed to assess the statistical support for the tree.

3.8 Soil Physico-Chemical Analysis

3.8.1 Soil texture

Soil texture was determined using soil texture triangle (Chopra and Kanwar, 1991) (www.usp.edu/geo/faculty/ritter/glossary/s_u/soil_texture_triangle.html).



Procedure

In the soil textural diagram, the points corresponding to percentage of silt and clay in soil were located on silt and clay lines respectively. Lines were then projected inward, parallel in the first case to the clay side of triangle and in the second case parallel to the side. The name of the compartment in which the two lines intersect is name of class of soil in question.

3.8.2 Water holding capacity

Water holding capacity was measured as per the method given by Black (1965). Circular brass boxes (keen boxes) of 5.6 cm internal diameter and 1.6 cm depth were taken which had 0.75 mm holes spaced 4 mm apart at the bottom. Each box is fitted with a brass lid.

Procedure

a) A filter paper strip of the size of the base of the keen box was cut.

b) The filter disc was weighed and placed in a petridish containing water for measuring the moisture absorbed by the filter paper.

c) The disc was placed at the bottom of the keen box and weighed followed by filling of the box with soil/fly ash. Each time the box was tapped to make a uniform soil column.

d) The box containing soil was weighed and kept in a petridish containing water for overnight saturation.

e) The box was removed the next day, wiped and weighed followed by overnight drying at 80º C in the oven in order to obtain constant weight.

f) The box containing oven-dry soil was weighed finally at room temperature.

Calculation

Weight of box+ filter paper = W1

Weight of the box +oven dry soil = W2

Weight of the box+ soil after moistening = W3

Weight of dry soil = W2-W1

Weight of moisture absorbed = W3-W2

Moisture absorbed by filter paper = W4

Moisture held by soil alone = W3-W2-W4

Water holding capacity of the soil = W3-W2-W4/W2-W1x100

3.8.3 Determination of Soil pH

a) 20 g of air dried soil was weighed and passed through 2 mm sieve into a 100 mL beaker.

b) 50 mL of distilled water was added to it and thoroughly stirred for 2-3 min using a glass rod. Further, it was kept in shaking condition (120 rpm) for 3 hr. Suspension was allowed to settle down for 30 min.

c) In the mean while, switched on the pH meter and after 10 min of warming up period, adjusted the pH meter reading to the pH of the buffer solution with the help of standardization knob.

d) The instrument was checked with two buffer solutions of known pH viz. one acidic and other alkaline.

e) The electrode was rinsed with distilled water and carefully wiped with filter paper.

f) The pH was measured of sample by immersing the electrode in supernatant solution.

g) pH value was recorded when the reading was stabilized (usually after 1 min).

3.8.4 Total Organic Carbon in soil (Walkley and Black, 1934)

a) Weighed 1 g of dried soil sample and transferred it into a 150 mL conical flask.

b) 10 mL of 1N $K_2Cr_2O_7$ was added and mixed well.

c) The blank was prepared in which all reagents except soil were added.

d) The conical flask was kept on teflon /asbestos sheet and 20 mL of concentrated H_2SO_4 was added from the sides of the flask with swirling of the flask during addition.

e) The flask was allowed to stand for 30 min and there after 70 mL of water was added.

f) These flasks were swirled thoroughly and allowed to settle the soil particles overnight.

g) The supernatant was decanted and read the color intensity using red filter at 660 nm.

h) For standard, weighed 0, 10, 20, 30, 40 and 50 mg of anhydrous sucrose crystals into a 100 mL volumetric flask and repeated the same procedure.

Calculation

The quantities of sucrose (0, 10, 20, 30, 40 and 50 mg) were multiplied by 0.4207 and found the quantities (%) of carbon present. Because 10 mg sucrose contained 4.207 mg C

and if suppose 4.207 mg C was found in 1000 mg of soil, it meant 100 mg soil contained = (4.207 x 100)/ 1000 mg carbon; Organic carbon (%) = Colorimetric reading x 0.0042. Because organic matter contains 58% carbon so, Organic matter = Organic carbon (%) x 100/ 58 or organic matter = org. C (%) x 1.724 (Van Bemmelen factor).

3.8.5 Available Phosphorus in Soil (Bray and Kurtz, 1945)

a) 2.5 g of soil sample was weighed in a 150 mL conical flask.
b) 50 mL of Bray's P-1 extractant (Appendix 1) was added and kept on reciprocating shaker for 5 min (soil- to- solution ratio 1:20).
c) It was filtered through Whatman No. 1 filter paper quickly so as to collect the filtrate within 10 min.
d) 5 mL of filtered aliquot was transferred into 50 mL volumetric flask and diluted to about 20 mL.
e) 8 mL of ascorbic acid (0.042 g) solution was added, made the volume 50 mL.
f) After 10 min, measured the color intensity at 882 nm.
g) Blank was prepared with the extracting solution also (without soil).
h) For standard, 0, 2, 5, 10, 15 and 20 mL were pipetted out of working phosphorus (KH_2PO_4) solution (1mg/L), in 6 different volumetric flasks and added 5 mL of extractant.
i) 8 mL of ascorbic acid solution was added and finally made the volume to 50 mL with distilled water. The phosphorus concentration of these solutions was 0.04, 0.1, 0.2, 0.3 and 0.4 µg/mL respectively.

Calculation

Available phosphorus (mg/kg) = (Q x V) / (A x S)

Q = quantity of phosphorus in µg read on X- axis against a sample reading; V = volume of extracting reagent (mL); A = volume of aliquot used (mL); S = weight of soil sample taken (g).

3.8.6 Total Nitrogen estimation in Soil (Modified Kjeldahl's method in Soil chemical analysis by Jackson, 1967)

Digestion of soil samples: The method employs a digestion to convert nitrogen present in soil to ammonium sulphate. The ammonium nitrogen was subsequently determined by distillation and titration.

Procedure:

1. Weighed and transferred 1g of finely ground (air dry < 0.25 mm) soil into a kjeldahl flask.
2. Added 10 ml conc. H_2SO_4 and mixed by swirling.
3. Heated at 200^0C in a digestion block until white vapors were released during digestion.
4. Added 1 Kjeldahl catalyst tablet and heated for 3-4 hours until tablet dissolves (200^0C)

   to get the precipitation.
5. Removed the digestion tubes from the block and allowed to cool for 5 minutes. "Didn't allow to cool in the heating block- NH_3 from $(NH_4)_2$ SO_4 formed by digestion will be lost"
6. Transferred the whole sample into volumetric flask and made the volume 50 ml.

ii) Determination: (Distillation- Titration method)

Procedure:

1. Transferred 5 ml of digested sample to the distillation apparatus.
2. Added 10 ml of 40% NaOH solution, formation of brown precipitates of ferric hydroxide, when the liquids were mixed indicated the neutralization of acid.
3. Took 10 ml of 4% boric acid and 2-3 drops of mixed indicator to a 100 ml Erlenmeyer flask and placed under the delivery tube of the condenser so that the tip was below the surface of the liquid.
4. Closed the sample inlet and drainage outlet and passed steam into the distillation flask. The liquid boiled and the indicator in the boric acid solution changed color from pink to green as soon as ammonia began to distill over.
5. After a minute or two, lowered the flask so that the tip of the delivery tube was clear of the liquid.
6. When about 20-25 ml of distillate had collected, rinsed the tip of the tube with little water and removed the flask.
7. Stopped the entry of steam. The distillation flask will empty automatically and the vacuum generated can be used to rinse the apparatus by immersing the tip of the delivery tube in distilled water.
8. Titrated the distillate against 0.01 N HCl to the pink color of the indicator.
9. Corrected titration for the reagent blank also.

Calculation:

$$\% \text{ Nitrogen in soil} = \frac{\text{Volume of 0.01 N HCl used (in ml)} \times 0.014 \times 50}{\text{Volume of aliquot used (in ml)}}$$

3.8.7 Determination of Mineral Elements by Atomic Absorption Spectrophotometer (AAS) (Page *et al*., 1982)

a) Took the 1 g dried soil/ in a 100 mL conical flask.

b) 15 mL of concentrated nitric acid was added and kept for 1 hour and then 5 mL of perchloric acid was added.

c) The solution was heated at about 100 ^{o}C for first one hour and then at 200 ^{o}C till the contents become colorless..

d) The heating was continued to reduced the acid content to about 2-3 mL. The solution was cooled filtered through Whatman No. 42 filter paper and the final volume was made to 20 mL with diluted HCl.

e) The concentration of mineral elements in the filtrate was measured using by atomic absorption spectrophotometer.

f) The calibration curve was prepared for each element by recording absorbance of a series of standard solutions of increasing concentrations.

Calculation

$$\text{Mineral ion (mg/g)} = C \times 20/W$$

C = concentration in the sample obtained on X-axis against the reading

W = weight of the soil taken

3.9 Enzyme Assays

3.9.1 Phosphatase Activity (Tabatabai and Bremner, 1969)

a) 10 g soil samples were placed in sterile 30 mL screw cap tubes with 4 mL of sterile modified universal buffer (Appendix I) solution.

b) 1 mL of filter sterilized 0.115 M disodium *p*-nitro phenyl phosphate solution was added

c) The contents were incubated at 37 ^{o}C in a water bath for 1 hr in the dark.

d) After 2 hr of incubation, 5 mL of 0.5N NaOH was added to stop the reaction.

e) The mixture was swirled and filtered through Whatman No. 1 filter paper.

f) The filtrate was transferred to glass cuvette and the yellow color was measured with UV-VIS spectrophotometer (Hitachi U-2001) at 410 nm.

g) Phosphatase activity was expressed as the amount of *p*-nitrophenol (PNP) released in the filtrate from the *p*-nitro phenyl phosphate substrate per gram of the soil. The *p*-nitrophenol (PNP) content was calculated with reference to a calibration graph plotted from the results obtained by standards containing 0, 10, 20, 30, 40 and 50 µg of *p*-nitrophenol. One unit of phosphatase activity is defined as 1 micro mole of PNP/g dry wt. soil/hr.

Calculation

Phosphatase activity (µM PNP/g dry wt. soil/hr) = Concentration of PNP (in µM / Dry weight of soil.

3.9.2 Cellulase enzyme assays from culture supernatant and soil samples (Bailey *et al.,* 1992)

a) The culture supernatants from cellulose grown culture were screened for the extracellular enzymes cellulase after centrifuging at 10,000 X *g* at 4 ℃ for 20 min.
b) Cellulase was assayed using culture supernatant (0.1 mL), 0.9 mL of 1.0% carboxymethyl cellulose (CMC) in 0.05 M phosphate buffer (pH 7.0) in a total volume of 1 mL and incubated at 37 ℃ for 1 hour.
c) The reducing sugar released was measured according to Miller (1959) in which 2 ml of DNSA reagent (Appendix 1) was added and incubated for 15 min in boiling water bath and expressed using glucose as the standard.

One unit of cellulase activity was defined as the enzyme amount necessary to release 1 μmol of glucose equivalent per min at 37 ^{o}C. All the experiments were performed in triplicates.

Calculations

Cellulase activity = (Glucose concentration x volume of enzyme used x dilution factor)/ Enzyme reaction period

3.10 Statistical analysis

3.10.1 Data analysis

Statistical analysis was performed by ANOVA and special effects were tested by the Newman Keuls test with a critical range of 5% using COSTAT software. Data characterized by the same letter were not significantly different.

Results

Chapter 4

Isolation, enumeration and screening of microbes improving soil organic matter

ISOLATION, ENUMERATION AND SCREENING OF MICROBES IMPROVING SOIL ORGANIC MATTER

The theme for sustainability in agriculture is prime and considered towards the diversification of wheat (*Triticum* spp.) crop with alternative crop of chickpea (*Cicer arietinum* L.) and/or sunflower (*Helianthus annuus*). Chickpea (*Cicer arietinum* L.) or Sunflower (*Helianthus annuus*) was grown as an alternative to wheat (*Triticum* spp.) during rabi season and followed by rice (*Oryza sativa*) during kharif season to study the microbial activities, population dynamics and nutritional status in following cropping pattern. Thus three different cropping patterns of chickpea-rice, wheat-rice and sunflower-rice were studied in field conditions using prescribed package of practices. The above crops were grown at farmer's field at four blocks of Patiala (PT), Rajpura (RJ), Derabassi (DB) and Samana (SM) in Patiala district (Punjab, India). Nutritional content of the soil was analyzed by quantifying the organic carbon, total nitrogen, available phosphorus and available potassium. The microbial population dynamics was quantified in term of total microbial count, phosphate solubilizers and cellulose degraders using plate count. Enzyme activity of cellulase, enzyme responsible for the formation of organic matter and phosphatase, responsible for phosphate solubilization, were assayed at the different stages of crop (viz sowing of crop, pod/ grain setting stage (midtime) and crop harvest).

4.1 Enumeration of microbial population in different study areas

4.1.1 Study area: Patiala

As shown in Fig 4.1, the total microbial population in PT-1 field at Patiala during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $149x10^6$ (cfu / g dry wt. of soil) which increased to $193x10^6$ (cfu / g dry

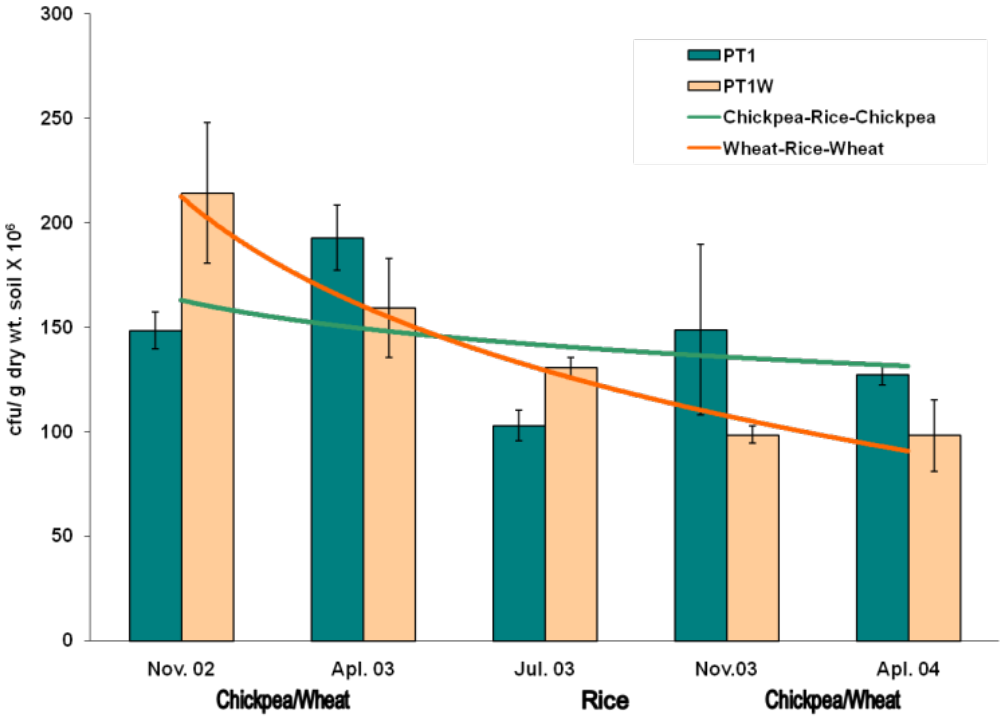


Fig 4.1: Microbial population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Patiala during three cropping seasons. The total microbial count was determined in the soil samples (Section 4.1.1) and analysed statistically.

wt. of soil) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the population was $103x10^6$ (cfu / g dry wt. of soil) which increased to $148x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season at the time of sowing of chickpea the microbial population decreased to 127 $x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The total microbial population in PT-1W field at Patiala during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat was $214x10^6$ (cfu / g dry wt. of soil) which decreased to $159x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was $130x10^6$ (cfu / g dry wt. of soil) which further decreased to $98x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season with wheat in the same field the population decreased to 97 $x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Patiala in different cropping

patterns showed relatively stable trend in total microbial population in chickpea-rice-chickpea cropping pattern as compared to decrease in wheat-rice-wheat cropping pattern.

4.1.2 Study area: Rajpura

As shown in Fig 4.2, the total microbial population in RJ-1 field at Rajpura during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $39x10^6$ (cfu / g dry wt. of soil) which increased to $149x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was $58x10^6$ (cfu / g dry wt. of soil) which increased to $87x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003).

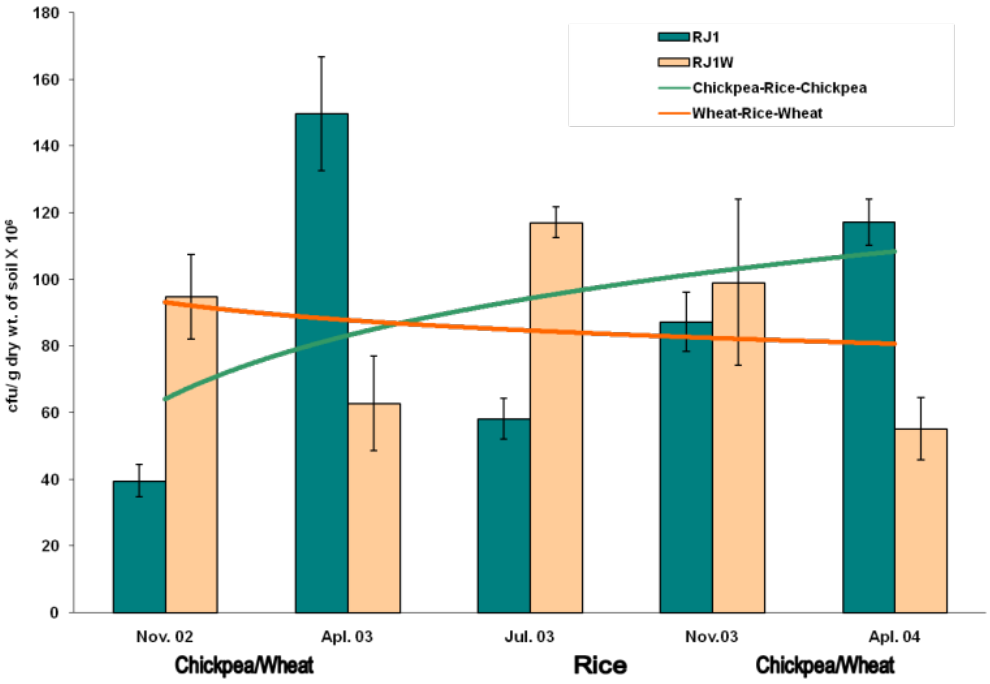


Fig 4.2: Microbial population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. The total microbial count was determined in the soil samples (Section 4.1.2) and analysed statistically.

In next cropping season of chickpea the microbial population decreased to 117 $x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The total

microbial population in RJ-1W field at Rajpura during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat was $94x10^6$ (cfu / g dry wt. of soil) which decreased to $62x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population increased to $116x10^6$ (cfu / g dry wt. of soil) which further decreased to $100x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field the population decreased to 55 $x10^6$ (cfu / g dry wt. of soil) during the time of harvest of wheat (April 2004). The overall trend at Rajpura in different cropping patterns showed relatively increased trend in total microbial population in chickpea-rice-chickpea cropping pattern as compared to decrease in wheat-rice-wheat cropping pattern.

4.1.3 Study area: Derabassi

As shown in Fig 4.3, the total microbial population in DB-1 field at Derabassi during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $104x10^6$ (cfu / g dry wt. of soil) which increased to $118x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was $74x10^6$ (cfu / g dry wt. of soil) which decreased to $71x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season of chickpea the microbial population decreased to 118 $x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The total microbial population in DB-1W field at Derabassi during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat was $143x10^6$ (cfu / g dry wt. of soil) which decreased to $81x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was $91x10^6$ (cfu / g dry wt. of soil) which further decreased to $78x10^6$ (cfu / g dry wt. of soil) during the rice

harvest (November 2003). In next cropping season of wheat in the same field decreased the population to 97 x10^6 (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Derabassi in different cropping patterns was observed to be relatively stable trend in total microbial population in chickpea-rice-chickpea cropping pattern as compared to decrease in wheat-rice-wheat cropping pattern.

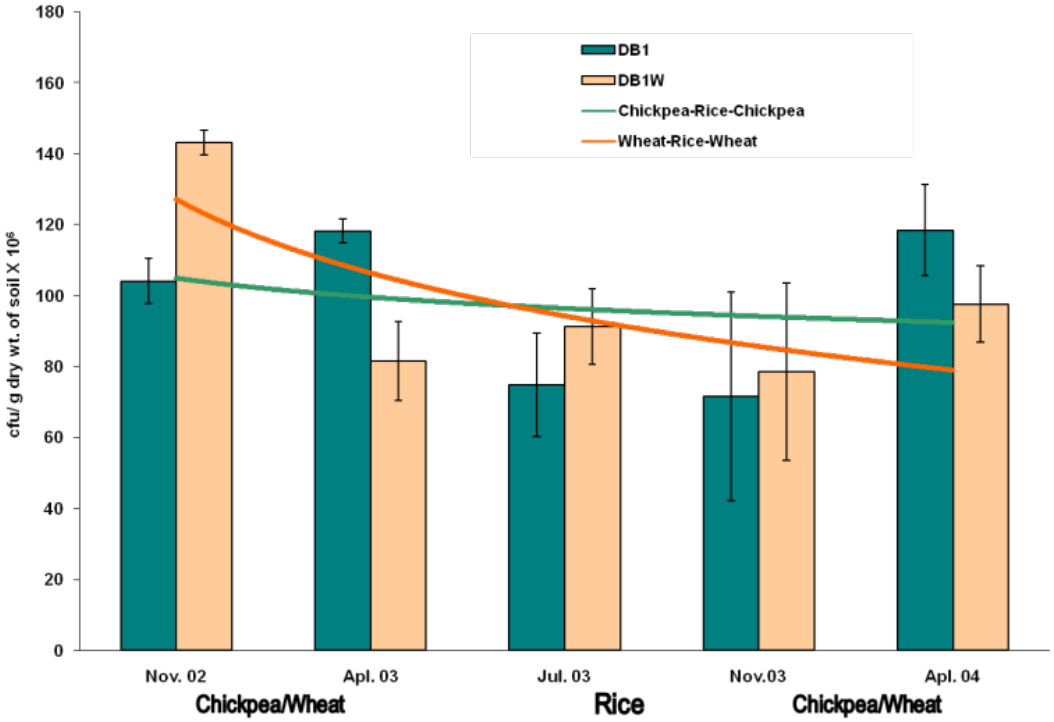


Fig 4.3: Microbial population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. The total microbial count was determined in the soil samples (Section 4.1.3) and analysed statistically.

4.1.4 Study area: Samana

As shown in Fig 4.4, he total microbial population in SM-5 field at Samana during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $37x10^6$ (cfu / g dry wt. of soil) which increased to $95x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was $97x10^6$ (cfu / g dry wt. of soil) which decreased to $37x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season of chickpea the microbial population increased to $86x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004).

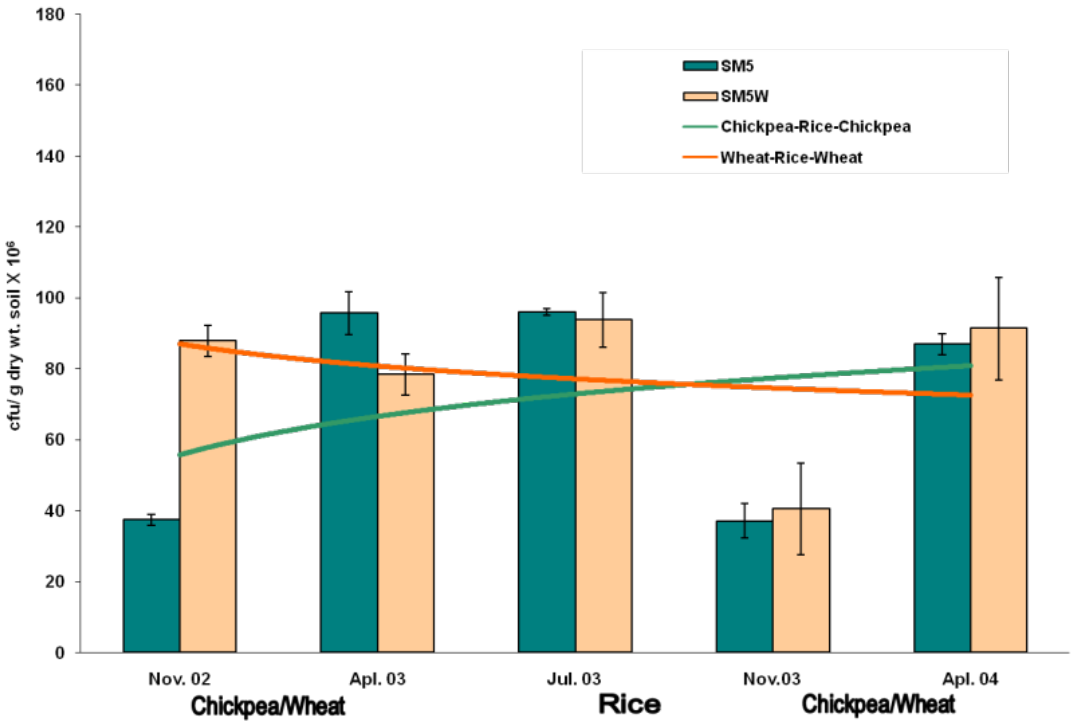


Fig 4.4: Microbial population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping. The total microbial count was determined in the soil samples (Section 4.1.4) and analysed statistically.

The total microbial population in SM-5W field at Samana during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat was $87x10^6$ (cfu / g dry wt. of soil) which decreased to $78x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the population was $93x10^6$ (cfu / g dry wt. of soil) which further decreased to $40x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field decreased the population to $91x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend in different cropping patterns showed increased trend in total microbial population during chickpea-rice-chickpea cropping pattern as compared to decrease in wheat-rice-wheat cropping pattern.

4.2 Isolation of cellulose degrading bacteria

Agricultural soil samples were collected from different locations of farmer's field at four blocks of Samana, Rajpura, Patiala and Derabassi in Patiala district (Punjab, India) during different cropping seasons and stages of crop growth and cellulose degrading bacteria were isolated. The cellulose degrading population from these samples varied from 40 to 200 ($x10^6$ cfu/ g dry wt. of soil).

4.3 Screening of cellulose degrading bacterial isolates

The various bacteria isolates were designated as MSK 1 to MSK 31 were screened for cellulase activity on CMC-ase media (Fig 4.5). Three isolates MSK1, MSK13 and MSK24 based on their high efficacy to degrade carboxy methyl cellulose (CMC) were chosen for further studies (Fig: 4.6) and showed the cellulase activity of 3.83, 4.21 and 4.52 mM glucose/ ml /hr respectively (Fig: 4.7).

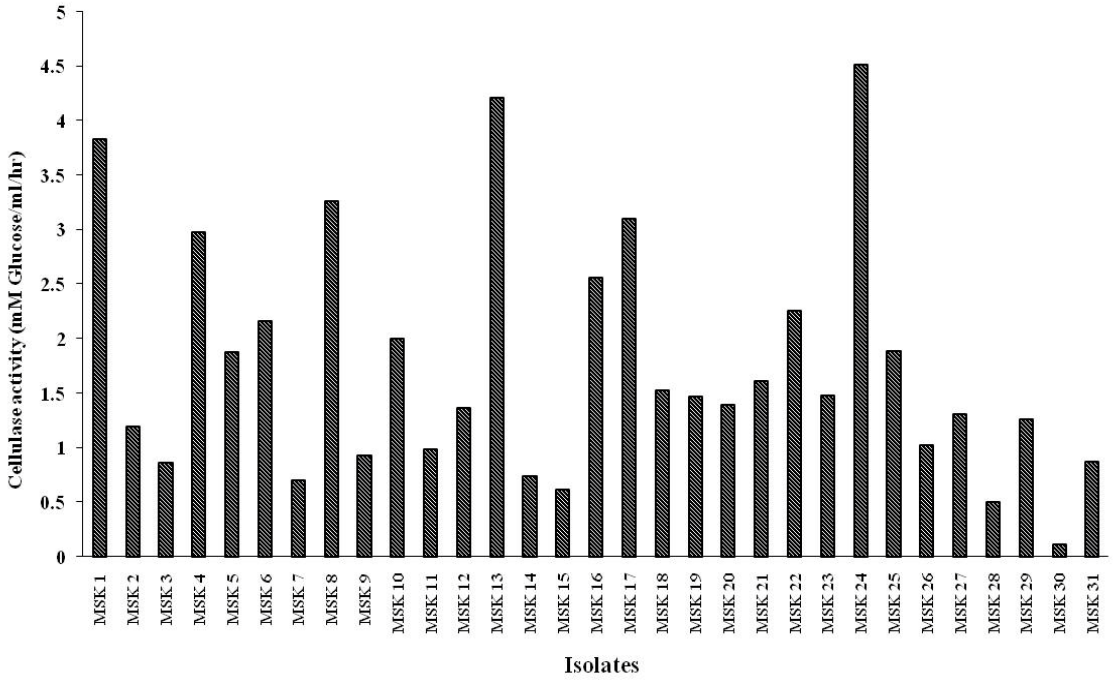


Fig 4.5: Screening of isolates for Cellulase (CMCase) activity from agricultural soils. Soil samples were collected from different locations and cellulose degrader were isolated (Section 4.2). Cellulase activity was determined (Section 4.3).

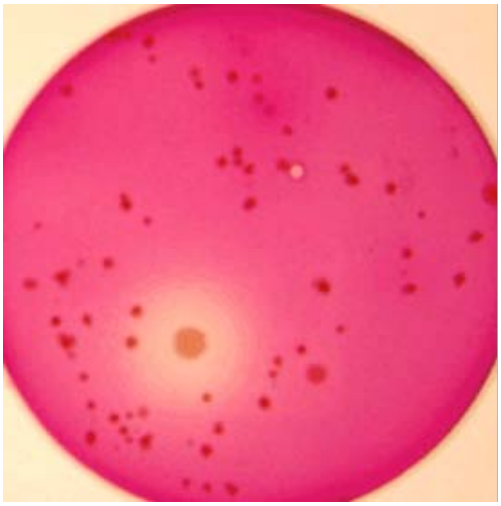


Fig. 4.6: Isolation of cellulase producing bacteria using agar medium containing 1 % (w/v) CMC. Samples were incubated at 37 ºC for 72 hours. The plates were stained with Congo red and destained with 1M NaCl solution. Clear zone indicated the hydrolysis of CMC as a result of cellulases production by MSK13.

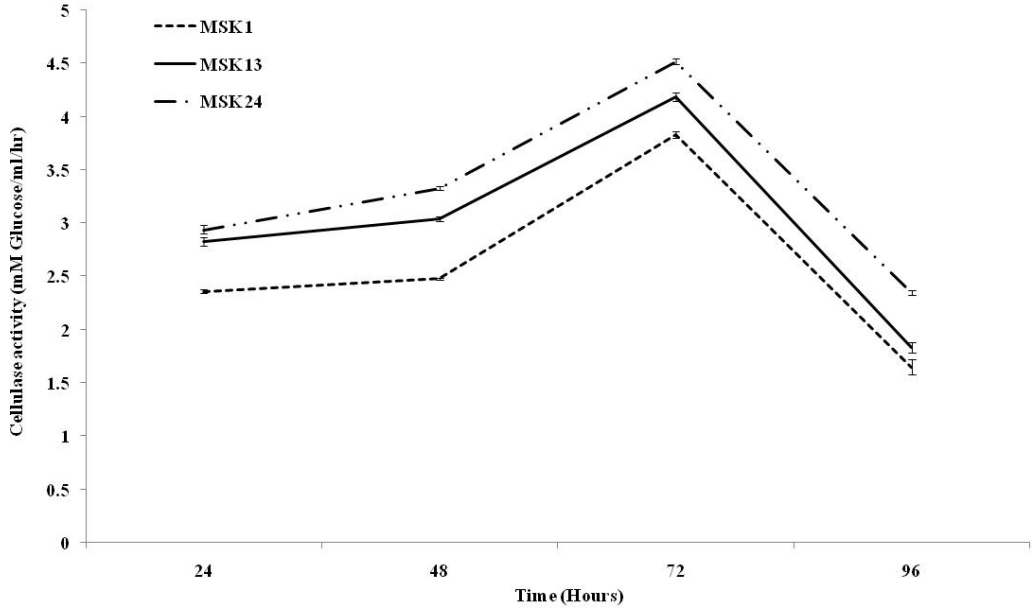


Fig 4.7: Estimation of Cellulase (CMCase) activity in MSK1 (*Serratia* sp.*)*, MSK13 (*Pseudomonas* sp.*)* and MSK24 (*Serratia* sp.*)*. MSK1, MSK13 and MSK24 were grown in 1% CMC in BHB media and incubated 37^{o}C at120rpm. Cellulase activity was determined at various times of growth of the isolates.

4.4 Biochemical characterisation of cellulose degrading bacterial isolates

The bacterial isolates had diverse morphotypes. The diversity found either in cell or in colony morphology and pigmentation was rather discrete. MSK1 appeared white, MSK13 had creamish white in color and MSK24 observed to be in pinkish white pigmentation. All three isolates showed gram negative reaction (Table 4.1). The isolates were non-sporulating. The MSK1 and MSK 24 were catalase positive, but MSK13 was negative for catalase test (Table 4.1). MSK1 and MSK 24 were oxidase negative, but MSK13 was oxidase positive (Table 4.1). The ability to reduce nitrate was a positive character for MSK13 isolates. All three isolates (MSK1, MSK13 and MSK 24) were found to hydrolyze xylan and carboxy methyl cellulose (CMC) extracellularly and were able to produce xylanase and cellulase in presence of substrates xylan and CMC respectively (Table 4.1). All bacterial isolates were checked for their sensitivity to different antibiotics (Section 3.6). The soil isolates,

MSK 1, MSK 13 and MSK 24 were found to be resistant to ampicillin (120µg/ml) antibiotic but were sensitive to gentamycin, tetracycline and kanamycin but were resistant to ampicillin (Table 4.1).

Table 4.1: Biochemical characterization of the screened bacterial isolates from agricultural soils.

Character	**MSK1**	**MSK13**	**MSK24**
Colony morphology	White	Creamy Yellow	Pinkish White
Gram stain	-	-	-
Spore	-	-	-
Catalase	+	-	+
Oxidase	-	+	-
Nitrate reduction	-	+	-
Growth at 25-37^{o}C	+	+	+
Growth at pH 6-8	+	+	+
CMCase	+	+	+
Xylanase	+	+	+
Ampicillin	R	R	R
Tetracyclin	S	S	S
Gentamycin	S	S	S
Kanamycin	S	S	S

+: positive reaction; -: negative reaction; R: Resistant at 120µg; S: Sensitive

4.5 Identification of cellulose degrading bacterial isolates

All bacterial isolates were subjected to 16S rDNA amplification using universal primers, and about 1.5 kb amplicon was observed in all isolates (Fig 4.8). The amplified products of different clones were subjected for restriction digestion enzyme analysis (*Eco*RI, *Hinf*I, *Rsa*I and *Mbo*I) to see the variation in the 16S rDNA region. The *Eco*RI restriction digestion banding pattern was unable to differentiate among the three isolates. On the other hand, *Hinf*I and *Rsa*I were unable to distinguish between the MSK1 and MSK 24, but the banding pattern was different of MSK 13 with respect to the other. Digestion with *Mbo*I showed genetic differentiation among all isolates (Fig 4.9). The 16Sr DNA was amplied and PCR product was cloned into the pDrive-cloning vector and transformed into *E. Coli* DH5α. The 16S rDNA products from three selected clones were then sequenced using Applied Biosystems automatic sequencer. Sequences were compared for the similarity in the GenBank DNA database using BlastN (NCBI) (Altschul *et al*., 1997). Pairwise alignment revealed that 16S rDNA of bacterial isolates had 99% similarity with the sequences of NCBI database. 16S rDNA sequence of the bacterial isolates and similar sequences were aligned using the Clustal W and dendrograms were generated by the distance neighbour-joining method using MEGA4 software (Tamura *et al.,* 2007). Numbers are percent gap values after 1000 bootstrap replications. Phylogenetic analysis revealed that all the isolates were related to the phylum Proteobacteria. MSK1 and MSK24 have shown similarity with *Serratia* sp., Whereas MSK13 has shown similarity with *Pseudomonas* sp. (Fig 4.10).

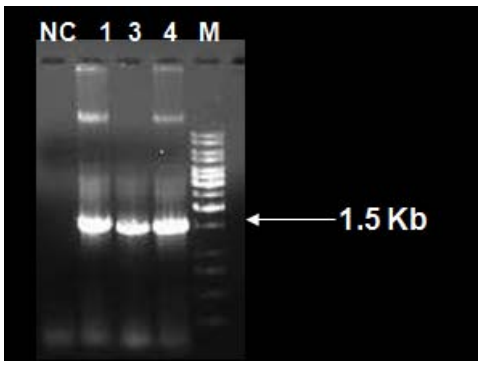


Fig 4.8 16S PCR amplification of known bacterial strains. Bacterial DNA of isolates MSK1, MSK13, and MSK24was amplified as described in section 3.7.4. Amplified DNA was analysed on 1.5% agarose gel. Lane 1, negative control; Lane 2, Bacterial strain MSK1; Lane 3, Bacterial strain MSK13; Lane 4, Bacterial strain MSK24; Lane 5, 1Kb Ladder.

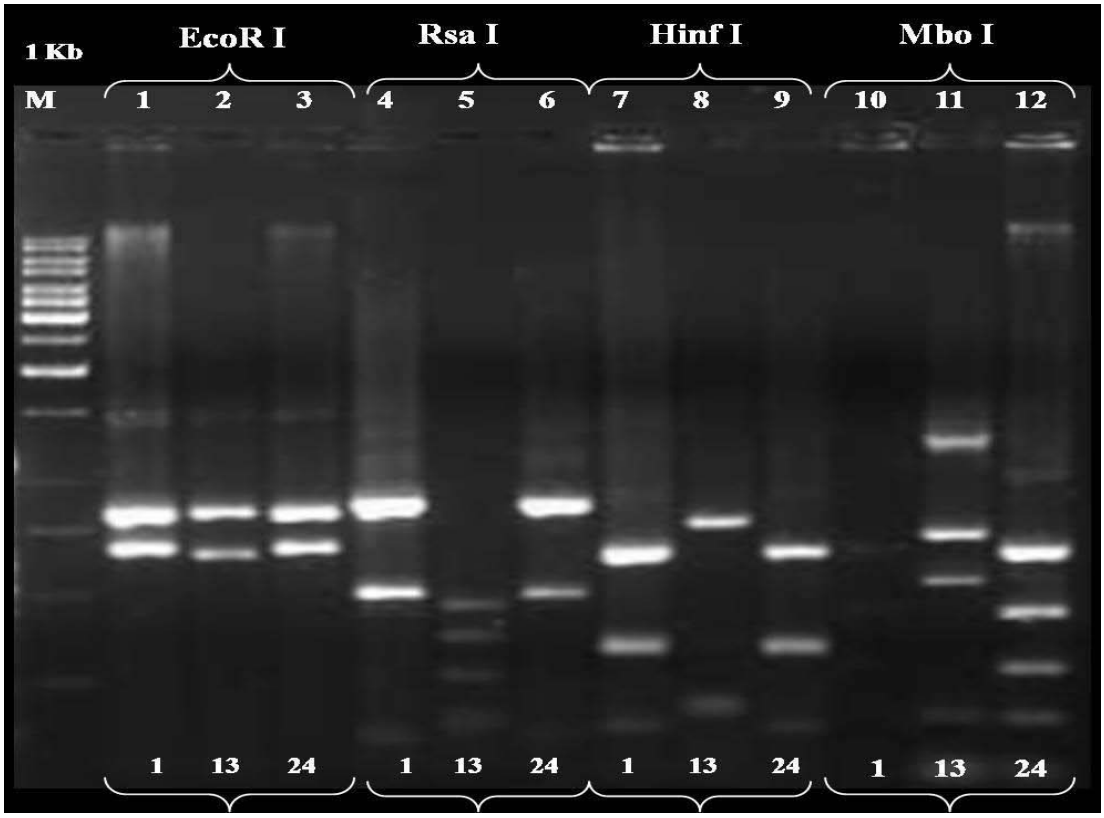


Fig 4.9 Amplified Ribosomal DNA Restriction Analysis ARDRA of bacterial isolates. PCR-amplified 16S rDNA of unknown bacterial isolates was digested with EcoR1, Rsa1, Hinf 1and Mbo 1and then analysed on 1.5% agarose gel. Lanes M:100bp Ladder (Fermentas), Lane 1-3: EcoR1 digest of MSK1, MSK13 & MSK24 respectively, Lane 4-6: Rsa1 digest of MSK1, MSK13 & MSK24 respectively, Lane 7-9 Hinf1 digest of MSK1, MSK13 & MSK24 respectively Lane 10-12: Mbo1 digest of MSK1, MSK13 & MSK24 respectively.

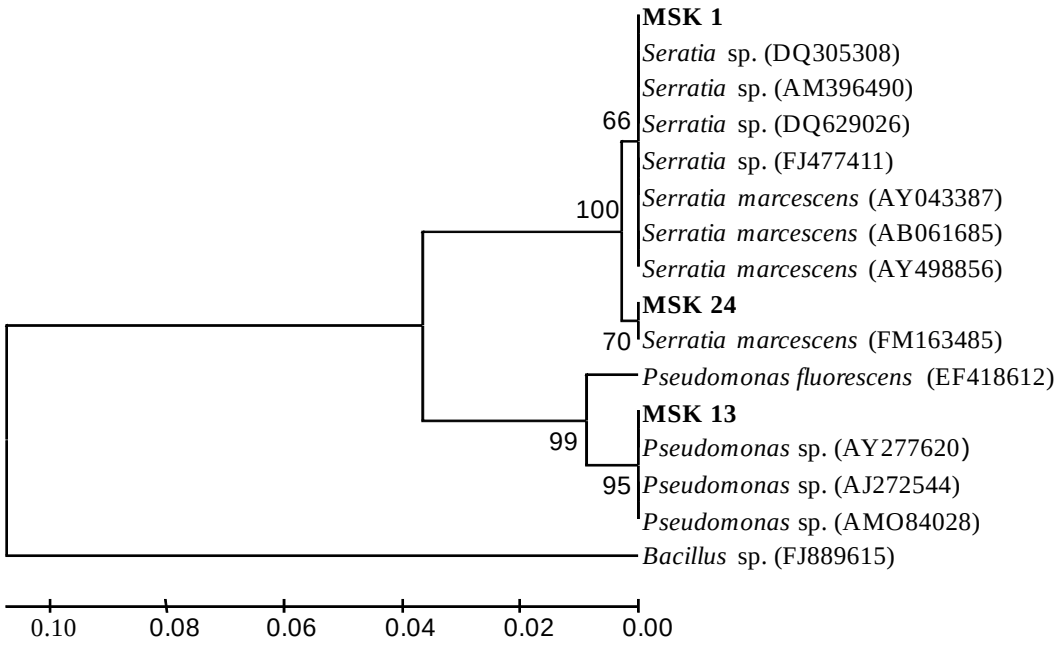


Fig 4.10 UPGMA tree based on bacterial 16S rDNA sequence data from different isolates of current study along with sequences available in GenBank database. Numerical values indicate bootstrap percentile from 1000 replicates.

Chapter 5

Estimation of microbial diversity in maintaining organic fertility of soil

ESTIMATION OF MICROBIAL DIVERSITY IN MAINTAINING ORGANIC FERTILITY OF SOIL

5.1 Estimation of phosphate solubilisers population, soil phosphatase activity and available phosphorus in maintaining organic fertility of soil in different study areas under diversified cropping pattern.

5.1.1 Study area: Patiala

As shown in Fig 5.1, the phosphate solubilisers population in PT-1 field at Patiala during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $85x10^6$ (cfu / g dry wt. of soil) which decreased to $60x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the sowing of rice (July 2003) the population was $28x10^6$ (cfu / g dry wt. of soil) which increased to $70x10^6$ (cfu / g dry wt. of soil) during the harvest (November 2003). In the next cropping season with chickpea the phosphate solubilisers population decreased to $18x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The phosphate solubilisers population in PT-1W field at Patiala during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be $65x10^6$ (cfu / g dry wt. of soil) which decreased to $50x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) the population increased to $52x10^6$ (cfu / g dry wt. of soil) which further decreased to $35x10^6$ (cfu / g dry wt. of soil) during the harvest (November 2003). In the next cropping season with wheat in the same field the population decreased to $15x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Patiala in different cropping patterns showed decreased phosphate solubilisers population in chickpea-rice-chickpea cropping pattern at PT-1 field as well as in wheat-

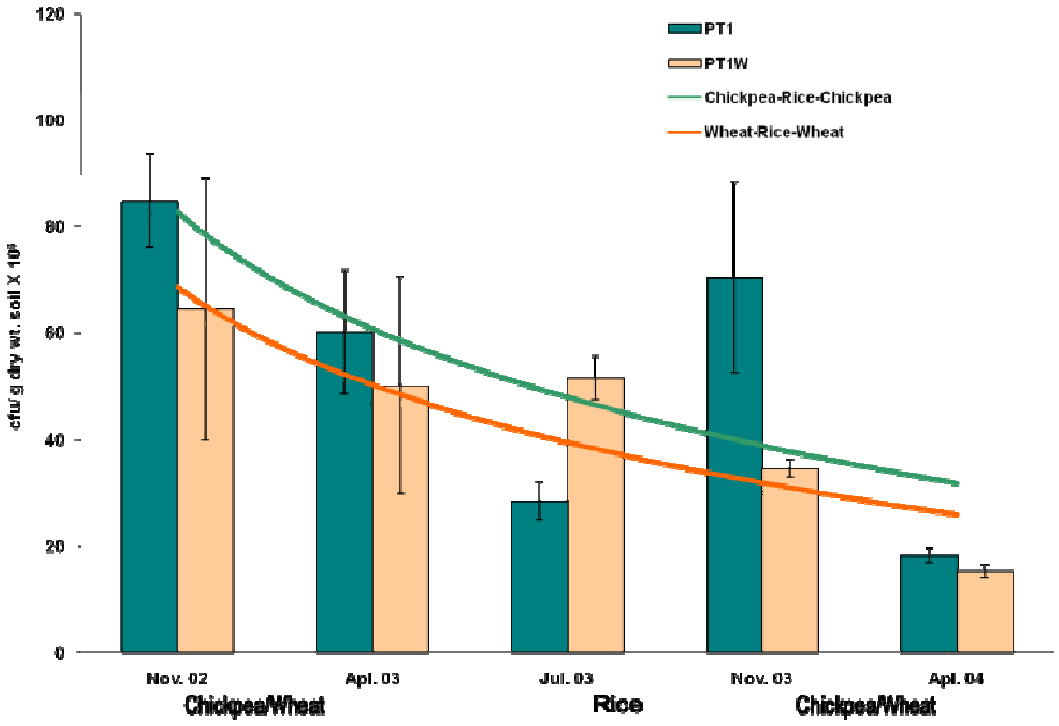


Fig 5.1: Phosphate solubilisers population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Patiala during three cropping seasons. Soil samples were collected from Patiala and phosphate solubilizers were enumerated (Section 5.1.1)

rice-wheat cropping pattern. As shown in Fig 5.2, the soil phosphatase activity in PT-1 field at Patiala during chickpea-rice-chickpea cropping pattern was 0.64 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), decreased slightly to 0.57 µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then further decreased to 0.12 µM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated phosphatase activity to 0.17 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 0.4 µM PNP/ g dry wt. soil/ hr at flowering (September 2003) and increased to 1.01 µM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In the next cropping season sowing of chickpea (mention time of the year) in the same field decreased the soil phosphatase activity from 1.01 to 0.77 µM PNP/ g dry wt. soil/ hr at flowering and activity increased to

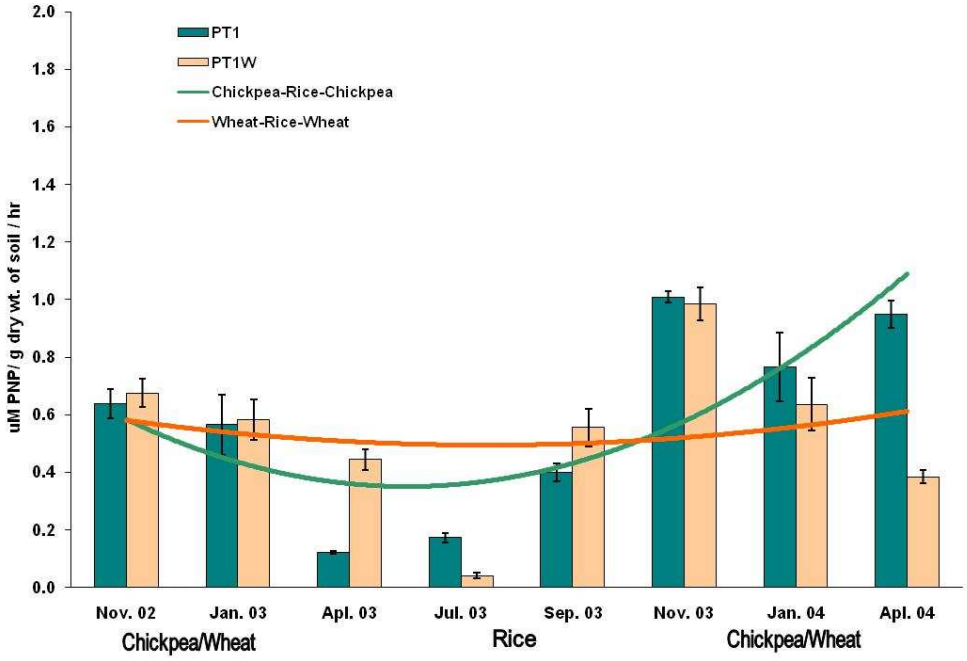


Fig 5.2: Phosphatase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Patiala during three cropping seasons. Soil samples were collected from Patiala and phosphatase activity was estimated (Section 5.1.1)

0.95 µM PNP/ g dry wt. of soil/ hr at harvesting of crop. The soil phosphatase activity in PT-1W field at Patiala during wheat-rice-wheat cropping pattern was 0.68 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), decreased to 0.58 µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then further decreased to 0.44 µM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field decreased the phosphatase activity to 0.04 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which increased to 0.56 µM PNP/ g dry wt. of soil/ hr at flowering (September 2003) and further increased to 0.99 µM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In the next cropping season sowing of chickpea (mention time of the year)

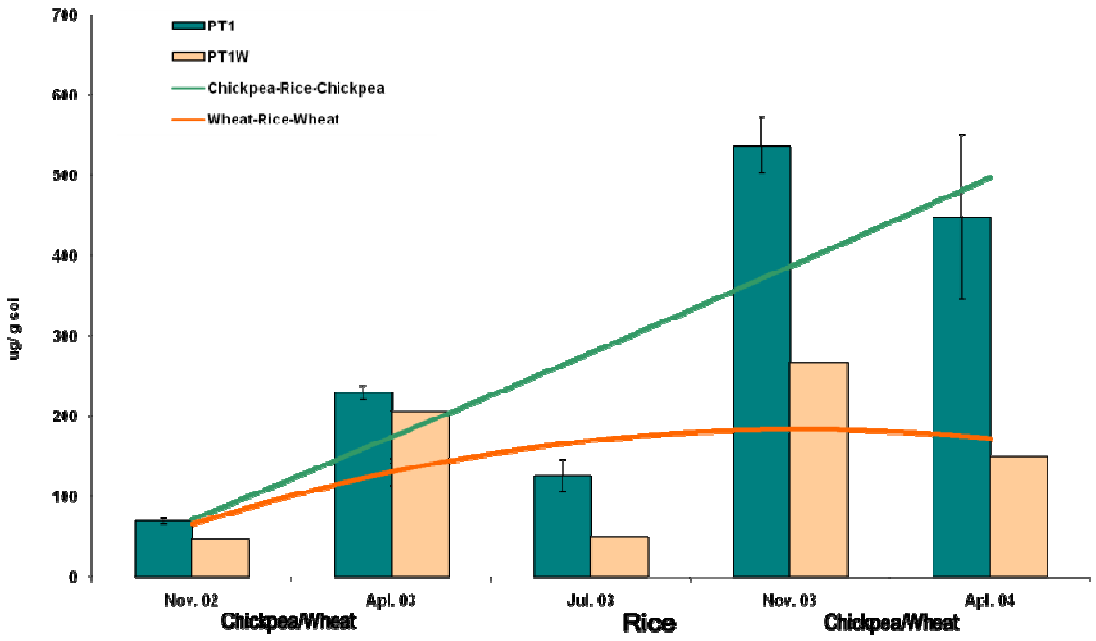


Fig 5.3: Available phosphorus in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Patiala during three cropping seasons. Soil samples were collected from Patiala and available phosphorus was estimated (Section 5.1.1)

in the same field decreased the soil phosphatase activity from 0.99 to 0.64 µM PNP/ g dry wt. of soil/ hr during sowing to flowering and activity decreased to 0.38 µM PNP/ g dry wt. of soil/ hr at harvesting of crop. The data (ref to Fig 5.2)shows an increased phosphatase activity in soil during chickpea-rice-chickpea cropping pattern in PT-1 field as compared to wheat-rice-wheat cropping pattern where no change was found in phosphatase activity at PT-1W field. As shown in Fig 5.3, the available phosphorus in PT-1 field at Patiala during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was 70 (mg/kg) which increased to 229 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) the available phosphorus was 126 (mg/kg) which increased to 537 (mg/kg) during the rice harvest (November 2003). In the next cropping season of chickpea the available phosphorus

decreased to 448 (mg/kg) at the time of harvest of chickpea (April 2004). The available phosphorus in PT-1W field at Patiala during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be 48 (mg/kg) which increased to 206 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) available phosphorus was 50 (mg/kg) which increased to 267 (mg/kg) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field the available phosphorus decreased to 150 (mg/kg) at the time of harvest of wheat (April 2004). The overall trend at Patiala in different cropping patterns showed increased available phosphorus in chickpea-rice-chickpea cropping pattern at PT-1 field as compared to wheat-rice-wheat cropping pattern at PT-1W field.

5.1.2 Study area: Rajpura

As shown in Fig 5.4, the phosphate solubilisers population in RJ-1 field at Rajpura during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $17x10^6$ (cfu / g dry wt. of soil) which increased to $66x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the sowing of rice (July 2003) the population was $22x10^6$ (cfu / g dry wt. of soil) which increased to $69x10^6$ (cfu / g dry wt. of soil) during the harvest (November 2003). In the next cropping season sowing with chickpea the phosphate solubilisers population decreased to $26x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The phosphate solubilisers population in RJ-1W field at Rajpura during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be $48x10^6$ (cfu / g dry wt. of soil) which decreased to $16x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the sowing of rice (July 2003) the population was increased to $27x10^6$ (cfu / g dry wt. of soil) which further

increased to $48x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In the next cropping season with wheat in the same field the population decreased to $8x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Rajpura in different cropping patterns showed increased phosphate solubilisers population in chickpea-rice-chickpea cropping pattern at RJ-1 field in comparision to decrease in wheat-rice-wheat cropping pattern in RJ-1W field. As shown in Fig 5.5, the soil phosphatase activity in RJ-1 field at Rajpura during chickpea-rice-chickpea cropping pattern was 0.89 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), decreased to 0.13 µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then increased to 0.19 µM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003).

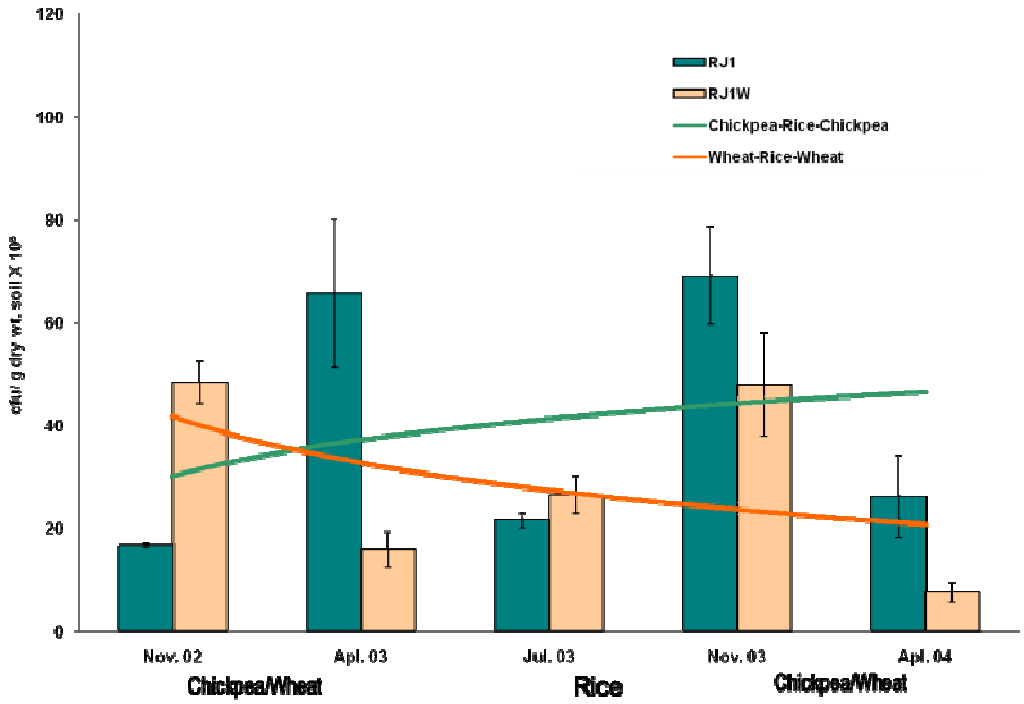


Fig 5.4: Phosphate solubilisers population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. Soil samples were collected from Rajpura and phosphate solubilizers were enumerated (Section 5.1.2)

Rice cropping in the same field stimulated phosphatase activity to 0.69 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 1.55 µM PNP/ g dry wt. of soil/ hr at flowering (September 2003) but decreased to 0.64 µM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In the next cropping season sowing of chickpea in the same field increased the soil phosphatase activity from 0.64 to 0.73 µM PNP/ g dry wt. of soil/ hr at flowering and activity decreased to 0.63 µM PNP/ g dry wt. of soil/ hr at harvesting of crop. The soil phosphatase activity in RJ-1W field at Rajpura during wheat-rice-wheat cropping pattern was 0.65 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), decreased to 0.34 µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then increased to 0.71 µM PNP/ g dry wt. of soil/ hr

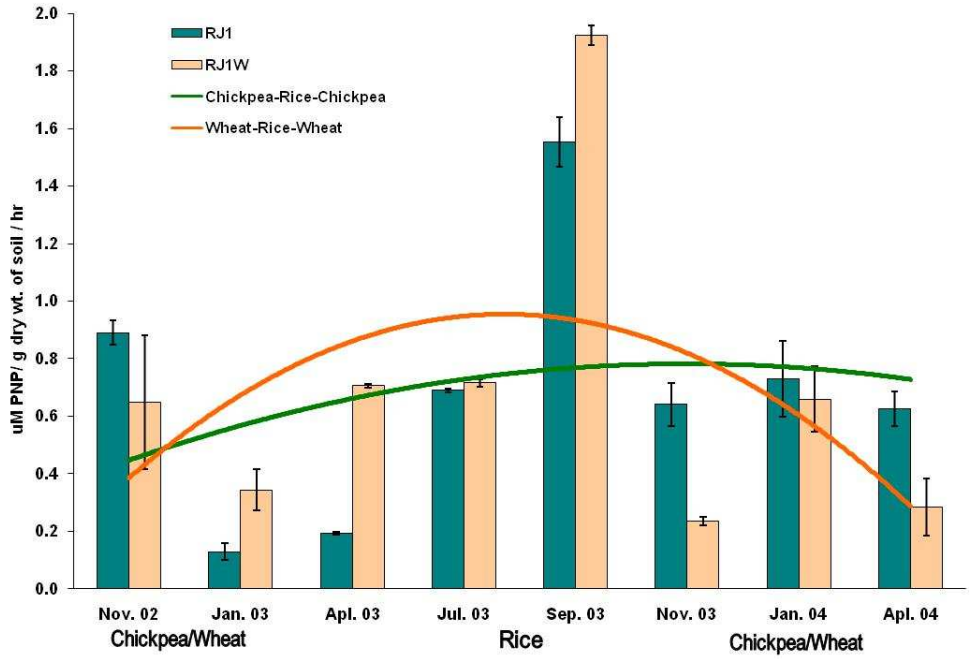


Fig 5.5: Phosphatase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. Soil samples were collected from Rajpura and phosphatase activity was estimated (Section 5.1.2)

at the time of harvest (April 2003). Rice cropping in the same field stimulated phosphatase activity to 0.72 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 1.92 µM PNP/ g dry wt. of soil/ hr at flowering (September 2003) and decreased to 0.23 µM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In the next cropping season sowing of chickpea in the same field increased the soil phosphatase activity from 0.23 to 0.66 µM PNP/ g dry wt. of soil/ hr during sowing to flowering and activity decreased to 0.29 µM PNP/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Rajpura shows an increased phosphatase activity in soil during chickpea-rice-chickpea cropping pattern in RJ-1 field as compared

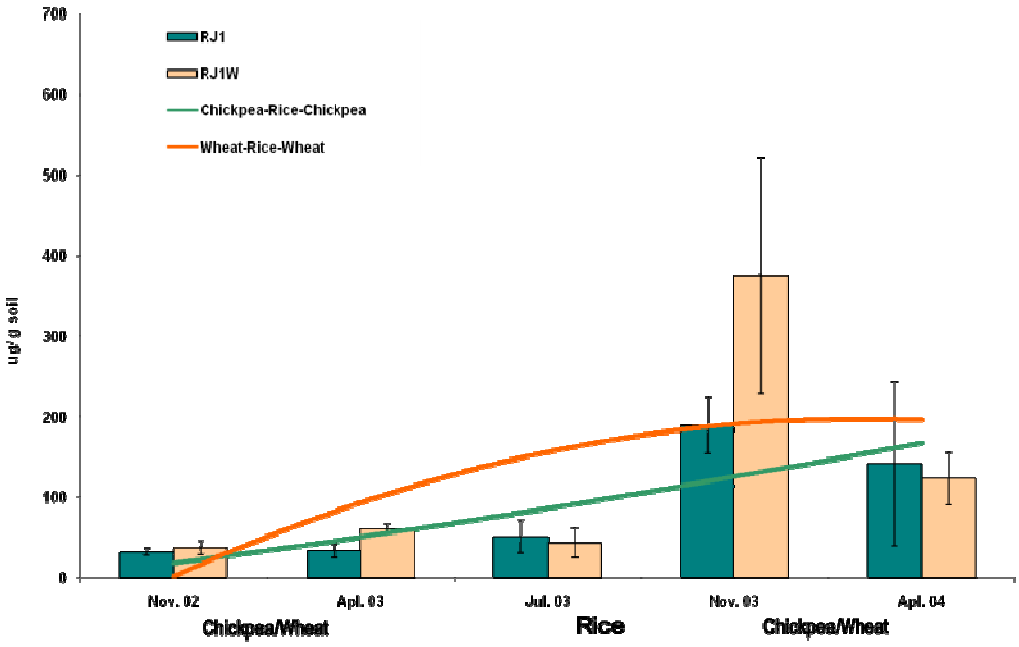


Fig 5.6: Available phosphorus in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. Soil samples were collected from Rajpura and available phosphorus was estimated (Section 5.1.2)

to decreased phosphatase activity at RJ-1W field during wheat-rice-wheat cropping pattern. As shown in Fig 5.6, the available phosphorus in RJ-1 field at Rajpura during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was 32 (mg/kg) which increased to 34 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) the available phosphorus was 51 (mg/kg) which further increased to 189 (mg/kg) during the rice harvest (November 2003). In the next cropping season of chickpea the available phosphorus decreased to 141 (mg/kg) at the time of harvest of chickpea (April 2004). The available phosphorus in RJ-1W field at Rajpura during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be 37 (mg/kg) which increased to 61 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) available phosphorus was decreased to 44 (mg/kg) which further increased to 375 (mg/kg) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field the available phosphorus decreased to 126 (mg/kg) at the time of harvest of wheat (April 2004). The overall trend at Rajpura in different cropping patterns showed increased available phosphorus in both chickpea-rice-chickpea as well as in wheat-rice-wheat pattern.

5.1.3 Study area: Derabassi

As shown in Fig 5.7, the phosphate solubilisers population in DB-1 field at Derabassi during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was $41x10^6$ (cfu / g dry wt. of soil) which decreased to $24x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the sowing of rice (July 2003) the population was $27x10^6$ (cfu / g dry wt. of soil) which increased to $33x10^6$

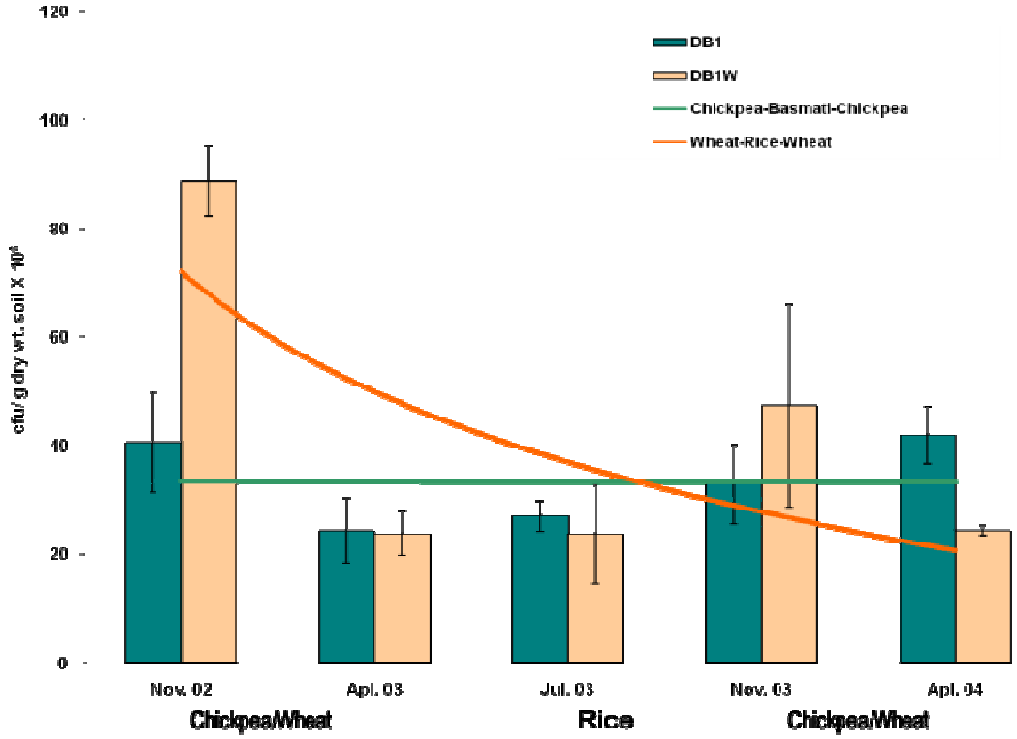


Fig 5.7: Phosphate solubilisers population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. Soil samples were collected from Derabassi and phosphate solubilizers were enumerated (Section 5.1.3)

(cfu / g dry wt. of soil) during the harvest (November 2003). In the next cropping season with chickpea further the phosphate solubilisers population increased to $42x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The phosphate solubilisers population in DB-1W field at Derabassi during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be $89x10^6$ (cfu / g dry wt. of soil) which decreased to $24x10^6$ (cfu / g dry wt. of soil) at the time of harvest(April 2003). At the sowing of rice (July 2003) the population was slightly decreased to $23x10^6$ (cfu / g dry wt. of soil) which further increased to $47x10^6$ (cfu / g dry wt. of soil) during the riceharvest (November 2003). In the next cropping season with wheat in the same field the population decreased to $24x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat

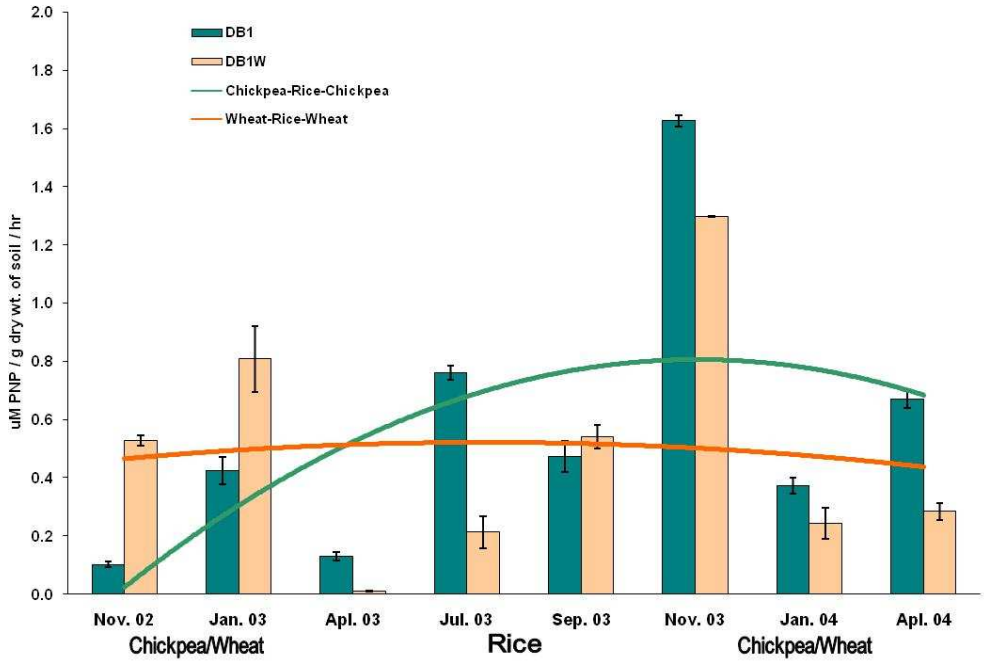


Fig 5.8: Phosphatase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. Soil samples were collected from Derabassi and phosphatase activity was estimated (Section 5.1.3)

(April 2004). The overall trend at Derabassi in different cropping patterns showed decreased phosphate solubilisers population in wheat-rice-wheat cropping pattern in DB -1W field with no overall significant change in chickpea-rice-chickpea cropping pattern at DB-1 field. As shown in Fig 5.8, the soil phosphatase activity in DB -1 field at Derabassi during chickpea-rice-chickpea cropping pattern was 0.1 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 0.43 µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then decreased to 0.13 µM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated phosphatase activity to 0.76 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further decreased to 0.47 µM PNP/ g dry wt. of soil/ hr at flowering(September 2003)

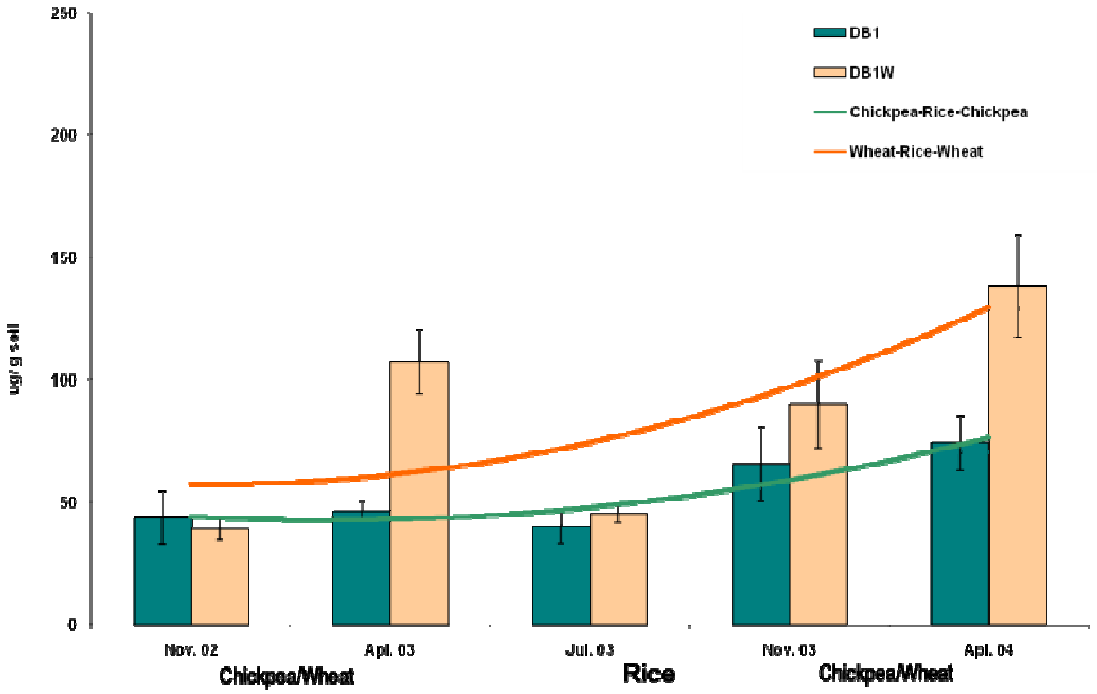


Fig 5.9: Available phosphorus in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. Soil samples were collected from Derabassi and available phosphorus was estimated (Section 5.1.3)

and increased to 1.63 µM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003).In the next cropping season sowing of chickpea in the same field decreased the soil phosphatase activity from 1.63 to 0.37 µM PNP/ g dry wt. of soil/ hr at flowering and activity increased to 0.67 µM PNP/ g dry wt. of soil/ hr at harvesting of crop. The soil phosphatase activity in DB -1W field at Derabassi during wheat-rice-wheat cropping pattern was 0.53 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 0.81 µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then highly decreased to 0.01 µM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003).Rice cropping in the same field stimulated phosphatase activity to 0.21 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 0.54 µM

PNP/ g dry wt. of soil/ hr at flowering (September 2003) and increased to 1.30 μM μM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field increased the soil phosphatase activity from 1.30 to 0.24 μM PNP/ g dry wt. of soil/ hr during flowering and activity increased to 0.28 μM PNP/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Derabassi shows an increased phosphatase activity in soil during chickpea-rice-chickpea cropping pattern in DB-1 field as compared to wheat-rice-wheat cropping pattern where no change was found in phosphatase activity at DB-1W field. As shown in Fig 5.9, the available phosphorus in DB-1 field at Derabassi during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was 44 (mg/kg) which increased to 46 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) the available phosphorus was 41 (mg/kg) which increased to 66 (mg/kg) during the rice harvest (November 2003). In the next cropping season sowing of chickpea further available phosphorus increased to 74 (mg/kg) at the time of harvest of chickpea (April 2004). The available phosphorus in DB-1W field at Derabassi during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be 39 (mg/kg) which increased to 107 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) available phosphorus was decreased to 45 (mg/kg) which increased to 90 (mg/kg) during the rice harvest (November 2003). In the next cropping season sowing of wheat in the same field further the available phosphorus increased to 138 (mg/kg) at the time of harvest of wheat (April 2004). The overall trend at Derabassi in different cropping patterns showed increased available phosphorus in chickpea-rice-chickpea cropping pattern at DB-1 field as well as in wheat-rice-wheat cropping pattern at DB-1W field.

5.1.4 Study area: Samana

As shown in Fig 5.10, the phosphate solubilisers population in SM-5 field at Samana during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $22x10^6$ (cfu / g dry wt. of soil) which decreased to $28x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the sowing of rice (July 2003) the population was $17x10^6$ (cfu / g dry wt. of soil) which increased to $18x10^6$ (cfu / g dry wt. of soil) during the harvest (November 2003). In the next cropping season sowing with chickpea the phosphate solubilisers population increased to $14x10^6$ (cfu / g dry wt. of

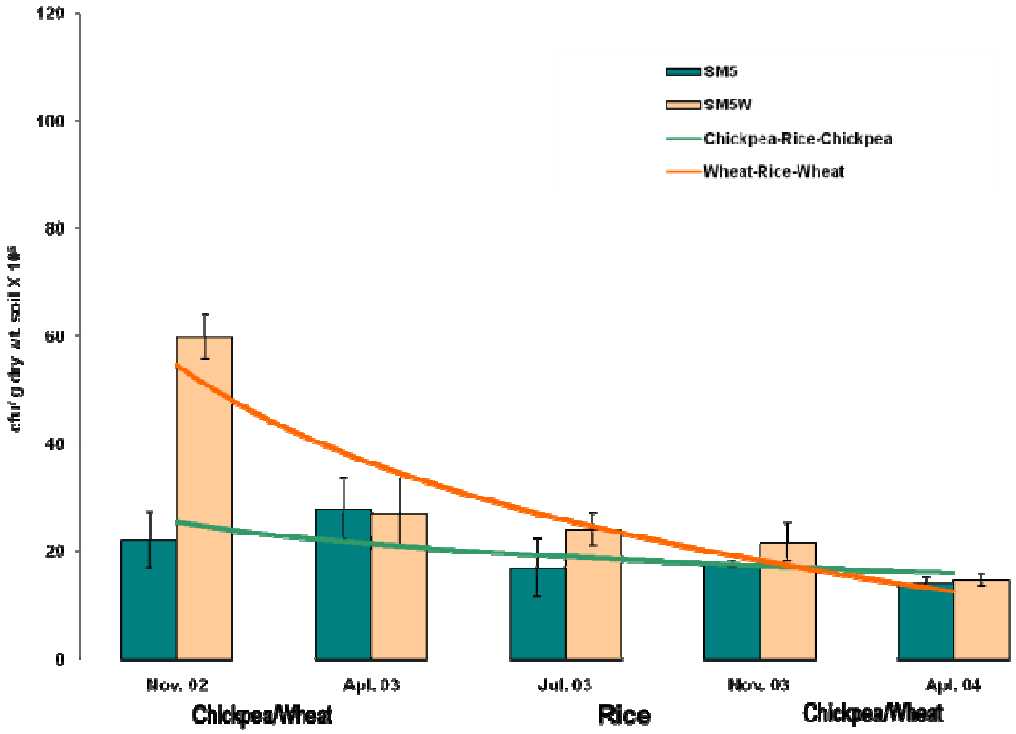


Fig 5.10: Phosphate solubilisers population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping seasons. Soil samples were collected from Samana and phosphate solubilizers were enumerated (Section 5.1.4)

soil) at the time of harvest of chickpea (April 2004). The phosphate solubilisers population in SM-5W field at Samana during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be $60x10^6$ (cfu / g dry wt. of soil) which decreased to $27x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). At the sowing of rice (July 2003) the population was slightly decreased to $24x10^6$ (cfu / g dry wt. of soil) which further increased to $22x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In the next cropping season with wheat in the same field the population decreased to $15x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Samana in different cropping patterns showed

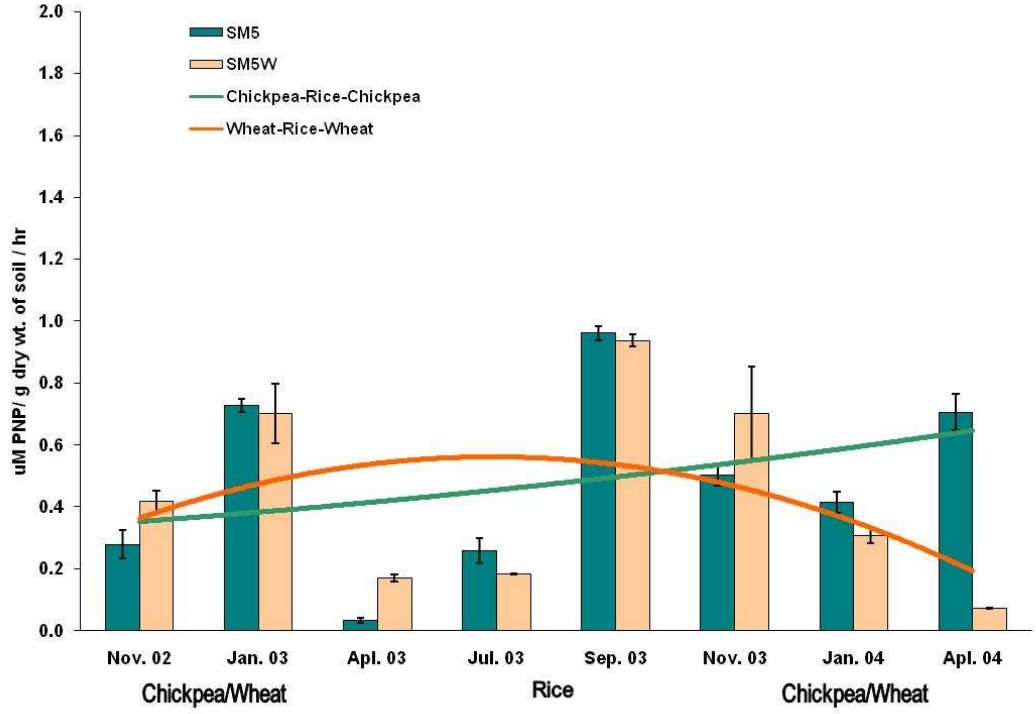


Fig 5.11: Phosphatase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping seasons. Soil samples were collected from Samana and phosphatase activity was estimated (Section 5.1.4)

decreased phosphate solubilisers population in wheat-rice-wheat cropping pattern in SM-5W field with no overall significant change in chickpea-rice-chickpea cropping pattern at SM-5 field field.As shown in Fig 5.11, the soilphosphatase activity in SM-5 field at Samana during chickpea-rice-chickpea cropping pattern was 0.28 µM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to µM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then decreased to 0.03 µM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated phosphatase activity to 0.26 µM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further decreased to 0.96 µM PNP/ g dry wt. of soil/ hr at flowering (September

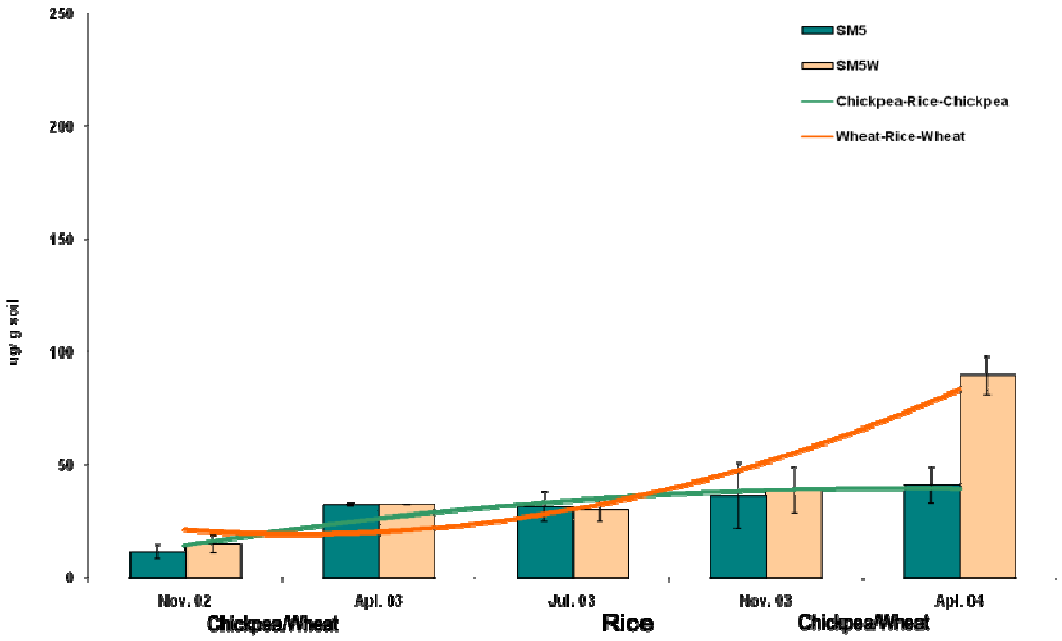


Fig 5.12: Available phosphorus in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping seasons. Soil samples were collected from Samana and available phosphorus was estimated (Section 5.1.4)

2003) and increased to 0.50 μM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field decreased the soil phosphatase activity from 0.50 to 0.42 μM PNP/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 0.71 μM PNP/ g dry wt. of soil/ hr at harvesting of crop. The soil phosphatase activity in SM-5W field at Samana during wheat-rice- wheat cropping pattern was 0.42 μM PNP/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 0.70 μM PNP/ g dry wt. of soil/ hr at flowering (January 2003) and then highly decreased to 0.17 μM PNP/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated phosphatase activity to 0.18 μM PNP/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 0.94 μM PNP/ g dry wt. of soil/ hr at flowering (September 2003) and decreased to 0.70 μM PNP/ g dry wt. of soil/ hr of soil at harvest (November 2003). In the next cropping season sowing of chickpea in the same field increased the soil phosphatase activity from 0.70 to 0.31 μM PNP/ g dry wt. of soil/ hr during flowering and activity decreased to 0.07 μM PNP/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Samana shows an increased phosphatase activity in soil during chickpea-rice-chickpea cropping pattern in SM-5 field as compared to wheat-rice-wheat cropping pattern where no change was found in phosphatase activity at SM-5W field. As shown in Fig 5.12, the available phosphorus in SM-5 field at Samana during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was 12 (mg/kg) which increased to 33 (mg/kg) at the time of harvest (April 2003). At the sowing of rice (July 2003) the available phosphorus was 32 (mg/kg) which increased to 36 (mg/kg) during the harvest (November 2003). In the next cropping season with chickpea the available phosphorus increased to 41 (mg/kg) at the time of harvest of chickpea (April

2004). The available phosphorus in SM-5W field at Samana during the wheat-rice-wheat cropping pattern at the time of sowing (November 2002) of wheat to be 15 (mg/kg) which increased to 33 (mg/kg) at the time of harvest (April 2003). At the time of sowing of rice (July 2003) available phosphorus was decreased to 30 (mg/kg) which increased to 39 (mg/kg) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field available phosphorus increased to 90 (mg/kg) at the time of harvest of wheat (April 2004). The overall trend at Samana in different cropping patterns showed increased available phosphorus in chickpea-rice-chickpea cropping pattern at SM-5 field as well as in wheat-rice-wheat cropping pattern at SM-5W field.

5.2 Estimation of cellulose degraders population, soil cellulase activity and organic carbon in maintaining organic fertility of soil in different study areas under diversified cropping pattern.

5.2.1 Study area: Patiala

As shown in Fig 5.13, the cellulose degraders population in PT-1 field at Patiala during the chickpea-rice-chickpea cropping pattern at the time of sowing (November 2002) of chickpea was $28x10^6$ (cfu / g dry wt. of soil) which increased to $60x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the population was $22x10^6$ (cfu / g dry wt. of soil) which increased to $66x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of chickpea further decreased the cellulose degraders population to $13x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The cellulose degraders population in PT-1W field at Patiala during the wheat-rice-wheat cropping pattern was observed at the time of sowing (November 2002) of wheat to be $98x10^6$ (cfu / g dry wt. of soil) which decreased to $42x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was slightly decreased to $25x10^6$ (cfu / g dry wt. of soil) which increased to $30x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field decreased the population to $7x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Patiala in different cropping patterns showed decreased cellulose degraders population in wheat-rice-wheat cropping pattern in PT-1W field with no overall significant change in chickpea-rice-chickpea cropping pattern at PT-1 field. As shown in Fig 5.14, the soil cellulase activity in PT-1 field at Patiala during

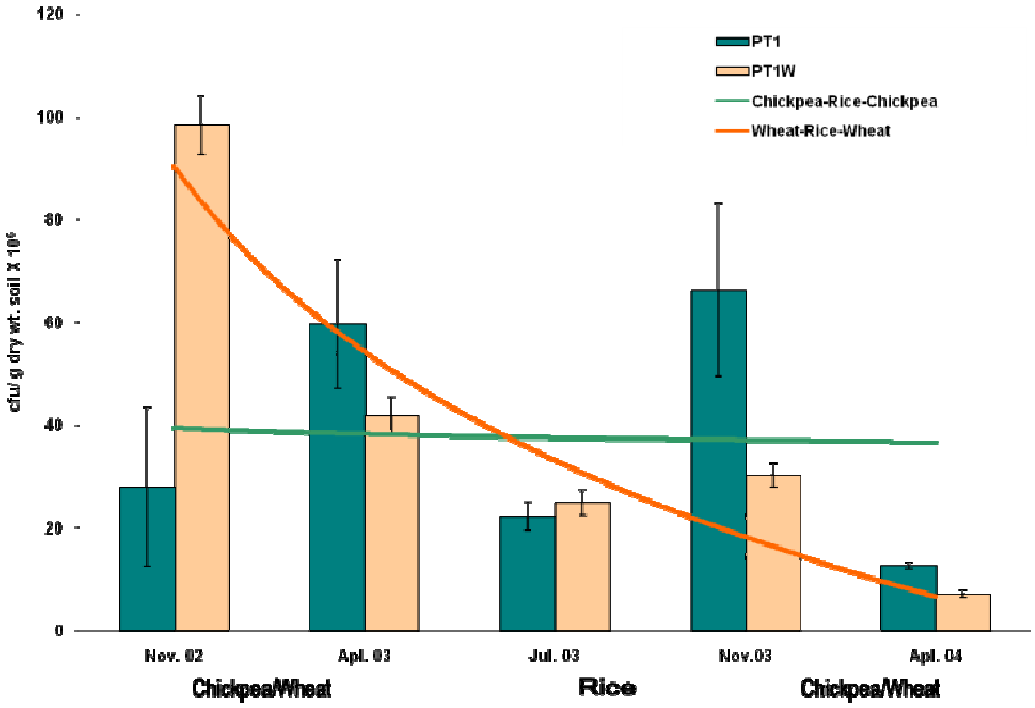


Fig 5.13: Cellulose degraders population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Patiala during three cropping seasons. Soil samples were collected from Patiala and cellulose degraders were enumerated (Section 5.2.1)

chickpea-rice-chickpea cropping pattern was 1.24 µM glucose/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 11.01 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then decreased to 9.04 µM glucose/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 21.52 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further decreased to 9.09 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but increased to 18.07 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field decreased the

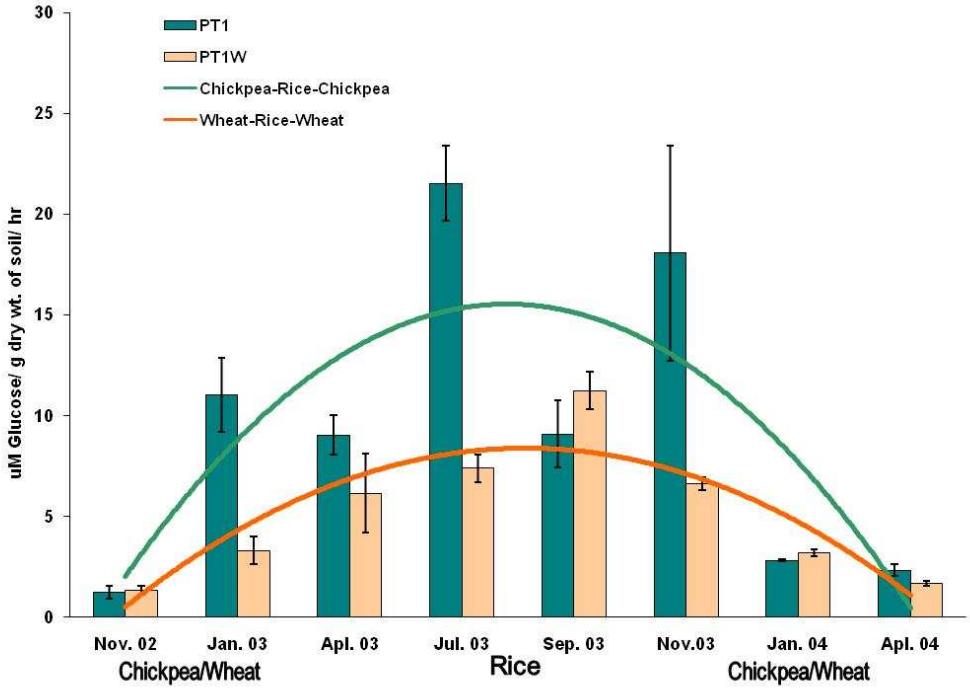


Fig 5.14: Cellulase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice wheat) at Patiala during three cropping seasons. Soil samples were collected from Patiala and cellulase activity was estimated (Section 5.2.1)

soil cellulase activity from 18.07 to 28.27 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 23.37 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The soil cellulase activity in PT-1W field at Patiala during wheat-rice-wheat cropping pattern was 1.33 µM glucose/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 3.32 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then highly further increased to 6.15 µM glucose/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 7.39 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 11.24 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003)

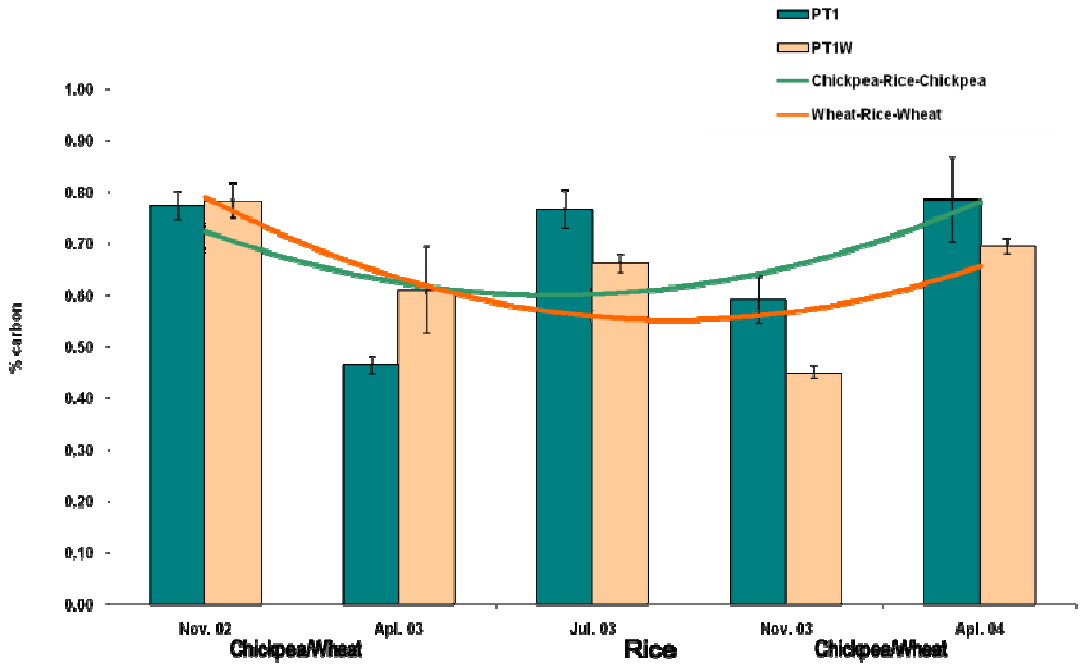


Fig 5.15: Organic carbon in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Patiala during three cropping seasons. Soil samples were collected from Patiala and organic carbon was estimated (Section 5.2.1)

but decreased to 6.62 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field increased the soil cellulase activity from 6.62 to 31.92 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 16.69 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Patiala shows an increased cellulase activity in soil during chickpea-rice-chickpea cropping pattern in PT-1 field as compared to wheat-rice-wheat cropping pattern where no change was found in cellulase activity at PT-1W field. As shown in Fig 5.15, the organic carbon in PT-1 field at Patiala during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was 0.77 (%) which increased to 0.47 (%) at the time of harvest (April

2003). Followed by sowing of rice (July 2003) the organic carbon was 0.77 (%) which increased to 0.59 (%) during the rice harvest (November 2003). In next cropping season sowing of chickpea further increased the organic carbon to 0.79 (%) at the time of harvest of chickpea (April 2004). The organic carbon in PT-1W field at Patiala during the wheat-rice-wheat cropping pattern was observed at the time of sowing (November 2002) of wheat to be 0.78 (%) which increased to 0.61 (%) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) organic carbon was decreased to 0.66 (%) which increased to 0.45 (%) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field further increased the organic carbon to 0.70 (%) at the time of harvest of wheat (April 2004). The overall trend at Patiala in different cropping patterns showed increased organic carbon in chickpea-rice-chickpea cropping pattern at PT-1 field as compared to wheat-rice-wheat cropping pattern at PT-1W field.

5.2.2 Study area: Rajpura

As shown in Fig 5.16, the cellulose degraders population in RJ-1 field at Rajpura during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was $5x10^6$ (cfu / g dry wt. of soil) which increased to $33x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the population was $20x10^6$ (cfu / g dry wt. of soil) which increased to $42x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of chickpea further decreased the cellulose degraders population to $14x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The cellulose degraders population in RJ-1W field at Rajpura during the wheat-rice-wheat cropping

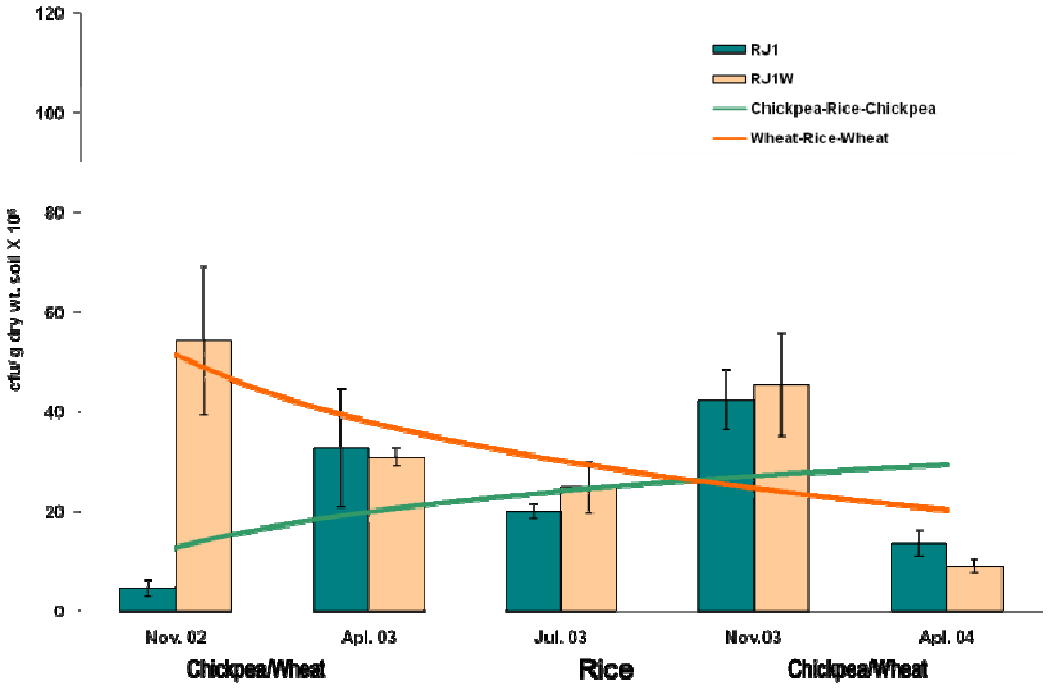


Fig 5.16: Cellulose degraders population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. Soil samples were collected from Rajpura and cellulose degraders were enumerated (Section 5.2.2)

pattern was observed at the time of sowing (November 2002) of wheat to be $54x10^6$ (cfu / g dry wt. of soil) which decreased to $31x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was slightly decreased to $25x10^6$ (cfu / g dry wt. of soil) which increased to $45x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field decreased the population to $9x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Rajpura in different cropping patterns showed decreased cellulose degraders population in wheat-rice-wheat cropping pattern in RJ-1W field with no overall significant change in chickpea-rice-chickpea cropping

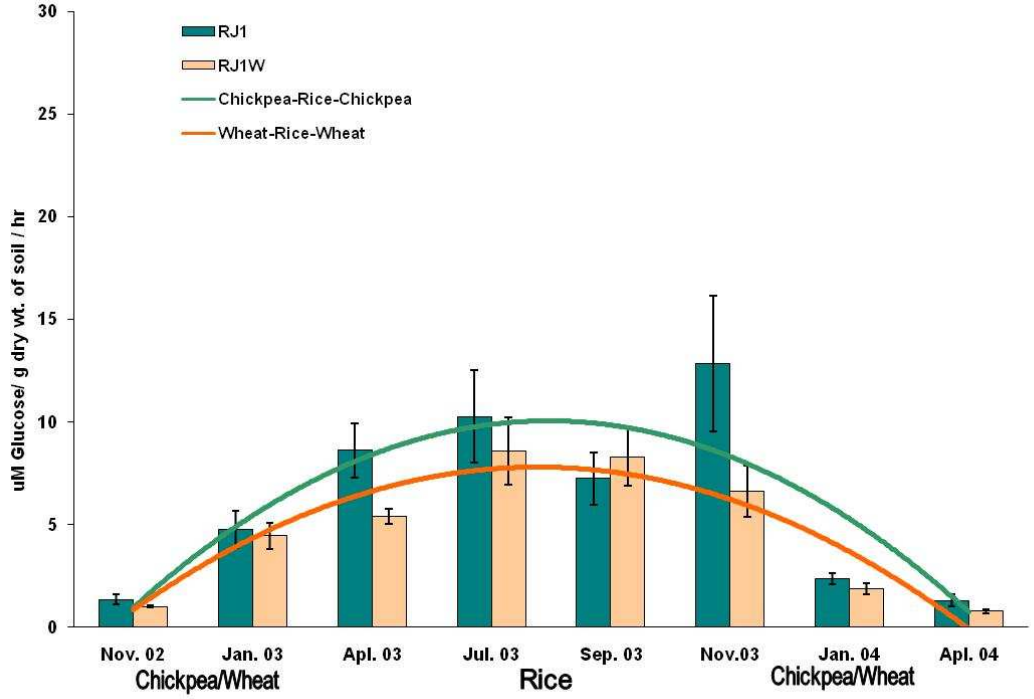


Fig 5.17: Cellulase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. Soil samples were collected from Rajpura and cellulase activity was estimated (Section 5.2.2)

pattern at RJ-1field. As shown in Fig 5.17, the soil cellulase activity in RJ-1 field at Rajpura during chickpea-rice-chickpea cropping pattern was 1.35 µM glucose/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 4.77 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then decreased to 8.61 µM glucose/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 10.26 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further decreased to 7.24 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but increased to 12.85 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). Innext cropping season sowing of chickpea in the same field decreased

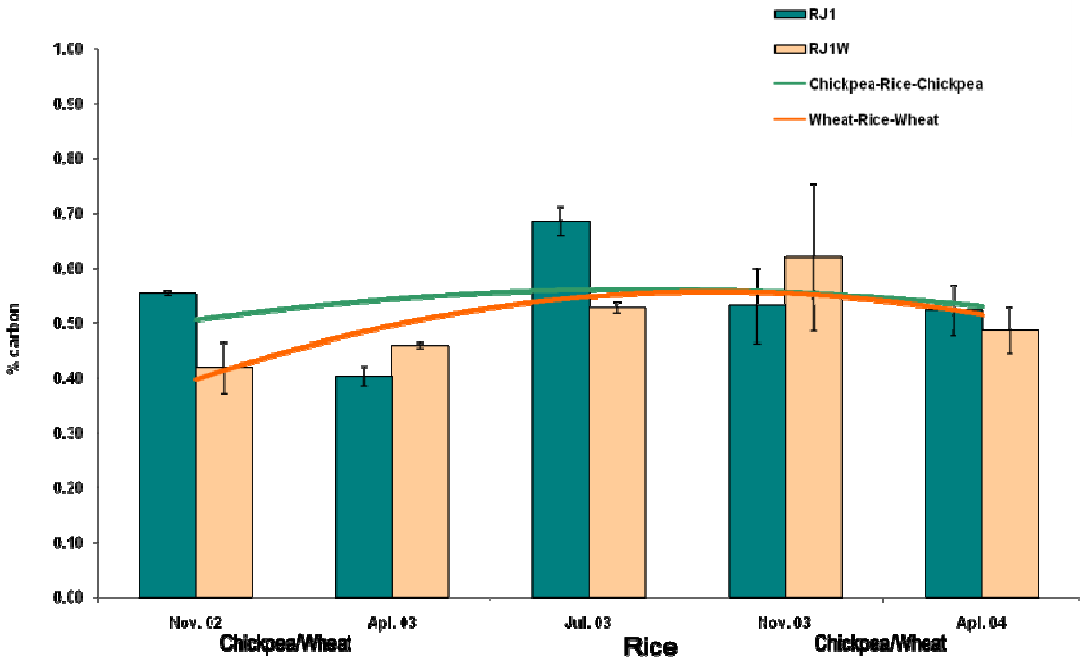


Fig 5.18: Organic carbon in different cropping pattern (Chickpea-Rice-Chickpea and Wheat-rice-wheat) at Rajpura during three cropping seasons. Soil samples were collected from Rajpura and organic carbon was estimated (Section 5.2.2)

the soil cellulase activity from 12.85 to 23.51 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 13.15 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The soil cellulase activity in RJ-1W field at Rajpura during wheat-rice-wheat cropping pattern was 1.02 µM glucose/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 4.45 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then highly further increased to 5.39 µM glucose/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 8.60 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 8.31 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but decreased to 6.61 µM glucose/ g dry wt. of soil/ hr of soil at

harvest (November 2003). In next cropping season sowing of chickpea in the same field increased the soil cellulase activity from 6.61 to 18.76 μM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 7.82 μM glucose/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Rajpura shows an increased cellulase activity in soil during chickpea-rice-chickpea cropping pattern in RJ-1field as compared to wheat-rice-wheat cropping pattern where no change was found in cellulase activity at RJ-1W field. As shown in Fig 5.18, the organic carbon in RJ-1field at Rajpura during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was 0.55 (%) which increased to 0.40 (%) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the organic carbon was 0.68 (%) which increased to 0.53 (%) during the rice harvest (November 2003). In next cropping season sowing of chickpea further increased the organic carbon to 0.52 (%) at the time of harvest of chickpea (April 2004). The organic carbon in RJ-1W field at Rajpura during the wheat-rice-wheat cropping pattern was observed at the time of sowing (November 2002) of wheat to be 0.42 (%) which increased to 0.46 (%) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) organic carbon was decreased to 0.53 (%) which increased to 0.62 (%) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field further increased the organic carbon to 0.49 (%) at the time of harvest of wheat (April 2004). The overall trend at Rajpura in different cropping patterns showed increased organic carbon in chickpea-rice-chickpea cropping pattern at RJ-1 field as well as in wheat-rice-wheat cropping pattern at RJ-1W field.

5.2.3 Study area: Derabassi

As shown in Fig 5.19, the cellulose degraders population in DB-1 field at Derabassi during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was $64x10^6$ (cfu / g dry wt. of soil) which increased to $65x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the population was $34x10^6$ (cfu / g dry wt. of soil) which increased to $32x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of chickpea further decreased the cellulose degraders population to $16x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The cellulose degraders population in DB-1W field at Derabassi during the wheat-rice-wheat cropping pattern was observed at the time of sowing (November 2002) of wheat to be $69x10^6$ (cfu / g dry wt. of soil) which decreased to $16x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was slightly decreased to $20x10^6$ (cfu / g dry wt. of soil) which increased to $23x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field decreased the population to $35x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Derabassi in different cropping patterns showed decreased cellulose degraders population in wheat-rice-wheat cropping pattern in DB-1W field with no overall significant change in chickpea-rice-chickpea cropping pattern at DB-1field. As shown in Fig 5.20, the soil cellulase activity in DB-1 field at Derabassi during chickpea-rice-chickpea cropping pattern was 1.78 μM glucose/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 4.37 μM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then decreased to 4.67 μM glucose

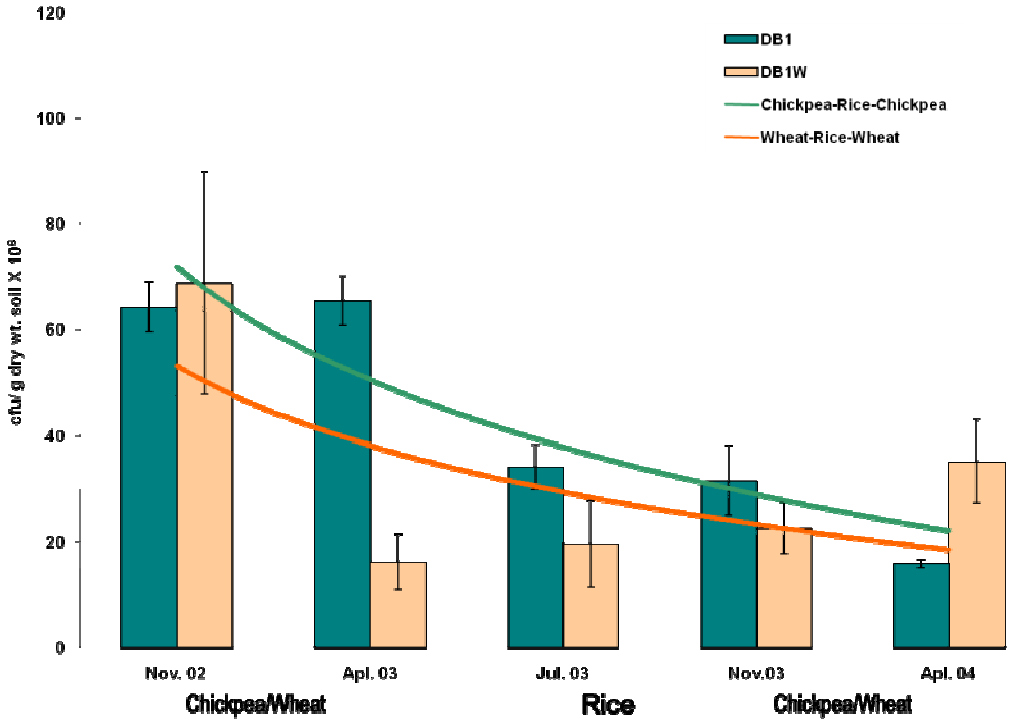


Fig 5.19: Cellulose degraders population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. Soil samples were collected from Derabassi and cellulose degraders were enumerated (Section 5.2.3)

g^{-1} dry wt. soil h^{-1} at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 4.66 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further decreased to 9.94 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but increased to 5.36 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field decreased the soil cellulase activity from 5.36 to 51.08 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 56.09 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The soil cellulase activity in DB-1W field at Derabassi during wheat-rice-wheat cropping pattern was 1.49 µM glucose/ g dry wt. of

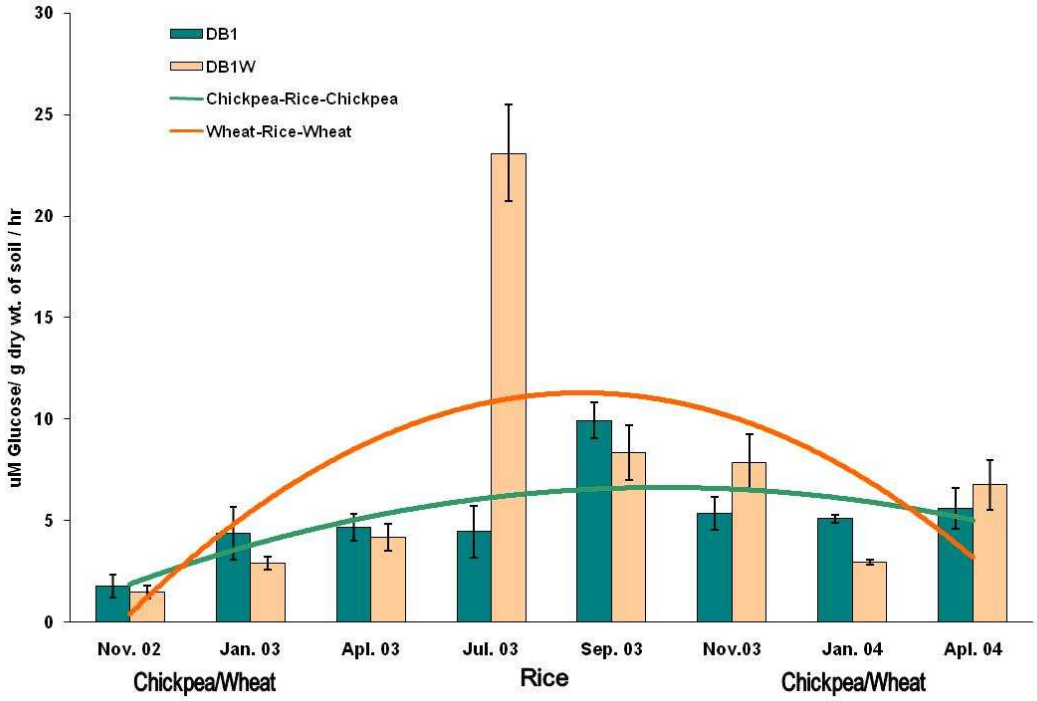


Fig 5.20: Cellulase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. Soil samples were collected from Derabassi and cellulase activity was estimated (Section 5.2.3)

soil/ hr at the time of sowing (November 2002), increased to 2.91 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then highly further increased to 4.19 µM glucose/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 23.10 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 8.36 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but decreased to 7.86 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field increased the soil cellulase activity from 7.86 to 29.62 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 67.68 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Derabassi shows

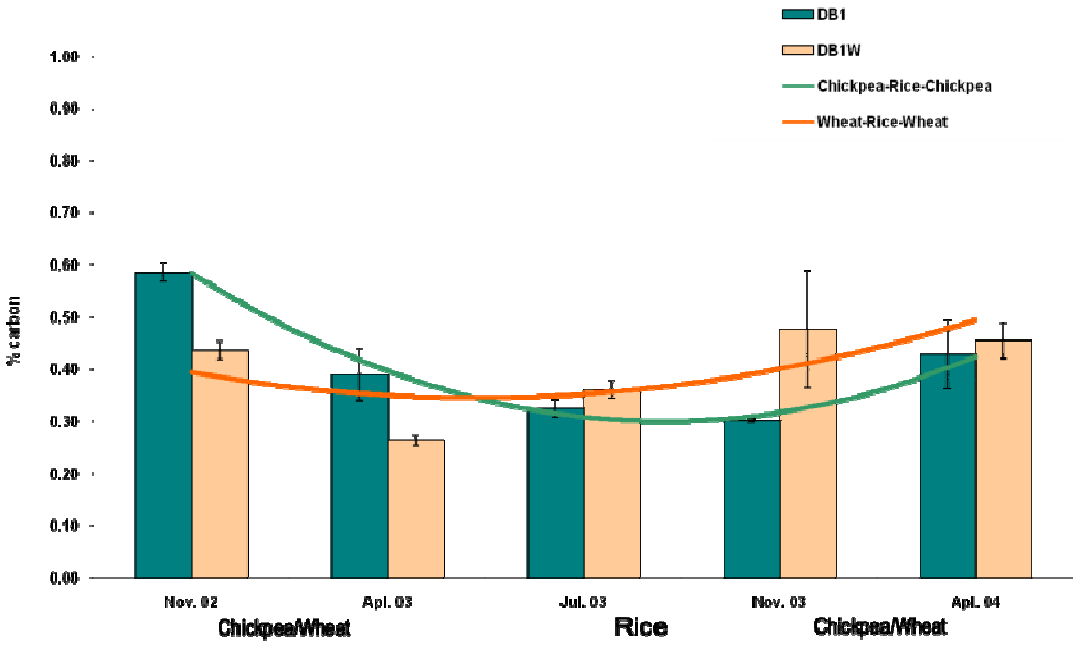


Fig 5.21 Organic carbon in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Derabassi during three cropping seasons. Soil samples were collected from Derabassi and organic carbon was estimated (Section 5.2.3)

an increased cellulase activity in soil during chickpea-rice-chickpea cropping pattern in DB-1 field as compared to wheat-rice-wheat cropping pattern where no change was found in cellulase activity at RJ-1W field. As shown in Fig 5.21, the organic carbon in DB-1field at Derabassi during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was 0.59 (%) which increased to 0.39 (%) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the organic carbon was 0.32 (%) which increased to 0.30 (%) during the rice harvest (November 2003). In next cropping season sowing of chickpea further increased the organic carbon to 0.43 (%) at the time of harvest of chickpea (April 2004). The organic carbon in DB-1W field at Derabassi during the wheat-rice-wheat cropping pattern was

observed at the time of sowing (November 2002) of wheat to be 0.44 (%) which increased to 0.26 (%) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) organic carbon was decreased to 0.36 (%) which increased to 0.48 (%) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field further increased the organic carbon to 0.46 (%) at the time of harvest of wheat (April 2004). The overall trend at Derabassi in different cropping patterns showed increased organic carbon in chickpea-rice-chickpea cropping pattern at DB-1field as well as in wheat-rice-wheat cropping pattern at DB-1W field.

5.2.4 Study area: Samana

As shown in Fig 5.22, the cellulose degraders population in SM-5 field at Samana during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was $5x10^6$ (cfu / g dry wt. of soil) which increased to $74x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the population was $18x10^6$ (cfu / g dry wt. of soil) which increased to $12x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of chickpea further decreased the cellulose degraders population to $25x10^6$ (cfu / g dry wt. of soil) at the time of harvest of chickpea (April 2004). The cellulose degraders population in SM-5W field at Samana during the wheat-rice-wheat cropping pattern was observed at the time of sowing (November 2002) of wheat to be $26x10^6$ (cfu / g dry wt. of soil) which decreased to $27x10^6$ (cfu / g dry wt. of soil) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) the population was slightly decreased to $14x10^6$ (cfu / g dry wt. of soil) which increased to $16x10^6$ (cfu / g dry wt. of soil) during the rice harvest (November 2003). In next cropping season sowing of wheat

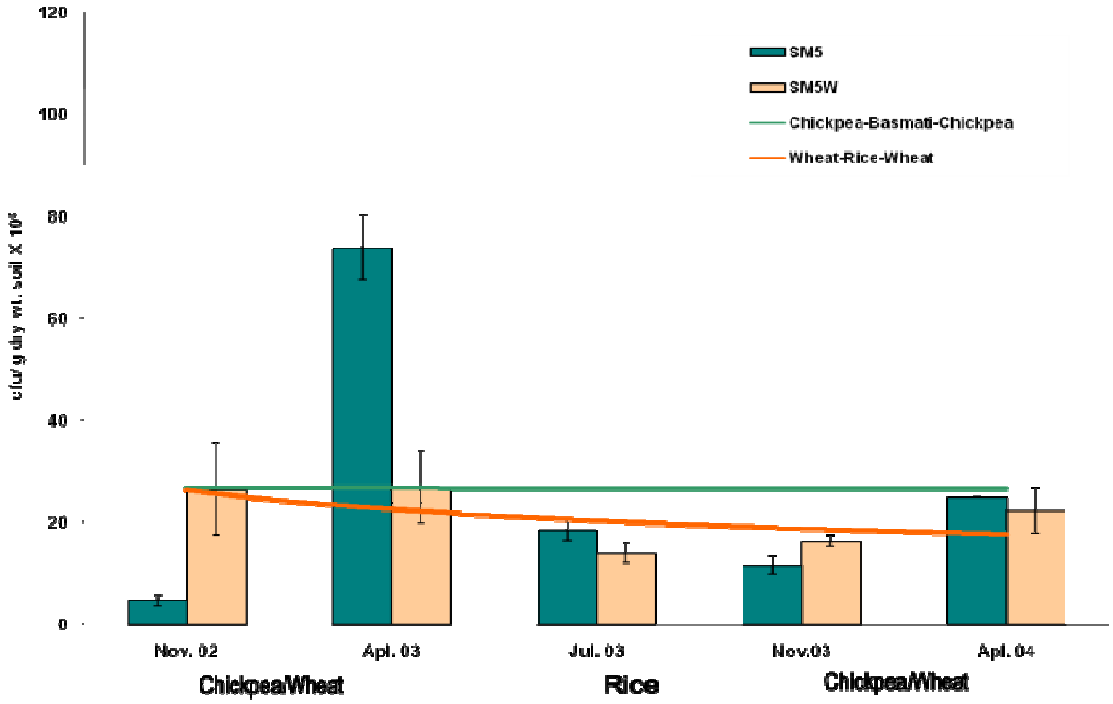


Fig 5.22: Cellulose degraders population in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping seasons. Soil samples were collected from Samana and cellulose degraders were enumerated (Section 5.2.4)

in the same field decreased the population to $22x10^6$ (cfu / g dry wt. of soil) at the time of harvest of wheat (April 2004). The overall trend at Samana in different cropping patterns showed decreased cellulose degraders population in wheat-rice-wheat cropping pattern in SM-5W field with no overall significant change in chickpea-rice-chickpea cropping pattern at SM-5field. As shown in Fig 5.23, the soil cellulase activity in SM-5 field at Samana during chickpea-rice-chickpea cropping pattern was 0.59 µM glucose/ g dry wt. of soil/ hr at the time of sowing (November 2002), increased to 3.65 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then decreased to 2.75 µM glucose/ g dry wt. of soil/ hr

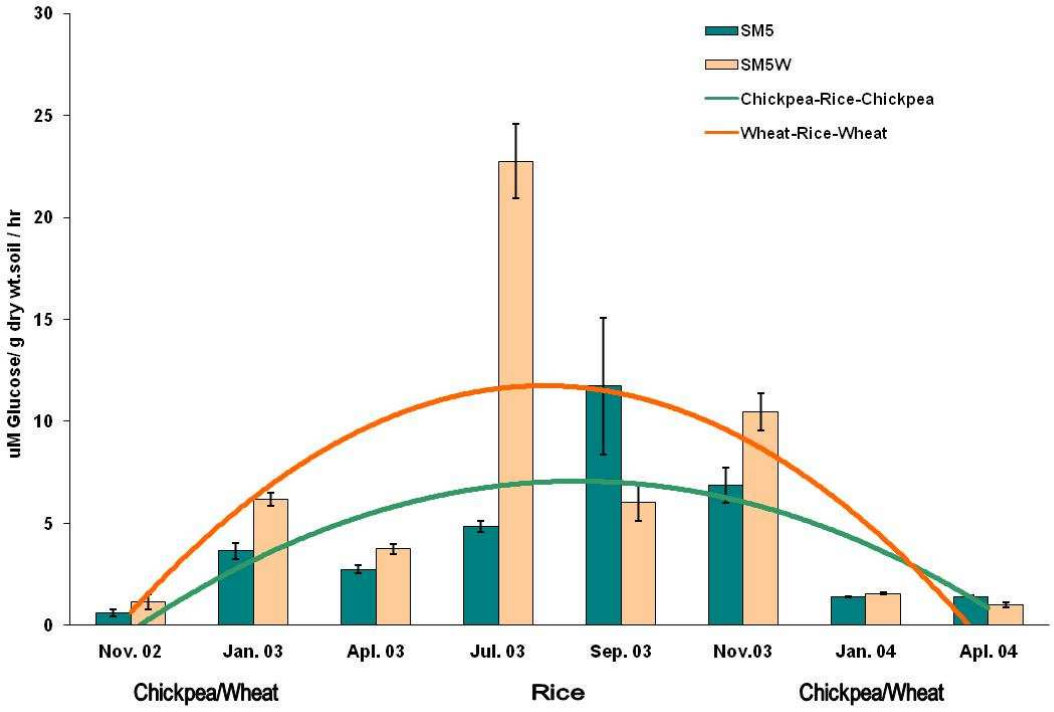


Fig 5.23: Cellulase activity in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping seasons. Soil samples were collected from Samana and cellulase activity was estimated (Section 5.2.4)

h^{-1} at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 4.84 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further decreased to 11.73 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but increased to 6.86 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field decreased the soil cellulase activity from 6.86 to 13.79 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 14.11 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The soil cellulase activity in SM-5W field at Samana during wheat- rice-wheat cropping pattern was 1.16 µM glucose/ g dry wt. of soil/ hr at

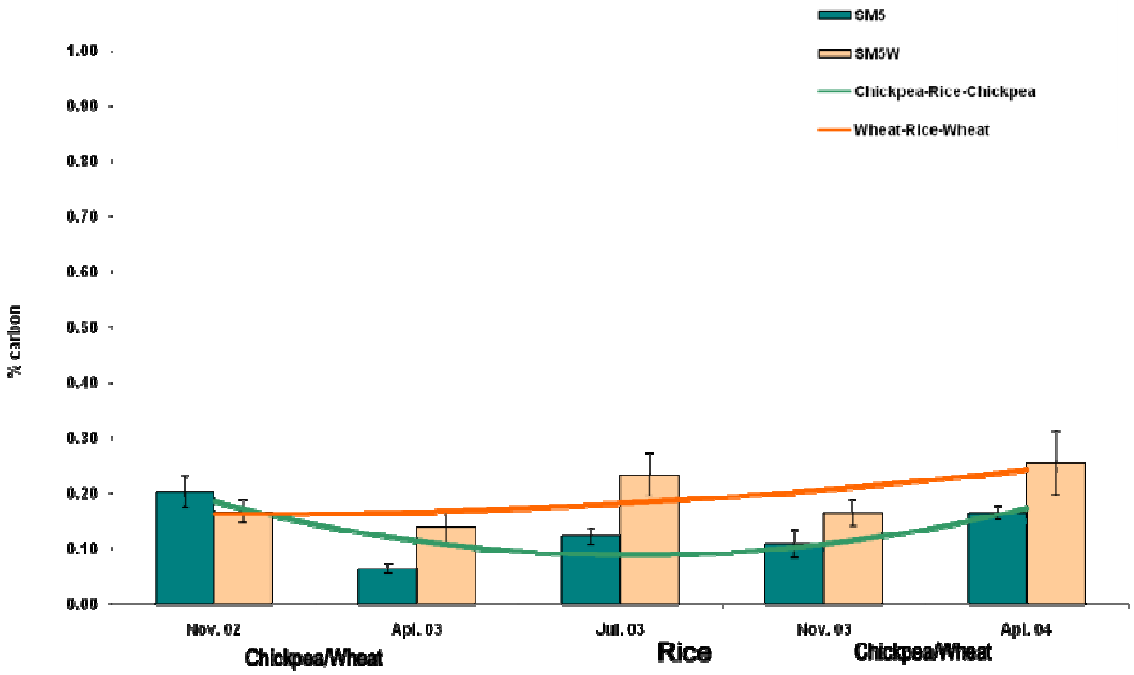


Fig 5.24: Organic carbon in different cropping pattern (Chickpea-rice-chickpea and Wheat-rice-wheat) at Samana during three cropping seasons. Soil samples were collected from Samana and organic carbon was estimated (Section 5.2.4)

the time of sowing (November 2002), increased to 6.19 µM glucose/ g dry wt. of soil/ hr at flowering (January 2003) and then highly further increased to 3.75 µM glucose/ g dry wt. of soil/ hr at the time of harvest (April 2003). Rice cropping in the same field stimulated cellulase activity to 22.75 µM glucose/ g dry wt. of soil/ hr during the sowing (July 2003) and which further increased to 6.02 µM glucose/ g dry wt. of soil/ hr at flowering (September 2003) but decreased to 10.48 µM glucose/ g dry wt. of soil/ hr of soil at harvest (November 2003). In next cropping season sowing of chickpea in the same field increased the soil cellulase activity from 10.48 to 15.68 µM glucose/ g dry wt. of soil/ hr during sowing to flowering and with the increased activity of 10.11 µM glucose/ g dry wt. of soil/ hr at harvesting of crop. The overall trend at Samana shows an increased cellulase activity in soil during chickpea-rice-chickpea cropping pattern in SM-

5 field as compared to wheat-rice-wheat cropping pattern where no change was found in cellulase activity at SM-5W field. As shown in Fig 5.24, the organic carbon in SM-5field at Samana during the chickpea-rice-chickpea cropping pattern was observed at the time of sowing (November 2002) of chickpea was 0.20 (%) which increased to 0.06 (%) at the time of harvest (April 2003). Followed by sowing of rice (July 2003) the organic carbon was 0.12 (%) which increased to 0.11 (%) during the rice harvest (November 2003). In next cropping season sowing of chickpea further increased the organic carbon to 0.17 (%) at the time of harvest of chickpea (April 2004). The organic carbon in SM-5W field at Samana during the wheat-rice-wheat cropping pattern was observed at the time of sowing (November 2002) of wheat to be 0.17 (%) which increased to 0.14 (%) at the time of harvest (April 2003). Followed by the sowing of rice (July 2003) organic carbon was decreased to 0.23 (%) which increased to 0.16 (%) during the rice harvest (November 2003). In next cropping season sowing of wheat in the same field further increased the organic carbon to 0.25 (%) at the time of harvest of wheat (April 2004). The overall trend at Samana in different cropping patterns showed increased organic carbon in chickpea-rice-chickpea cropping pattern at SM-5field as well as in wheat-rice-wheat cropping pattern at SM-5W field.

5.3 Influence of diversified cropping pattern on microbial activities and population dynamics in agricultural soil.

Comparative field studies were conducted at Village: Khera Gajju in Rajpura in field conditions using prescribed package of practices. The soil at the site was silt loam (26.91% sand, 54.69% silt and 18.40% clay) soil with organic carbon, total nitrogen and available phosphorus of 0.50±0.02 %, 0.014±0.005 % and 35.52±2.48 mg/kg respectively. Either Chickpea (*Cicer arietinum* L.), wheat (*Triticum* spp.), sunflower (*Helianthus annuus*) were grown during rabi season which was followed by rice (*Oryza sativa*) during kharif season. Therefore the three different cropping patterns were chickpea-rice, wheat-rice and sunflower-rice. The total microbial population observed at the time of sowing of chickpea was $39x10^6$ which increased to 1.49 $x10^8$ at the harvest. With Rice in the same field the population at sowing was 58 $x10^6$ which increased to 87 $x10^6$ during rice harvest. In the other field with wheat at time of sowing, the total microbial population was 94 $x10^6$ which decreased to 62 $x10^6$ during the harvest of the crop. With Rice in the same field the population at sowing was 117 $x10^6$ which then decreased to 99 $x10^6$ at harvest time of rice. In the corresponding field of sunflower at the time of sowing the total microbial population was 102 $x10^6$ which decreased to 70 $x10^6$ during the harvest. With rice in the same field the population was 71 $x10^6$ at sowing while at the time of harvest it was 73 $x10^6$ (Fig. 5.25, 5.26 and 5.27). The overall trend in different cropping patterns as observed showed significant increase in total microbial population in chickpea-rice cropping pattern as compared to decrease in both sunflower-rice and wheat-rice cropping pattern. Cellulose degrading population during the chickpea sowing was 4.6 $x10^6$ which increased to 32 $x10^6$ at the time of crop harvest. Sowing of

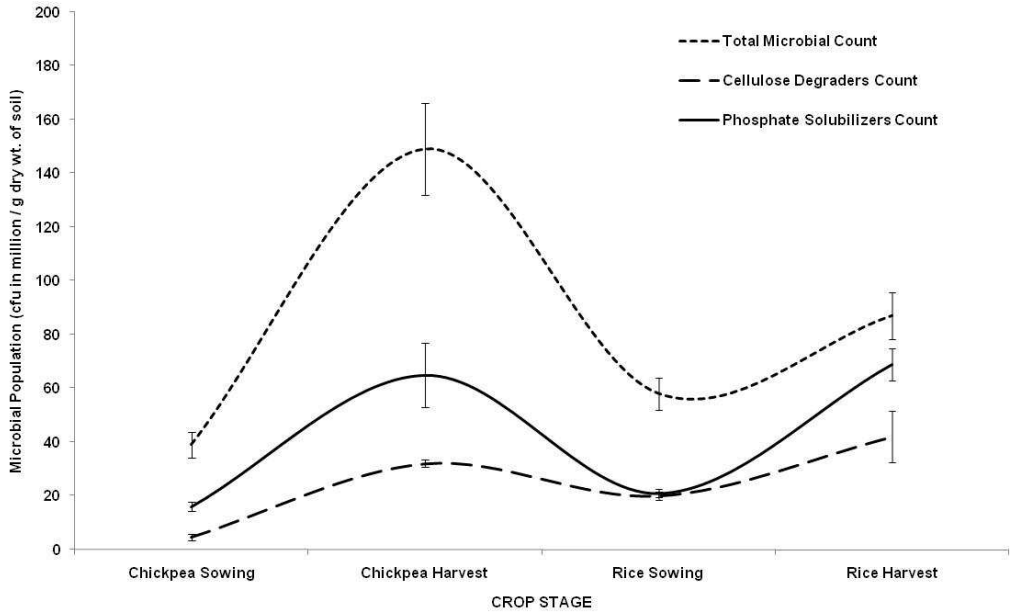


Fig 5.25: Population dynamics in chickpea-rice cropping pattern at various stages of crop growth. Total microbial count, cellulose degraders and phosphate solubilizers were estimated at different stages of chickpea and rice crop, as described in section 5.3.

rice in the same field showed an increase in cellulose population to 42 $x10^6$ during the rice crop. In the other field of wheat cellulose degrading population was 54 $x10^6$ at sowing which decreased to 30 $x10^6$ at harvest time and then increased to 45 $x10^6$ when rice was grown in these fields. With sunflower the cellulose degrading population at sowing was 17 $x10^6$, and increased to 35 $x10^6$ at harvest time. Sowing of rice in the same field decreased the population to 22 $x10^6$ at rice harvest (Fig. 5.25, 5.26 and 5.27). The overall trend in different cropping patterns as observed showed an increase in cellulose degrading population in all the three chickpea-rice, sunflower-rice and wheat-rice cropping pattern but the increase was not significant at various stages of crop growth. Phosphate solubilizers population showed an increase from 16 $x10^6$ to 65 $x10^6$ during the chickpea growth. Growth of rice in the same field did not increase the phosphate solubilizers population, it remaining at 69 $x10^6$ at harvest of rice. In fields where wheat

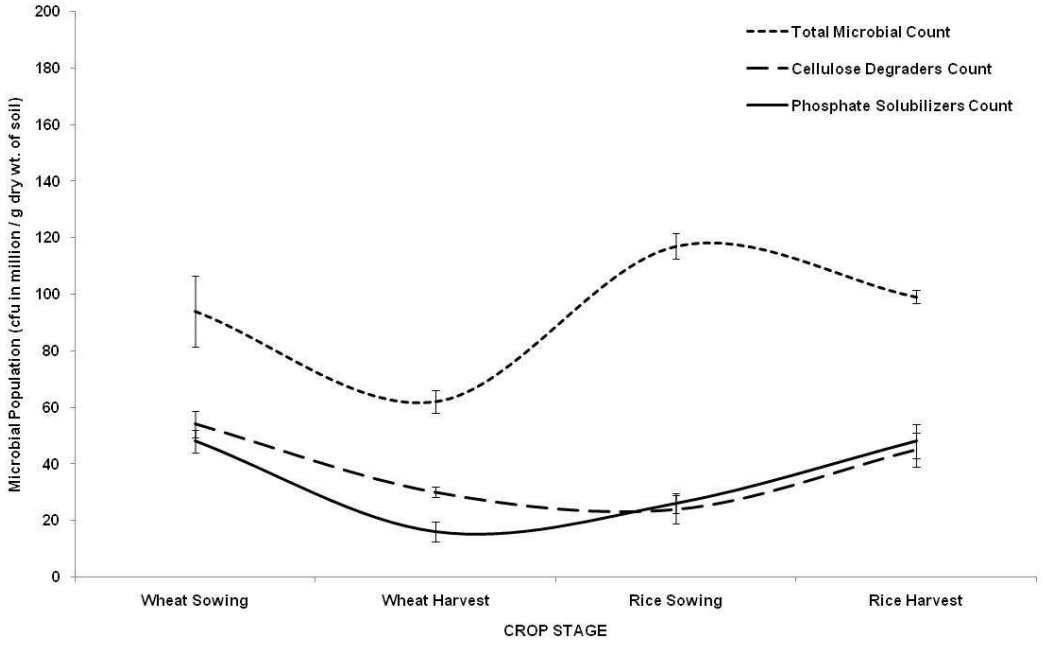


Fig 5.26: Population dynamics in wheat-rice cropping pattern at various stages of crop growth. Total microbial count, cellulose degraders and phosphate solubilizers were estimated at different stages of wheat and rice crop, as described in section 5.3.

was grown phosphate solubilizers population decreased from 48 $x10^6$ at the time of sowing to 16 $x10^6$ at harvest. Growth of rice did not change the population much with an increase to 48 $x10^6$ at rice harvesting. In sunflower –rice combination phosphate solubilizers population decreased from 50 $x10^6$.at the time of sowing of sunflower to 8 $x10^6$ at sunflower harvest. Rice growth in the same field increased the population to 32 $x10^6$ at rice harvest. (Fig. 5.25, 5.26 and 5.27). The results showed the increase in phosphate solubilizers population in chickpea-rice as well as sunflower-rice cropping pattern as compared to decrease in wheat –rice cropping pattern. The overall trend in different cropping patterns showed no significant differences in the phosphate solubilizers population at each stage of crop growth. Our results showed that total

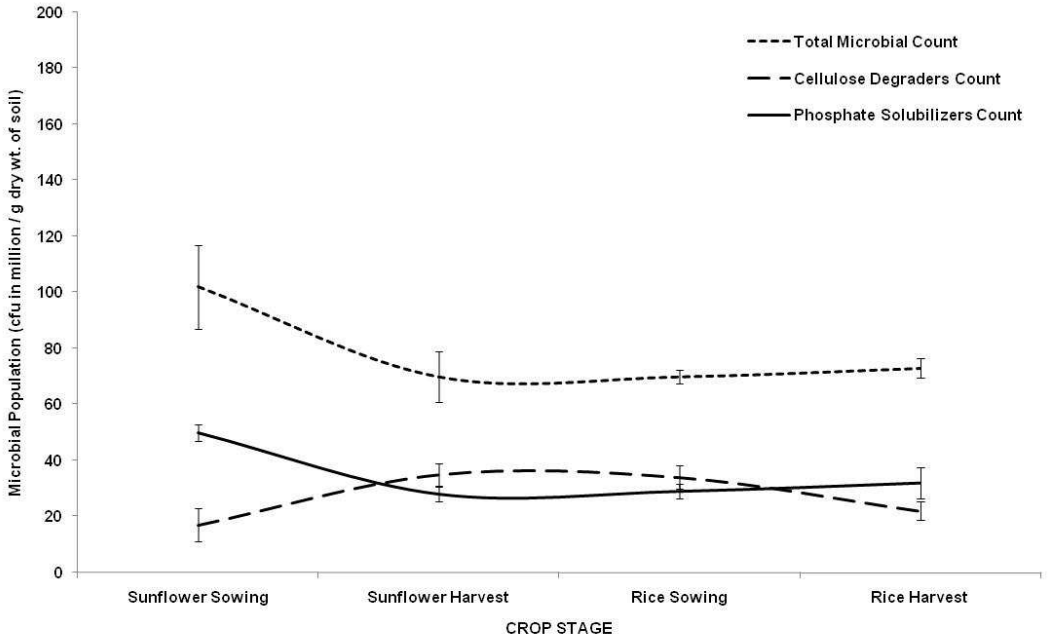


Fig 5.27: Population dynamics in sunflower-rice cropping pattern at various stages of crop growth. Total microbial count, cellulose degraders and phosphate solubilizers were estimated at different stages of sunflower and rice crop, as described in section 5.3.

microbial count was promoted significantly in chickpea-rice grown field as compared toother treatments, which showed its dependence on its physical, chemical, microbiological and biochemical properties. The soil phosphatase activity during chickpea cropping was the highest at the time of sowing (0.89 μM PNP/ g dry wt. of soil/ hr), decreased at flowering (0.13 μM PNP/ g dry wt. of soil/ hr) and then increased slightly to 0.19 μM PNP/ g dry wt. of soil/ hr at harvest. Rice cropping in the same field stimulated phosphatase activity to 0.69 μM PNP/ g dry wt. of soil/ hr at sowing, increased to 1.55 μM PNP/ g dry wt. of soil/ hr at flowering but the decreased to 0.64 μM PNP/ g dry wt. of soil/ hr at harvest. In the wheat crop, the soil phosphatase activity varies from 0.65 to 0.34 μM PNP/ g dry wt. of soil/ hr at sowing and harvesting with

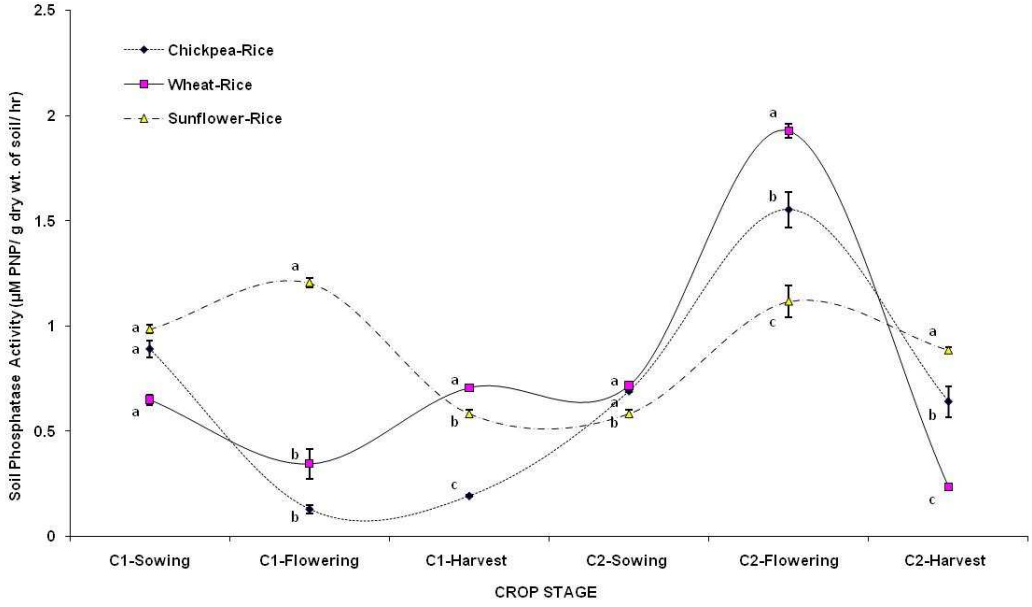


Fig 5.28: Soil Phosphatase activity in different cropping patterns at various stages of crop growth. Soil Phosphatase activity was estimated at different stages of various crops, as described in section 5.3. In Chickpea- Rice C1 is chickpea and C2 is rice, in Wheat-Rice C1 is wheat and C2 is Rice while in Sunflower-Rice C1 is sunflower and C2 is Rice.

the activity of 0.70 µM PNP/ g dry wt. of soil/ hr during the flag-leaf of crop. When rice was grown in the same field after wheat, the phosphatase activity increased to 0.71 µM PNP/ g dry wt. of soil/ hr at sowing, increased to 1.92 µM PNP/ g dry wt. of soil/ hr at midtime of crop but then decreased to 0.23 µM PNP/ g dry wt. of soil/ hr at rice harvest. With sunflower- rice cropping, the soil phosphatase activity increased with both sunflower and rice from 0.98 to 1.20 µM PNP/ g dry wt. of soil/ hr at sowing and flowering of sunflower and then decreasing to 0.58 µM PNP/ g dry wt. of soil/ hr at harvest. With rice also the activity decreased to 0.58 µM PNP/ g dry wt. of soil/ hr at sowing, increased to 1.11 µM PNP/ g dry wt. of soil/ hr at flowering of crop, but then decreased to 0.88 µM PNP/ g dry wt. of soil/ hr at harvest (Fig. 5.28). Among the three cropping patterns both chickpea-rice and wheat-rice showed increase in phosphatase

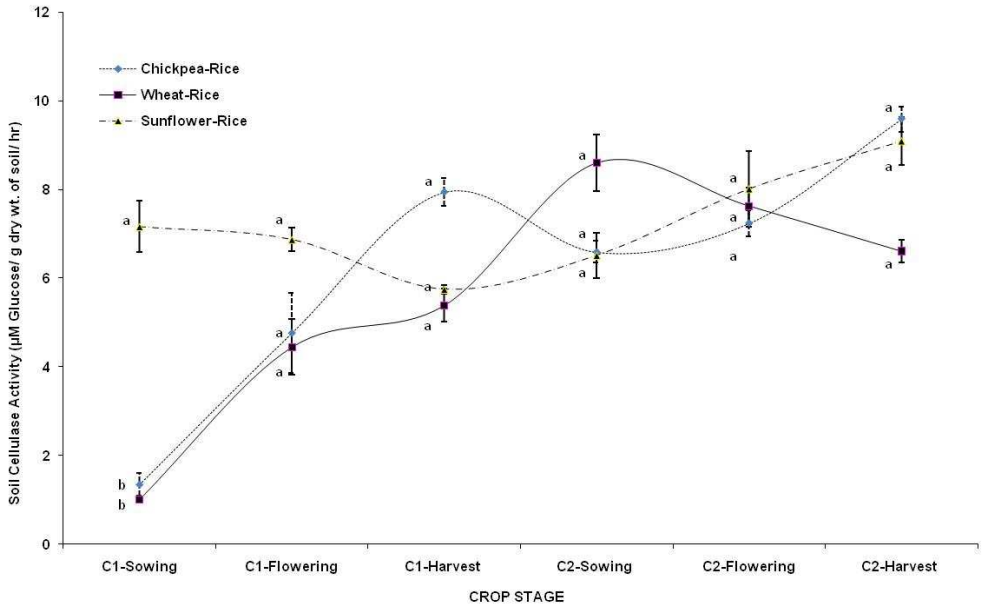


Fig 5.29: Soil cellulase activity in different cropping pattern at various stages of crop growth. Soil Cellulase activity was estimated at different stages of various crops, as described in section 5.3. In Chickpea- Rice C1 is chickpea and C2 is rice, in Wheat-Rice C1 is wheat and C2 is Rice while in Sunflower-Rice C1 is sunflower and C2 is Rice.

activity with rice crop while with sunflower-rice crops maximum phosphatase activity was at flowering in both sunflower and rice. Soil cellulase activity with chickpea-rice cropping showed an increase from sowing to harvest, increasing from 1.35 at sowing to 4.76 µM glucose/ g dry wt. of soil/ hr at flowering and then to 6.61 µM glucose/ g dry wt. of soil/ hr at harvest. When rice was sown after chickpea cellulose activity decreased from 10.29 µM glucose/ g dry wt. of soil/ hr at sowing to 7.24 µM glucose/ g dry wt. of soil/ hr at flowering and then increased to 12.84 µM glucose/ g dry wt. of soil/ hr at harvest. In wheat – rice cropping, with wheat cellulose activity showed an increase from 1.02 at sowing to 4.44 µM glucose/ g dry wt. of soil/ hr at flowering and then to 5.39 µM glucose/ g dry wt. of soil/ hr at harvest. Followed by rice, cellulose activity showed no increase from sowing to flowering (8.60 µM glucose/ g dry wt. of soil/ hr at sowing to

8.31 μM glucose/ g dry wt. of soil/ hr at flowering) and then decreased to 6.61 μM glucose/ g dry wt. of soil/ hr at harvest. During the sunflower cropping, the soil cellulase activity varied from 7.16 to 5.75 μM glucose/ g dry wt. of soil/ hr at sowing and maturity respectively with a transient increase at flowering (10.21 μM glucose/ g dry wt. of soil/ hr) In soils with rice crop after sunflower, cellulose activity showed continuous increase from sowing to flowering and then maturity respectively (6.51 μM glucose/ g dry wt. of soil/ hr to 8.35 to 10.76 μM glucose/ g dry wt. of soil/ hr). In all the three different cropping patterns, an increase in cellulose activity was observed with not much significant difference between various stages of growth (Fig. 5.29). Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme better so that it may be used more regularly as a predictive tool in our soil fertility programmes. During the above comparative field experiments, the overall scenario in the results showed as positive correlation with r-value of 0.766 among the soil cellulase activity and cellulose degraders population during the chickpea-rice cropping, as compared to wheat-rice and sunflower-rice with r-value of 0.655 and 0.354 respectively (Fig 5.30, 5.31 and 5.32). On the other hand no correlation was observed among the soil phosphatase and phosphate solubilisers population in all three cropping pattern of chickpea-rice, wheat-rice and sunflower-rice with r-value of 0.038, 0.007 and 0.002 respectively (Fig 5.33, 5.34 and 5.35).

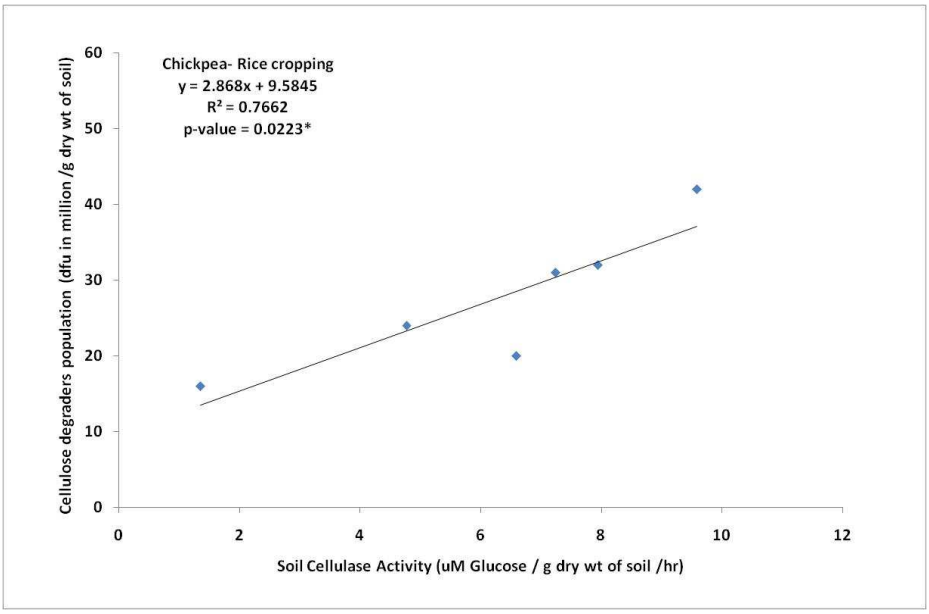


Fig 5.30: Correlation between the cellulose degraders population and soil cellulase activity during Chickpea-Rice rotation. Cellulose degrader population and cellulase activity were estimated as described in section 3.4 and 3.9.2. * significant at $p<0.05$

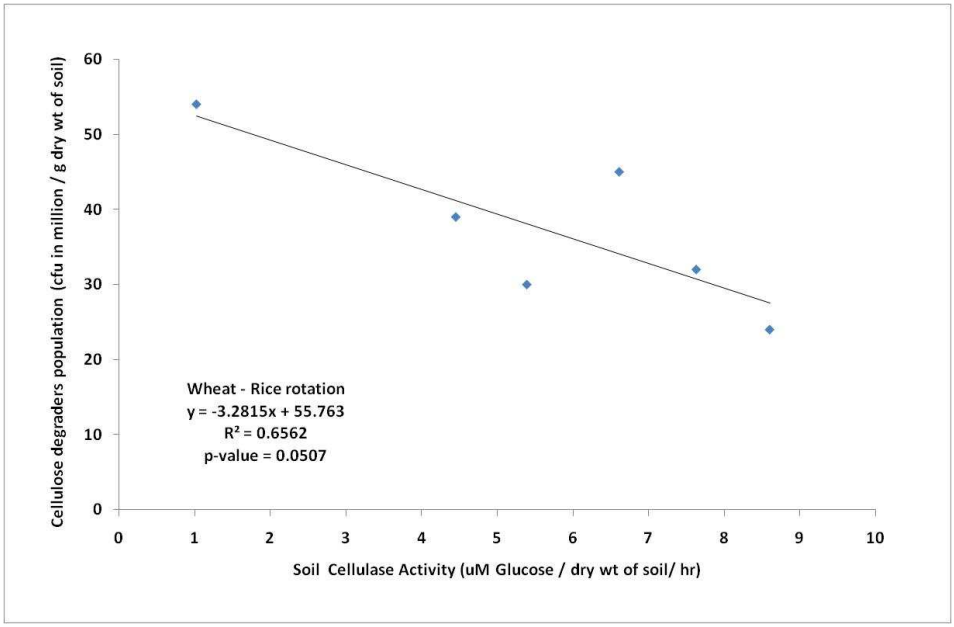


Fig 5.31: Correlation between the cellulose degraders population and soil cellulase activity during Wheat-Rice rotation. Cellulose degrader population and cellulase activity were estimated as described in section 3.4 and 3.9.2.

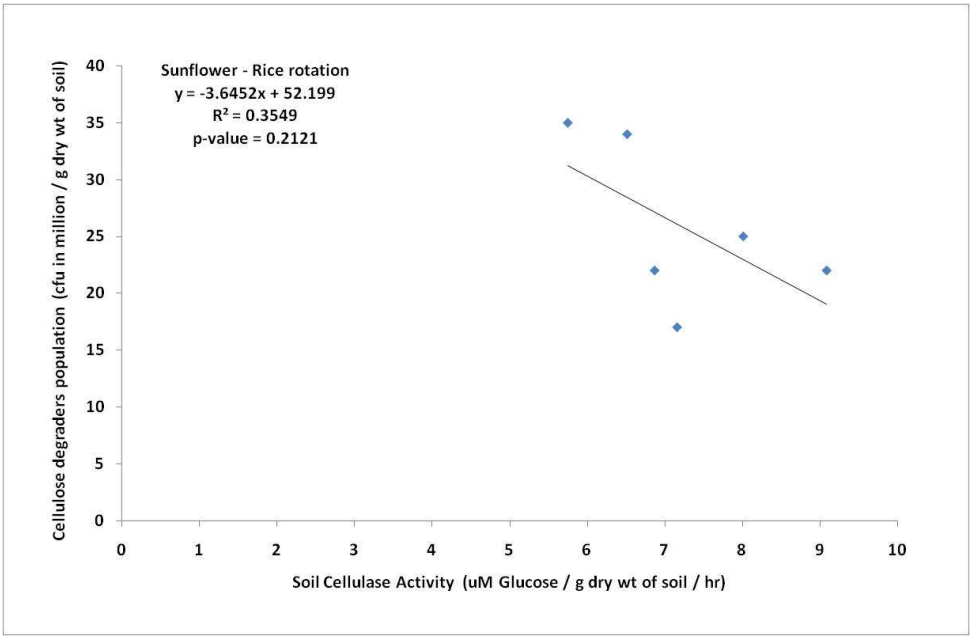


Fig 5.32: Correlation between the cellulose degraders population and soil cellulase activity during Sunflower-Rice rotation. Cellulose degrader population and cellulase activity were estimated as described in section 3.4 and 3.9.2.

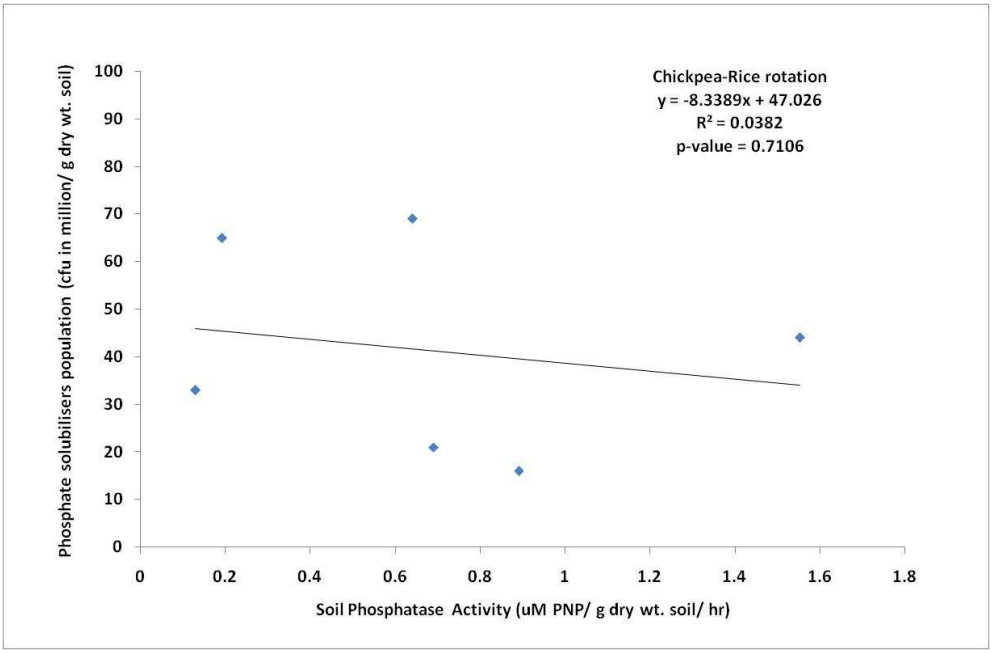


Fig 5.33: Correlation between the phosphate solubilisers population and soil phosphatase activity during Chickpea-Rice rotation. Phosphate solubilizers population and phosphatase activity were estimated as described in section 3.4 and 3.9.1.

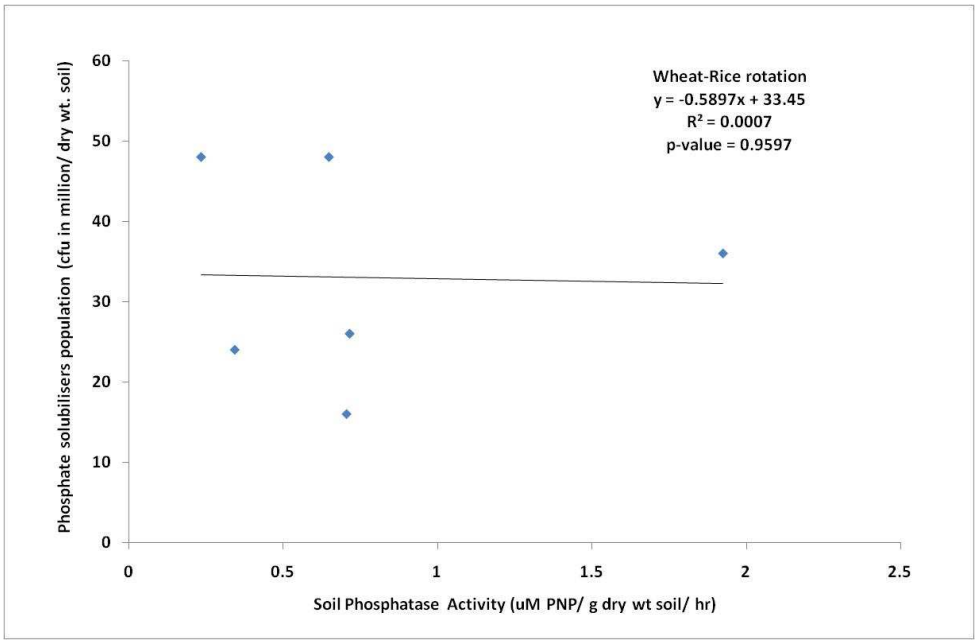


Fig 5.34: Correlation between the phosphate solubilisers population and soil phosphatase activity during Wheat-Rice rotation. Phosphate solubilizers population and phosphatase activity were estimated as described in section 3.4 and 3.9.1.

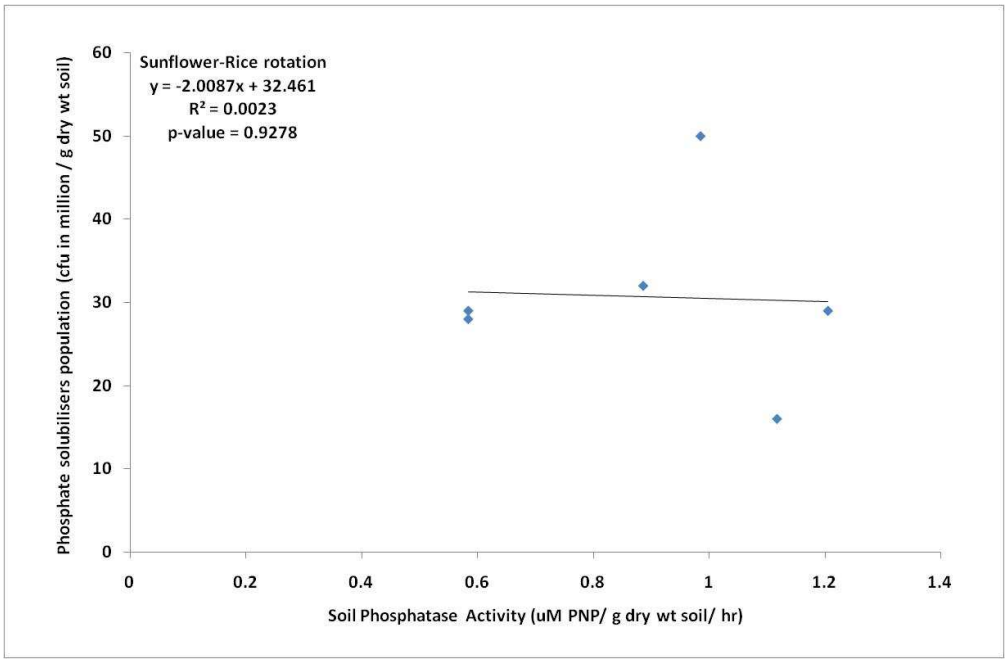


Fig 5.35: Correlation between the phosphate solubilisers population and soil phosphatase activity during Sunflower-Rice rotation. Phosphate solubilizers population and phosphatase activity were estimated as described in section 3.4 and 3.9.1.

Chapter 6

Development of molecular marker for population dynamics of the cellulose degrading microbes

DEVELOPMENT OF MOLECULAR MARKER FOR POPULATION DYNAMICS OF THE CELLULOSE DEGRADING MICROBES

6.1 Development of molecular markers for tracking the fate of bacteria

Genotypic relationships among microorganisms have been determined by analyzing the genomic DNA with PCR-based methods. Bacterial strains can be differentiated by DNA fingerprints based on ERIC (enterobacterial repetitive intergenic consensus)-PCR. ERIC-PCR has been used successfully to generate DNA fingerprints to distinguish between genetically unrelated isolates and closely related bacterial strains. DNA fingerprinting based on the ERIC-PCR of these isolates showed 10 to 14 amplified bands with sizes ranging from 11,000 bp to 100 bp of varying intensity (Fig. 6.1a). DNA fingerprints of various isolates were compared with inoculated bacterial strains. In the present study, unique ERIC PCR genotypic fingerprints of different strains isolated from agricultural soil samples were standardized and used for the enumeration of the introduced cellulolytic bacterial isolates (Fig. 6.1a). Survival of inoculated bacteria was estimated based on their ERIC-PCR banding pattern similarity (Fig. 6.1b).

6.2 Survival studies of bacterial strains inoculated in Chickpea plot trails

The cellulolytic bacteria *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 were resistant to ampicillin, thus ampicillin was selected as marker for population survival along with ERIC-PCR method. In plot studies, ampicillin resistance plate count of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 showed 15, 13 and15 ($x10^7$cfu / g dry wt. soil) respectively at the time of

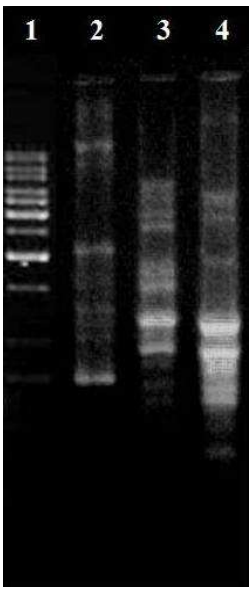


Figure 6.1-a. ERIC-PCR fingerprint of unknown bacterial isolates on 1.5% agarose gel. Bacterial colonies were isolated from soil in the plots. Pure colonies were picked, heat lysed, and whole genomic DNA was subjected to ERIC-PCR to get the fingerprint. **Lane 1:** 1Kb ladder **Lane 2:** Standard bacterial strain of *Pseudomonas* sp. MSK13, **Lane 3:** Standard bacterial strain of *Serratia* sp. MSK1, **Lane 4:** Standard bacterial strain of *Serratia* sp. MSK24.

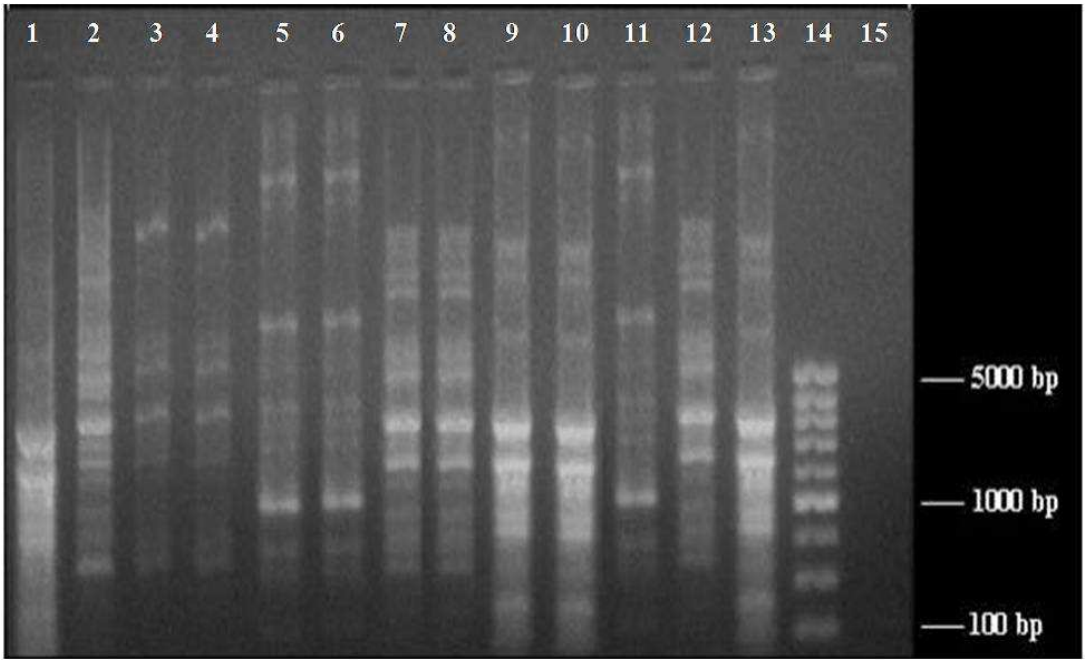


Figure 6.1-b ERIC-PCR fingerprint of unknown bacterial isolates on 1.5% agarose gel. Bacterial colonies were isolated from soil in the plots. Pure colonies were picked, heat lysed, and whole genomic DNA was subjected to ERIC-PCR to get the fingerprint. **Lanes 1-10:** fingerprints of different isolates obtained from selected plates containing 120ug/ml of Ampicillin, **Lane 11:** Standard bacterial strain of *Pseudomonas* sp. MSK13, **Lane 12:** Standard bacterial strain of *Serratia* sp. MSK1, **Lane 13:** Standard bacterial strain of *Serratia* sp. MSK24, **Lane 14:** 500bp ladder and **Lane 15:** Negative Control.

inoculation, the population decreased gradually to 10, 8.8 and 11.5 ($x10^7$cfu / g dry wt. soil) during 8th week or midtime of crop growth for *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 respectively which further decreased to 8.0, 6.0 and 9.0 ($x10^7$cfu / g dry wt. soil) at the time of crop harvest (Table 6.1). Microbial population based on LA + ampicillin plates in control plot decreased from 2.2 to 1.3 ($x10^7$cfu / g dry wt. soil) after 16 week of chickpea harvest. Population dynamic studies based on ERIC-PCR showed that population of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 were 11.7, 10.2 and 13.4 ($x10^7$cfu / g dry wt. soil) at the time of inoculation during germinating or 2-3 leaflet stage of chickpea, and then decreased to 4.7, 3.5 and 7.6 ($x10^7$cfu / g dry wt. soil) at the end of 16th week. (Table 6.1) thus showing a survival of 40.2, 34.4 and 56.8 % respectively after 16 weeks (Table 6.1). *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 with soil cellulase activity of 7.51, 9.02 and 10.25 respectively, these could not be detected in the soil at zero time (before application of the bacterial inoculums) and in control as well as test plots. These results clearly demonstrated that enumeration of the introduced cellulolytic bacterial isolates by antibiotic resistance method always showed higher level of survival as compared to that by ERIC-PCR. Since ERIC-PCR enumerates the population of the introduced bacterial isolates without any interference from other bacteria, this method presents a very accurate estimation of the survival of the introduced bacteria. The various soil parameters such as population of cellulose degraders as well as phosphate solubilisers, cellulase activity, phosphatase activity, organic carbon, total nitrogen content and available phosphorus in the experimental plots was also observed and it found to be enhanced by the introduction of the inoculants in the plots in comparison to the control plot. The soil cellulase activity in control plot increased from 0.784 (at

the time of inoculation) to 4.49 μM glucose/ g dry wt. soil/ hr while in *Serratia* sp. MSK1 inoculated plot cellulase activity was 1.40 which increased to 7.51 μM glucose/ g dry wt. soil/ hr. Similarly the cellulose activity was 1.28 at inoculation and was 9.02 μM glucose/ g dry wt. soil/ hr at harvest in the *Pseudomonas* sp. MSK13 inoculated plot and 1.27 at inoculation and was 10.25 μM glucose/ g dry wt. soil/ hr at harvest in the *Serratia* sp. MSK24 inoculated plot (Table 6.2). The cellulose degraders population in control, *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 inoculated plots was10.04, 9.64, 10.88 and 12.87 ($x10^6$cfu/ g dry wt. soil) respectively at the time of inoculation, The population increased gradually to 32.61, 56.11, 58.64 and 73.58 ($x10^6$cfu/ g dry wt. soil) during 16^{th} week or at the time of crop harvest (Table 6.3). The organic carbon also increased from 0.34 to 0.55 % in the control plot, 0.39 to 0.66 % in the *Serratia* sp. MSK1 plot, 0.36 to 0.71% in the *Pseudomonas* sp. MSK13 plot and 0.37 to 0.83% in the *Serratia* sp. MSK24 plot (Table 6.4). The soil phosphatase activity in control plot increased from 0.17 (at the time of inoculation) to 0.31 μM PNP/ g dry wt. of soil/ hr, 0.17 to 0.70 μM PNP/ g dry wt. of soil/ hr in the *Serratia* sp. MSK1 inoculated plot, 0.16 to 0.73 μM PNP/ g dry wt. of soil/ hr in the *Pseudomonas* sp. MSK13 inoculated plot and 0.16 to 0.88 μM PNP/ g dry wt. of soil/ hr in the *Serratia* sp. MSK24 inoculated plot during 8^{th} week or midtime of crop growth. The soil phosphatase activity increased gradually during 16^{th} week or harvesting of crop to 0.66 μM PNP/ g dry wt. of soil/ hr in control plot, 0.86 μM PNP/ g dry wt. of soil/ hr in the *Serratia* sp. MSK1 inoculated plot, 1.41 μM PNP/ g dry wt. of soil/ hr in the *Pseudomonas* sp. MSK13 inoculated plot and 1.49 μM PNP/ g dry wt. of soil/ hr in the *Serratia* sp. MSK24 inoculated plot (Table 6.5). The phosphate solubilisers population in control, *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 inoculated plots showed 13.00, 17.09, 14.09 and

14.37 (x10^6cfu/ g dry wt. soil) respectively at the time of inoculation, The population increased gradually to 26.76, 40.37, 44.99 and 53.14 (x10^6cfu/ g dry wt. soil) during 16th week or at the time of crop harvest (Table 6.6). The available phosphorus in control plot increased from 44 (at the time of inoculation) to 70.33 mg/kg, 47.00 to 98.66 mg/kg in the *Serratia* sp. MSK1 inoculated plot, 48.66 to 96.66 mg/kg in the *Pseudomonas* sp. MSK13 inoculated plot and 48.00 to 99.66 mg/kg in the *Serratia* sp. MSK24 inoculated plot during 16th week or at the time harvest of crop (Table 6.7). The total nitrogen in control plot increased from 0.082 (at the time of inoculation) to 0.169 %, 0.081 to 0.234 % in the *Serratia* sp. MSK1 inoculated plot, 0.085 to 0.245 % in the *Pseudomonas* sp. MSK13 inoculated plot and 0.086 to 0.% in the *Serratia* sp. MSK24 inoculated plot during 16th week or at the time harvest of crop (Table 6.8).

During plot experiments, a significantly high value of soil cellulase activity as well as organic carbon was seen in *Serratia* sp. MSK24 inoculated plots as compared to the plots inoculated with the other two isolates after 16 weeks of crop harvest (Table 6.1). Mass inoculations of bacterial culture in the plots resulted in a significantly enhancement of cellulose degraders population which could be positively correlated (R^2=0.906) to the higher soil cellulase activity (Fig.6.2). The overall scenario in the experiment results showed an increased organic carbon in the treated plots, which was observed as a positive correlation (R^2=0.916) between the soil cellulase activity and soil organic carbon during the chickpea plot trails (Fig. 6.3). A positive correlation (R^2=0.853) was also observed among soil phosphatase activity and phosphate solubilisers population (Fig 6.4), which was also positively correlated (R^2=0.730) to available phosphorus (Fig 6.5).

Table 6.1: Survival of introduced population during growing chickpea crop in plots. Soil suspension diluted in physiological saline was plated on LA-ampicillin plate and genomic DNA was amplified by ERIC-PCR.

Colony forming units (X 10^7cfu/g of dry wt. soil)						
Time (weeks)		**0**	**4**	**8**	**12**	**16**
Control	Ampicillin count	2.2	1.8	1.5	1.7	1.3
	PCR count	0	0	0	0	0
***Serratia* sp. MSK1**	Ampicillin count	15.0	13.0	10.0	8.5	8.0
	PCR count	11.7	10.4	7.2	5.8	4.7
***Pseudomonas* sp. MSK13**	Ampicillin count	13.0	12.0	8.8	7.0	6.0
	PCR count	10.2	9.9	5.6	5.1	3.5
***Serratia* sp. MSK24**	Ampicillin count	15.0	14.0	11.5	10.5	9.0
	PCR count	13.4	12.0	9.3	8.7	7.6

Table 6.2: The soil cellulase activity (µM glucose/ g dry wt. soil/ hr) during growing chickpea crop in plots.

Plot	0 week	8 week	16 week
Control	0.78±0.05b	2.01±0.17c	4.49±0.32d
Serratia sp. MSK1	1.40±0.10a	4.10±0.20b	7.51±0.39c
Pseudomonas sp. MSK13	1.28±0.01a	5.18±0.37b	9.02±0.23b
Serratia sp. MSK24	1.27±0.08a	7.24±0.71a	10.25±0.39a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.3: The soil cellulose degraders population (x10^6cfu/ g dry wt. soil) during growing chickpea crop in plots.

Plot	0 week	16 week
Control	10.04±0.93a	32.61±2.57b
Serratia sp. MSK1	9.64±1.56a	56.11±6.64a
Pseudomonas sp. MSK13	10.88±0.88a	58.64±6.83a
Serratia sp. MSK24	12.87±1.41a	73.58±6.35a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.4: The soil organic carbon (%) during growing chickpea crop in plots.

Plot	0 week	16 week
Control	0.34±0.03a	0.55±0.01c
Serratia sp. MSK1	0.39±0.03a	0.66±0.02b
Pseudomonas sp. MSK13	0.36±0.03a	0.71±0.04ab
Serratia sp. MSK24	0.37±0.03a	0.83±0.04a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.5: Soil phosphatase activity (µM PNP / g dry wt. soil/ hr) during growing chickpea crop in plots.

Plot	0 week	8 week	16 week
Control	0.17±0.01a	0.31±0.01c	0.66±0.06c
Serratia sp. MSK1	0.17±0.01a	0.70±0.01b	0.86±0.06b
Pseudomonas sp. MSK13	0.16±0.01a	0.73±0.02b	1.41±0.06a
Serratia sp. MSK24	0.16±0.01a	0.88±0.03a	1.49±0.02a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.6: Phosphate solubilizers population (x 10^6 cfu/ g dry wt. soil) during growing chickpea crop in plots.

Plot	0 week	16 week
Control	13.00 ±0.96a	26.76 ±1.43b
Serratia sp. MSK1	17.09 ±1.28a	40.37 ±5.41ab
Pseudomonas sp. MSK13	14.09 ±2.10a	44.99 ±4.38ab
Serratia sp. MSK24	14.37 ±0.71a	53.14 ±6.28b

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.7: The soil available phosphorus (mg/kg) during growing chickpea crop in plots.

Plot	0 week	16 week
Control	44.00±5.68a	70.33±6.11a
Serratia sp. MSK1	47.00±6.24a	98.66±7.05a
Pseudomonas sp. MSK13	48.66±4.97a	96.66±14.31a
Serratia sp. MSK24	48.00±8.50a	99.66±11.89a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.8: The total nitrogen (%) in soil during growing chickpea crop in plots.

Plot	0 week	16 week
Control	0.082±0.005a	0.169±0.009b
Serratia sp. MSK1	0.082±0.006a	0.234±0.020ab
Pseudomonas sp. MSK13	0.085±0.006a	0.245±0.022ab
Serratia sp. MSK24	0.087±0.008a	0.273±0.024a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

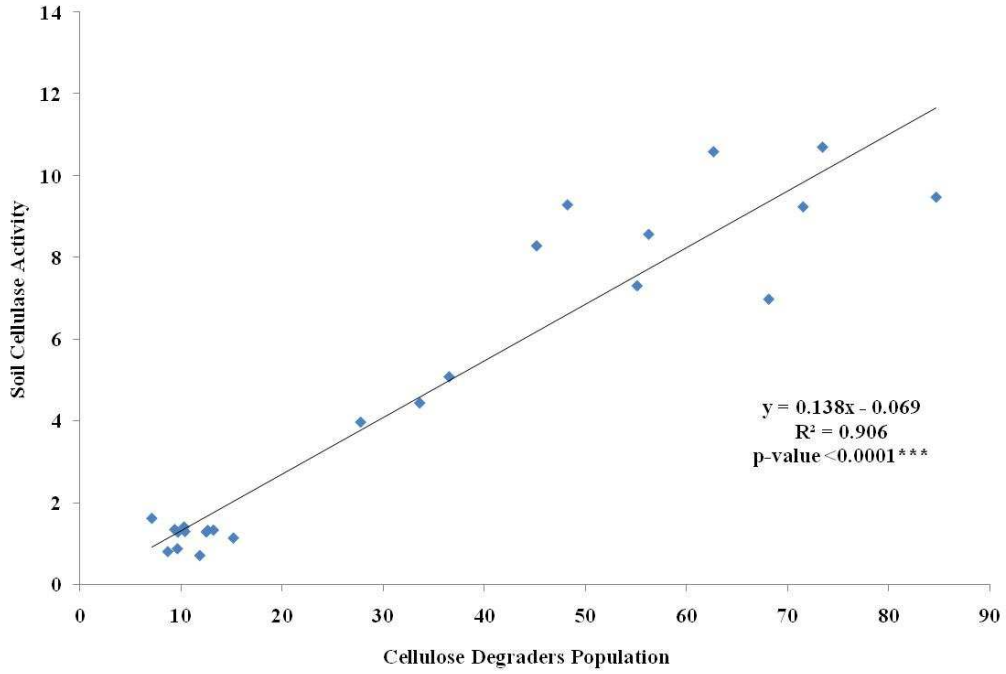


Fig 6.2: Correlation between the cellulase activity & cellulose degraders population in soil during the chickpea plot experiments.

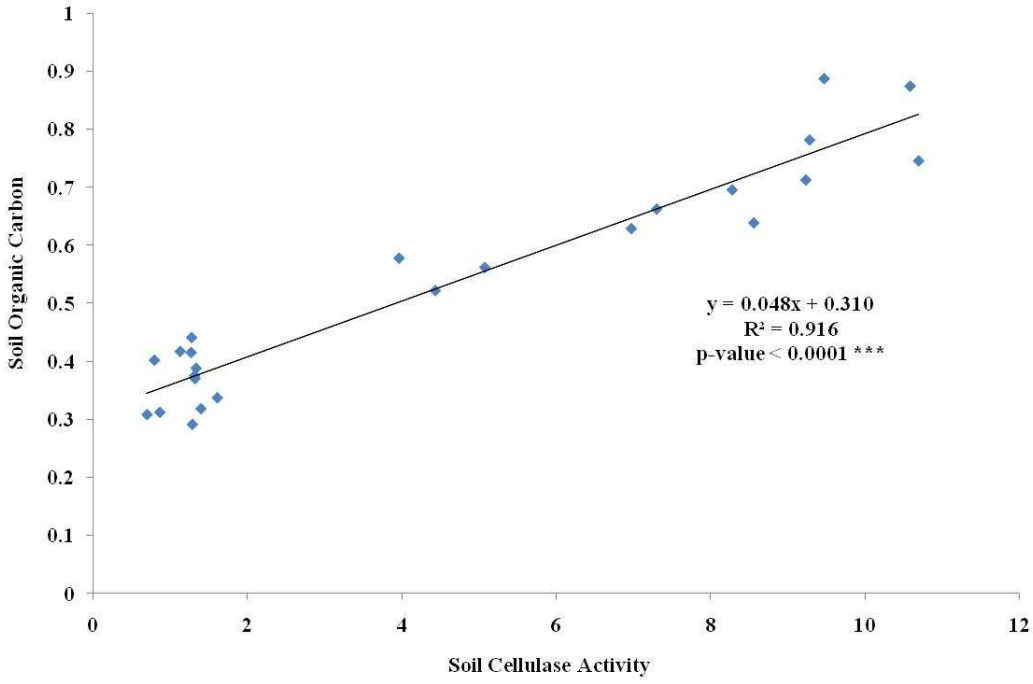


Fig 6.3: Correlation between the cellulase activity & organic carbon in soil during the chickpea plot experiments.

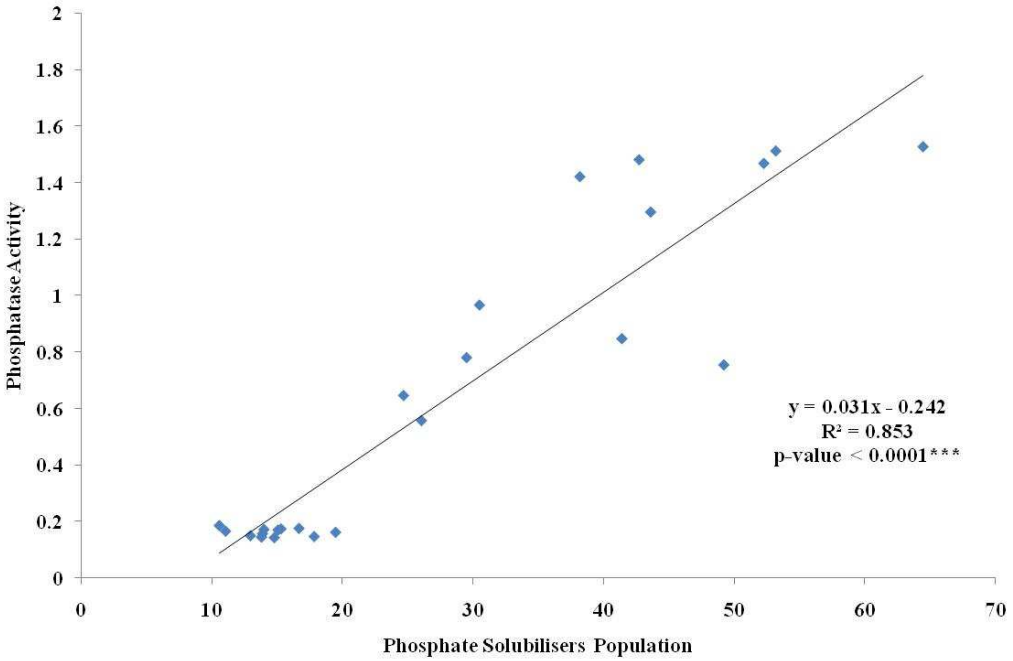


Fig 6.4: Correlation between the phosphatse activity & phosphate solubilisers population in soil during the chickpea plot experiments.

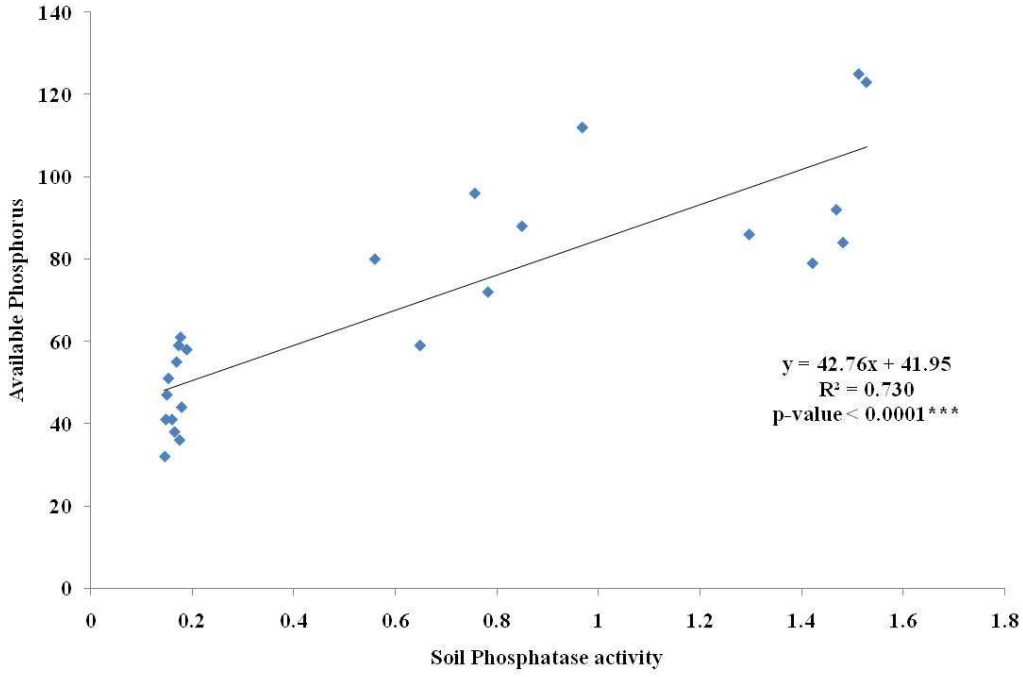


Fig 6.5: Correlation between the phosphatase activity & available phosphorus in soil during the chickpea plot experiments.

6.3 Survival studies of bacterial strains inoculated in Sunflower plot trails

In plot studies, ampicillin resistance plate count of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 was 13.3, 13 and13.5 ($x10^7$cfu / g dry wt. soil) respectively at the time of inoculation, The population decreased gradually to 9.8, 8.7 and 10.4 ($x10^7$cfu / g dry wt. soil) during 4^{th} week crop growth for *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 respectively which further decreased to 8.4, 6.9 and 7.8 ($x10^7$cfu / g dry wt. soil) during 8^{th} week. The population further decreased to 6.3, 3.8 and 5.9 ($x10^7$cfu / g dry wt. soil) during 12^h week or at the time of crop harvest for *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 respectively (Table 6.9). Microbial population based on LA + ampicillin plates in control plot decreased from 1.8 to 1.0 ($x10^7$cfu / g dry wt. soil) after 12 week of sunflower ie. at harvest. Population dynamic studies based on ERIC-PCR showed that population of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 was 10.5, 9.8 and 10.8 ($x10^7$cfu / g dry wt. soil) at the time of inoculation during germinating stage of sunflower, and then decreased to 5.5, 3.0 and 3.4 ($x10^7$cfu / g dry wt. soil) at the end of 12^{th} week. (Table 6.9) thus showing a survival of 52.4, 30.7 and 31.5 % respectively after 12 weeks (Table 6.9). Cellulose degrading *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 with soil cellulase activity of 4.62, 5.07 and 5.89 respectively, these could not be detected in the soil at zero time (before application of the bacterial inoculums) and in control as well as test plots. These results clearly demonstrated that enumeration of the introduced cellulolytic bacterial isolates by antibiotic resistance method always shoed higher level of survival as compared to that by ERIC-PCR. Since ERIC-PCR enumerates the population of the introduced bacterial isolates without any interference from other bacteria, this method presents a very accurate estimation of the survival of

the introduced bacteria. The various soil parameters such as population of cellulose degraders as well as phosphate solubilisers, cellulase activity, phosphatase activity, organic carbon, total nitrogen content and available phosphorus in the experimental plots was also observed and it found to be enhanced by the introduction of the inoculants in the plots in comparison to the control plot. The soil cellulase activity in control plot increased from 3.25 (at the time of inoculation) to 4.32 μM glucose/ g dry wt. soil/ hr , while in *Serratia* sp. MSK1 inoculated plot cellulase activity was 3.73 which increased to 4.62 μM glucose/ g dry wt. soil/ hr Similarly the cellulase activity was 4.05 at inoculation and was 5.07 μM glucose/ g dry wt. soil/ hr at harvest in the *Pseudomonas* sp. MSK13 inoculated plot and 3.99 at inoculation and was to 5.89 μM glucose/ g dry wt. soil/ hr at harvest the *Serratia* sp. MSK24 inoculated plot(Table 6.10). The cellulose degraders population in control, *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 inoculated plots was 11.71, 11.86, 13.07 and 11.79 ($x10^6$cfu/ g dry wt. soil) respectively at the time of inoculation, The population increased gradually to 17.78, 18.82, 28.30 and 32.70 ($x10^6$cfu/ g dry wt. soil) during 12^{th} week or at the time of crop harvest (Table 6.11). The soil phosphatase activity in control plot increased from 0.40 (at the time of inoculation) to 0.16 μM PNP/ g dry wt. of soil/ hr, 0.42 to 0.24 μM PNP/ g dry wt. of soil/ hr in the *Serratia* sp. MSK1 .inoculated plot, 0.42 to 0.25 μM PNP/ g dry wt. of soil/ hr in the *Pseudomonas* sp. MSK24 inoculated plot and 0.41 to 0.38 μM PNP/ g dry wt. of soil/ hr in the *Serratia* sp. MSK24 inoculated plot during 12^{th} week or harvesting of crop (Table 6.12). The phosphate solubilisers population in control, *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 inoculated plots showed 33.92, 30.49, 31.66 and 35.53 ($x10^6$cfu/ g dry wt. soil) respectively at the time of inoculation, The population increased gradually to 15.82, 18.70, 23.72 and 30.17

(x10^6cfu/ g dry wt. soil) during 12^{th} week or at the time of crop harvest (Table 6.13). The available phosphorus in control plot increased from 96.7 (at the time of inoculation) to 60.33 mg/kg, 96.53 to 70.66 mg/kg in the *Serratia* sp. MSK1 inoculated plot, 87.73 to 61.0 mg/kg in the *Pseudomonas* sp. MSK24 inoculated plot and 92.0 to 73.33 mg/kg in the *Serratia* sp. MSK24 inoculated plot during 12^{th} week or at the time harvest of crop (Table 6.14). The organic carbon also increased from 0.38 to 0.32 % in the control plot, 0.40 to 0.34 % in the *Serratia* sp. MSK1 plot, 0.40 to 0.33% in the *Pseudomonas* sp. MSK24 plot and 0.37 to 0.35% ^{in} the *Serratia* sp. MSK24 plot (Table 6.15). The total nitrogen in control plot increased from 0.076 (at the time of inoculation) to 0.039 %, 0.075 to 0.043 % in the *Serratia* sp. MSK1 inoculated plot, 0.080 to 0.051 % in the *Pseudomonas* sp. MSK24 inoculated plot and 0.084 to 0.065 % in the *Serratia* sp. MSK24 inoculated plot during 12^{th} week or at the time harvest of crop (Table 6.16).

During plot experiments, a significantly high value of soil cellulase activity as well as organic carbon was seen in *Serratia marcescens* inoculated plots as compared to the plots inoculated with the other two isolates after 12 weeks of crop harvest (Table 6.9). Mass inoculations of bacterial culture in the plots resulted in a significantly enhancement of cellulose degraders population which could be mild positively correlated (R^2=0.723) to the higher soil cellulase activity (Fig.6.6). The overall scenario in the experiment results showed a decreased organic carbon in the treated plots, which was observed as a negative correlation (R^2=0.145) between the soil cellulase activity and soil organic carbon during the sunflower plot trails (Fig. 6.7). A negative correlation (R^2=0.280) was also among soil phosphatase activity and phosphate solubilisers population (Fig 6.8), which was also negatively correlated (R^2=0.570) to available phosphorus (Fig 6.9).

Table 6.9: Survival of introduced population during growing sunflower crop in plots. Soil suspension diluted in physiological saline was plated on LA-ampicillin plate and genomic DNA was amplified by ERIC-PCR.

Colony forming units (X 10^7cfu/g of dry wt. soil)					
Time (weeks)		**0**	**4**	**8**	**12**
Control	Ampicillin count	1.8	1.3	1.2	1.0
	PCR count	0	0	0	0
Serratia sp.	Ampicillin count	13.3	9.8	8.4	6.3
	PCR count	10.5	8.2	7.0	5.5
Pseudomonas sp.	Ampicillin count	13.0	8.7	6.9	3.8
	PCR count	9.8	7.5	5.5	3.0
Serratia marcescens	Ampicillin count	13.5	10.4	7.8	5.9
	PCR count	10.8	8.8	6.5	3.4

Table 6.10: The soil cellulase activity (µM glucose/ g dry wt. soil/ hr) during growing sunflower crop in plots.

Plot	0 week	6 week	12 week
Control	3.25±0.05c	6.41±0.20c	4.32±0.48a
Serratia sp. MSK1	3.73±0.05b	7.28±0.10b	4.62±0.25a
Pseudomonas sp. MSK13	4.05±0.04a	8.05±0.37b	5.07±0.38a
Serratia sp. MSK24	3.99±0.10a	9.25±0.42a	5.89±0.70a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.11: The soil cellulose degraders population (x10^6cfu/ g dry wt. soil) during growing sunflower crop in plots.

Plot	0 week	12 week
Control	11.71±0.31a	17.78±0.67b
Serratia sp. MSK1	11.86±0.88a	18.82±3.08b
Pseudomonas sp. MSK13	13.07±1.14a	28.30±2.15a
Serratia sp. MSK24	11.79±0.80a	32.70±1.85a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.12: Soil phosphatase activity (µM PNP / g dry wt. soil/ hr) during growing sunflower crop in plots.

Plot	0 week	6 week	12 week
Control	0.40±0.02a	1.06±0.01c	0.16±0.01b
Serratia sp. MSK1	0.42±0.02a	1.25±0.03bc	0.24±0.01b
Pseudomonas sp. MSK13	0.42±0.04a	1.35±0.07b	0.25±0.01b
Serratia sp. MSK24	0.41±0.03a	1.76±0.08a	0.38±0.04a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.13: Phosphate solubilizers population ($x10^6$cfu/ g dry wt. soil) during growing sunflower crop in plots.

Plot	0 week	12 week
Control	33.92±1.89a	15.82±2.03c
Serratia sp. MSK1	30.49±3.87a	18.70±0.47bc
Pseudomonas sp. MSK13	31.66±2.60a	23.72±1.03b
Serratia sp. MSK24	35.53±3.07a	30.17±3.11a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.14: The soil available phosphorus (mg/kg) during growing sunflower crop in plots.

Plot	0 week	12 week
Control	96.73±6.39a	60.33±10.71a
Serratia sp. MSK1	96.53±15.04a	70.66±8.41a
Pseudomonas sp. MSK13	87.73±7.42a	61.00±7.00a
Serratia sp. MSK24	92.00±14.35a	73.33±14.76a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.15: The soil organic carbon (%) during growing sunflower crop in plots.

Plot	0 week	12 week
Control	0.38±0.02a	0.32±0.01a
Serratia sp. MSK1	0.40±0.01a	0.34±0.02a
Pseudomonas sp. MSK13	0.40±0.03a	0.33±0.01a
Serratia sp. MSK24	0.37±0.04a	0.35±0.02a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

Table 6.16: The total nitrogen (%) in soil during growing sunflower crop in plots.

Plot	0 week	12 week
Control	0.076±0.005a	0.039±0.003b
Serratia sp. MSK1	0.075±0.003a	0.043±0.003b
Pseudomonas sp. MSK13	0.080±0.011a	0.051±0.002b
Serratia sp. MSK24	0.084±0.010a	0.065±0.003a

#Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)

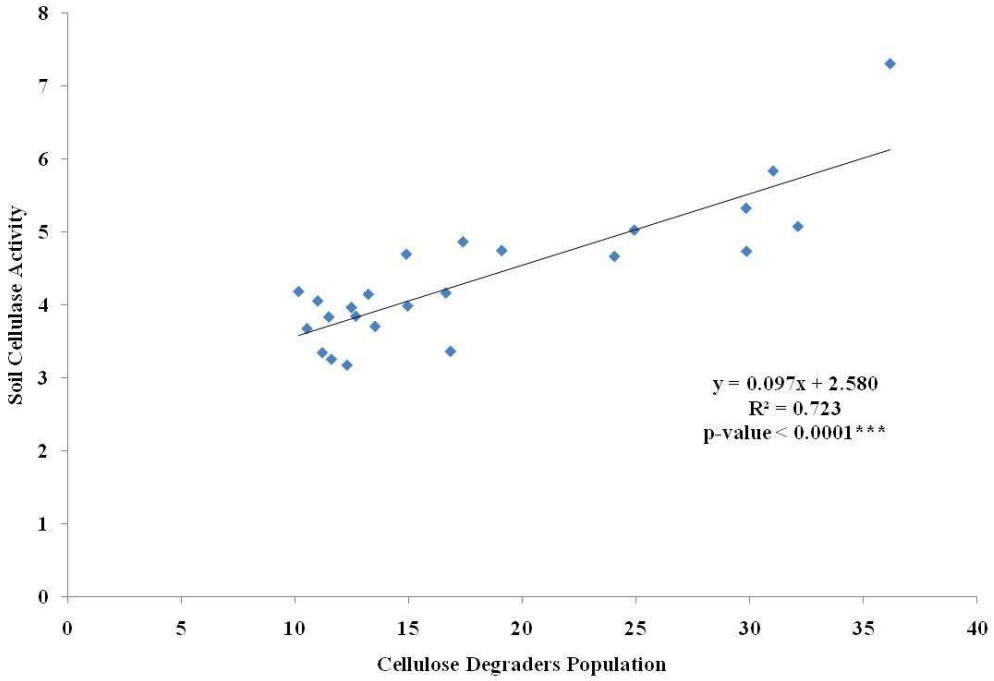


Fig 6.6: Correlation between the cellulase activity & cellulose degraders population in soil during the sunflower plot experiments.

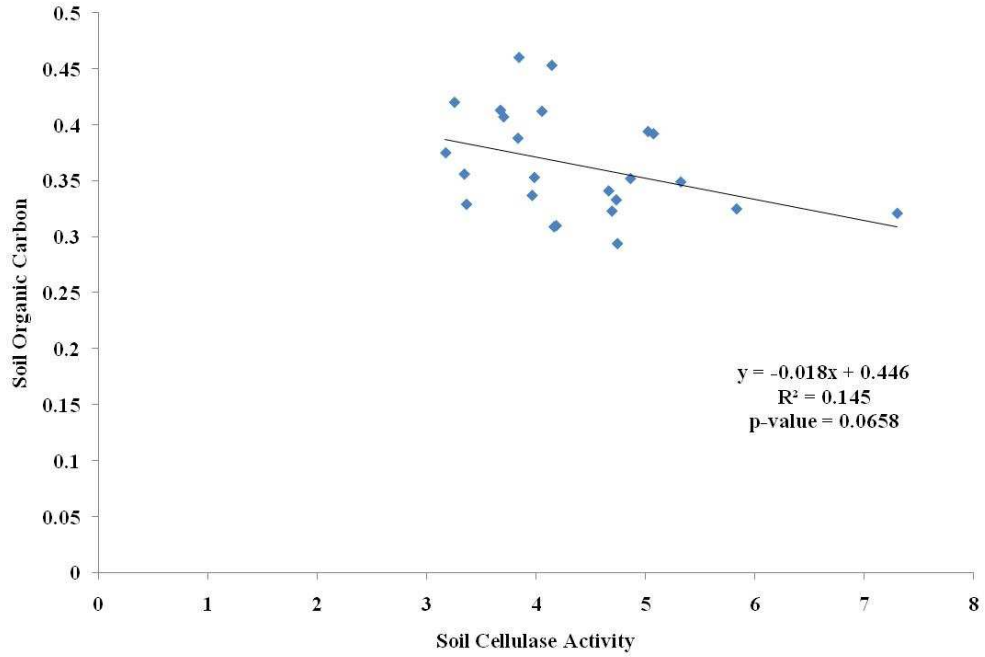


Fig 6.7: Correlation between the cellulase activity & organic carbon in soil during the sunflower plot experiments.

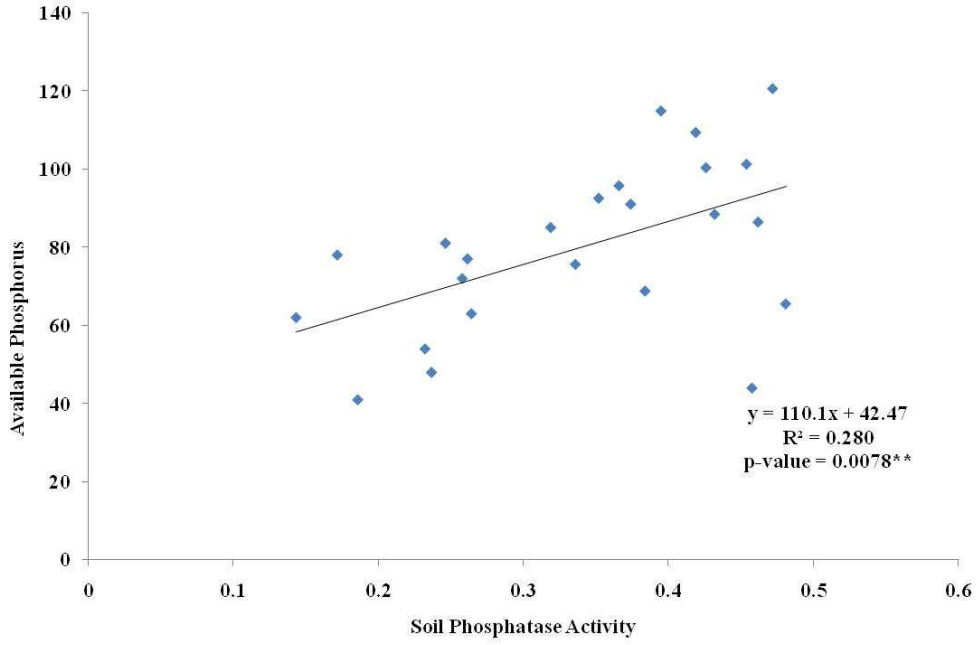


Fig 6.8: Correlation between the phosphatase activity & available phosphorus in soil during the sunflower plot experiments.

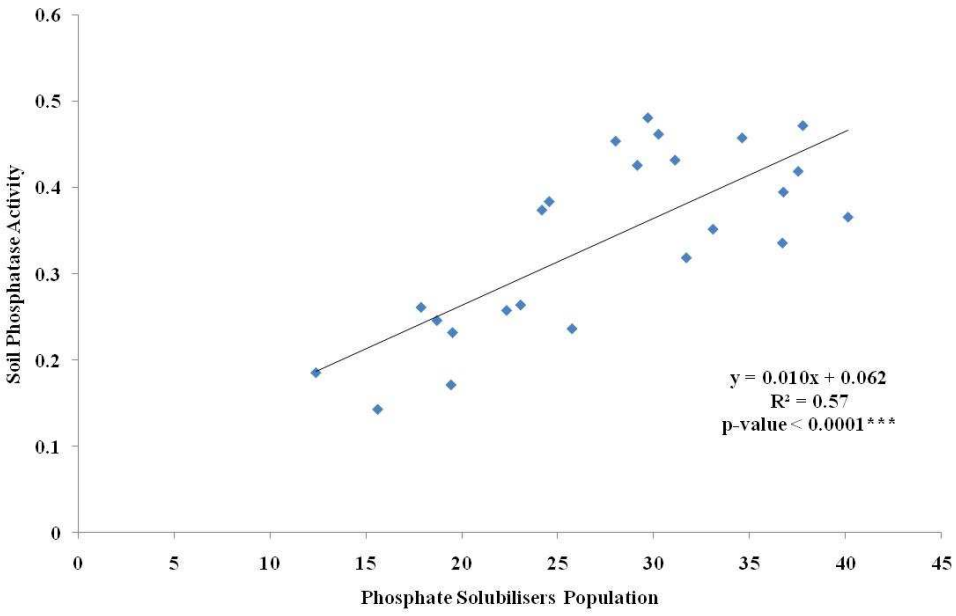


Fig 6.9: Correlation between the phosphatase activity & phosphate solubilisers population in soil during the sunflower plot experiments.

Chapter 7

Economic analysis of diversified cropping

ECONOMIC ANALYSIS OF DIVERSIFIED CROPPING

The agricultural scenario in Punjab is changing rapidly as a land of agricultural bounty in country. But of late, there's been a growing concern over a possible collapse of agriculture in the state due to excessive exploitation of land and water resources. The common notion is that the collapse is almost certain, until the cropping pattern is suitably modified and diversified. In the present study we estimated the profits and economic gain of the farmers by following the three different cropping patterns of chickpea-rice, wheat-rice and sunflower-rice using prescribed package of practices under management strategies, using both estimates based on expenditure on inputs in terms of cost of quality seeds, fertilizers, tillage, pesticides, weeding, irrigation and harvesting along with production in terms of economic output i.e. yields and current market rates offered to the farmers in agricultural market.

Table: 7.1 The total expenditure per acre during the various cropping during the field trails (in ₹). The table represents the yield during 2002-03 and 2003-04.

Inputs Costs	**Chickpea**	**Sunflower**	**Rice**	**Wheat**
Cost of Seed	1200	625	300	400
Cost of Fertilizer	650	1100	1200	1000
Cost of Tillage	800	1000	1000	400
Cost of Pesticide	600	0	650	800
Cost of Weeding	1000	0	600	0
Cost of Irrigation	250	600	1200	600
Cost of Harvesting	400	1000	400	400
Total Expenditure	**4900**	**4325**	**5350**	**3600**
Year	**Yield (Quintal / acre)**			
2002-03	4 – 7.5	9.5	10-11	15 – 19
2003-04	4 – 11.5	10	10-13	16 - 19

As described in Table 7.1, maximum inputs are provided, in terms of investment, to chickpea and sunflower as compared to wheat during the chickpea-rice, wheat-rice and sunflower-rice cropping patterns as rabi crops (November- May) with rice as common crop during kharif (June-October). Data from farmers' fields in Patiala district showed that yield of chickpea varies from 4 – 7.5 quintal/ acre during 2002-03, which increased to 4 – 11 quintal/ acre during 2003-04**.** The highest yield of chickpea was observed at Rajpura. The yields of sunflower vary from 9.5 - 10 quintal/ acre and that of wheat from 15 – 19 quintal/ acre during rabi season. The kharif basmati rice crop yields were 15 – 19 quintal/ acre during 2002-03 and similarly 16 – 19 quintal/ acre during year 2003-04. The yield variation as recorded in farmers field is quite high, as various factor such as soil texture, nutritional status, microbial population and activities played a crucial role despite similar inputs and management practices.

The adoption of low water consuming chickpea crop are directly linked to the water saving as it requires two irrigation during the crop cycle, one while sowing followed by second during the grain setting stage. On the contrary wheat requires five irrigations during the crop cycle. Thus there is net saving of three irrigation which contributes towards the sustainability in water usage as well as being less labour intensive. In case of chickpea, the variations in yield are much high from 4.5 to 11 quintal/ acre. As described in Table 7.2, maximum yield of 11.5 quintal/ acre fetched the rate of ₹ 1800 per quintal which showed chickpea cropping adoption lead to the generation of income of ₹ 20700. As compared to ₹ 16000 in sunflower (oil crop) cropping with limitation of selling market. On the other hand wheat only generated the ₹ 11970 on the maximum yield of 19 quintals. The overall scenario proved more

profitable to the farmer with the adaption of chickpea and sunflower during diversification from wheat cropping.

Table 7.2: The economical returns under different cropping systems (in ₹) during the year 2003-2004 as per agricultural marketing rates.

	Chick pea	**Sunflower**	**Rice**	**Wheat**
Total Expenditure (₹/Qt)	4900	4325	5350	3600
Av. Yield (Qt.)	4-11.5	10	10-13	15-19
Rate (₹/Qt)	1800	1600	1300	630
Gross Income (₹/Qt)	7200-20700	16000	13000-17000	9450-11970
Net Income (₹/Qt)	2300-15800	11675	7650-11550	5850-8370
Benefit: Cost Ratio	**1.47-4.22**	**3.70**	**2.43-3.18**	**2.63-3.33**

Table 7.3: The economical returns under different cropping systems (in ₹) during the current fiscal year 2008-2009 as per agricultural marketing rates.

	Chick pea	**Sunflower**	**Rice**	**Wheat**
Total Expenditure (₹/Qt)	7100	6200	7500	5400
Av. Yield (Qt.)	4-11.5	10	10.13	15-19
Rate (₹/Qt)	3400	3100	2100	1100
Gross Income (₹/Qt)	13600-39100	31000	21000-27300	16500-20900
Net Income (₹/Qt)	6500-32000	24800	13500-19800	11100-15500
Benefit: Cost Ratio	**1.91-5.50**	**5.00**	**2.80-3.64**	**3.06-3.87**

The suitability of a cropping system is ultimately determined by the monetary gain. Data (Table 7.2 & 7.3) reveal that there were pronounced differences among the various rice based cropping system under three different cropping patterns of

chickpea-rice, wheat-rice and sunflower-rice in terms of net income per acre. As shown in Table 7.2 during year 2003-04, the net income of different cropping patterns of chickpea-rice, sunflower-rice and wheat-rice were recorded as ₹ 2300 to 15800, 11675 and 5850 to 8370 per acre respectively. The benefit-cost ratio was assessed among all the three cropping pattern with dividing gross income with total expenditure. The benefit-cost ratios of three rice-based cropping systems were recorded and vary with yield as 1.47 to 4.22, 3.70 and 2.63 to 3.33 for chickpea, sunflower and wheat respectively as against 2.43 to 3.18 for rice. On the other hand same results can extrapolated with current agricultural commodity rates as observed in Table 7.3 during year 2008-09, the net income of farmers under chickpea-rice, sunflower-rice and wheat-rice cropping patterns of were recorded as ₹ 6500 to 32000, 24800 and 13500 to 19800 per acre respectively. The benefit-cost ratio was assessed among all the three cropping pattern with dividing gross income with total expenditure. The benefit-cost ratios of three rice-based cropping systems were recorded and vary with yield as 1.91 to 5.50, 5.00 and 3.06 to 3.87 for chickpea, sunflower and wheat respectively as against 2.80 to 3.64 for rice.

The results further indicate that chickpea-rice cropping system gave the highest profit with benefit to cost ratio of 4.22 in year 2003-04 and similar trend was observed in current year 2008-09 with 5.50. This cropping pattern was found to be more sustainable in terms of yield, water saving, organic matter, microbial activities, economic analysis and prosperity of farmers. From the agricultural and ecological viewpoints, the aims will be achieved towards sustainable plant productivity, while maintaining environmental quality.

Chapter 8

Discussion

DISCUSSION

Sustainable agriculture integrates three main goals-environmental health, economic profitability, and socio and economic equity. A variety of philosophies, policies and practices have contributed to these goals. Sustainability rests on the principle that we must meet the needs of the present without compromising the ability of future generations to meet their own needs. Making the transition to sustainable agriculture is a process. For farmers, the transition to sustainable agriculture normally requires a series of small, realistic steps. Family economics and personal goals influence how fast or how far participants can go in the transition. The key to moving forward is the will to take the next step with good economic returns.

Agriculture, a primary sector in the country plays a significant role in the development of the nation since its main aim is to raise crop production for the nourishment of the increasing population. In the country there has been a major emphasis on one or two crops due to the concept of minimum support price and the subsidy on farm products. Diversification of crops is need of the hour for sustainable agricultural maintenance of the nutritional status in soil, which makes farms more economically and ecologically resilient. While monoculture farming has advantages in terms of efficiency and ease of management, the loss of the crop in any one year could put a farm out of business and/or seriously disrupt the stability of a community dependent on that crop. By growing a variety of crops, farmers spread economic risk and are less susceptible to the radical price fluctuations associated with changes in supply and demand. In annual cropping systems, crop rotation can be used to suppress weeds, pathogens and insect pests.

Microbial community in soils plays an important role in agricultural practices. They have major roles in nutrient cycling, and in pathogenic and beneficial

interactions with crop plants. Switching away from chemical fertilizer inputs towards inputs such as compost, manures, and green manures, sometimes leads to a decrease in yields (Prabhu *et al.*, 1998). This may be because the right populations of microbes for breaking down cellulose that are necessary to release the nutrients into the soil are not present in sufficient numbers. Thus there is a need to study differences between conventional and sustainable agricultural practices, concentrating on soil bacterial and fungal population and diversity. Soil health is closely linked to microbial diversity, and that relatively minor change in environmental conditions can dramatically alter population structures. Therefore there is the need to understand the role of biodiversity in maintenance of soil fertility, be able to recognize and measure its key components, and develop reliable monitoring methods.

Punjab's agriculture has a far-reaching impact on its economy. The plains of Punjab were formed by the deposition of alluvium. It has deep and fertile soils. The major crops in Punjab are wheat and rice. Though Punjab is a one of the smallest state with a total land area of only 0.33% of the world and 1.6% of the country with 2.6 percent of it's cropped area yet it contributes to 1% of rice, 2% of the wheat and 2% of the cotton in the total world production vis-à-vis 42% rice, 55% wheat, and 24% cotton production in the country (Mcguirk and Mundlak, 1991). In Punjab this has lead to virtual monoculture practices, which is leading to degradation of nutritional status of the soils and thus the productivity. In the nineties Punjab saw a fall in the growth of agricultural output. More serious than the fall in the growth was the stagnation of yield of many crops, some crops declining even below the national average. One of the factors affecting the decline in the growth and stagnation of agricultural output was the availability of water resource. Therefore shrinking water resources in northwest India calls for diversification from a rice–wheat cropping

system to alternative cropping pattern and sustainable agriculture system particularly in Punjab state. In Punjab alternative to rice-wheat cropping cycle has to be demonstrated so as to convince the farmer of the viability of the alternative system.

Chickpea because of its lower water demand and irrigation requirement and sunflower as oil crop has been identified as a suitable alternate crop to wheat. Earlier, chickpea was grown in the states of Punjab, Haryana and Rajasthan (North India) as a rain-fed rabi crop only for subsistence and not as a commercial crop. Under rain-fed conditions, chickpea often suffered from water deficits due to low and uncertain rainfall, resulting in low yields. A number of field experiments were conducted in the late seventies by various researchers (Mathur *et al.*, 1973; Sandhu *et al.*, 1978; Mandal *et al.*, 1979) on differential effects of soil moisture storage at sowing and post-plant irrigations on chickpea yield and a new irrigation regime was suggested for chickpea. The objectives of the present study was to assess the microbial diversity and soil enzymatic response to chickpea on different soil in a chickpea-rice-chickpea and wheat-rice-wheat rotation from the data generated using a developed model after its validation. Thus chickpea was grown in 4 blocks of the farmer's field in Patiala, Derabassi, Rajpura and Samana. These farmers were provided the quality seed, fertilizers, as well as biocontrol agentsand were advised about the various crop practices. Nutritional status of the soil with the quantification of organic carbon, total nitrogen, available phosphorus and the microbial population and soil enzyme activities were used for the development of the model.

In this work, we attempted to understand the microbial responses to different soil management practices performed in different sites under field conditions. Experimental field sites were different in soil texture, nutritional status and microbial load / activities were strongly influenced in both amount and composition by site and

origin of sampling. In the present field sites on a soil characteristics basis at Patiala and Samana with sandy loam soil, Rajpura and Derabassi with silt loams (three cropping seasons, respectively) were selected to ensure stabilized but contrasting soil-ecological and nutritional conditions.

The yield data reveal that there were pronounced differences among the three different cropping patterns of chickpea-rice, wheat-rice and sunflower-rice in terms of net income per acre. During year 2003-04, the benefit-cost ratios of three rice-based cropping systems were observed as 1.55 to 4.45, 3.70 and 2.43 to 3.18 for chickpea, sunflower and wheat respectively as against 2.63 to 3.33 for rice, which were 2.00 to 5.75, 5.00 and 3.06 to 3.87 for chickpea, sunflower and wheat respectively as against 2.80 to 3.64 for rice with current agricultural commodity rates for year 2008-09, The results indicate that chickpea-rice cropping system gave the highest profit with benefit to cost ratio of 4.45 in year 2003-04 and similar trend was observed in current year 2008-09 with 5.75.

The finding reveals that the total microbial population decreased during wheat- rice -wheat cropping in all the four location but showed either no change or increase during chickpea- rice -chickpea cropping. The total microbial population as well as soil enzyme activities increased by diversifying the cropping pattern from conventional wheat-rice-wheat to chickpea-rice chickpea cropping pattern. In addition, the study reveals that cultivation of chickpea provides about two times higher economic returns to the growers compared to wheat and can, thus, be a good alternative. Chickpea also improves soil fertility by capturing nitrogen from air and fixing it in the soil and requires only two-fifth the quantity of water for successful growth. The overall average yield of chickpea was 4 to 11 Q/acre with input expenditure was 22% higher as compared to wheat. However, the net returns were

approx. ₹ 5000 more per acre over wheat. The major benefit apart from the increased returns was water conservation. Chickpea crop requires only two irrigation as compared to five in case of wheat cropping thus reducing the consumption of water. During sunflower field trails the average yield was 10 Q/acre with 13% higher input expenditure as compared to wheat, but due to higher selling price the net returns were 31% higher over wheat.

Enzymes in soils originate from animal, plant and microbial sources. Assessment of soil enzyme activities would provide a measure of microbial activity in soils (Ladd, 1972). Speir and Ross (1978) have demonstrated that microorganisms supply most of the soil enzyme activity, with their large biomass, high metabolic activity and short lifetime under favourable conditions. Soil enzyme activities are influenced by management practices because they are also related to microbial biomass which is sensitive to different treatments. The impact of diversified cropping pattern on soil enzymes (phosphatases and cellulases) as well as the microbial populations involved with these enzymes as studied for a duration of three years, at varying stages of crop growth. The main emphasis was on the field agroecosystems with a rotation of two cropping patterns one conventional (wheat-rice-wheat) and other (chickpea-rice-chickpea). Most of the biological parameters were influenced by the type of soil and practices. There have been fewer investigations concerning ecological agriculture and enzyme activity.

The trend in total microbial population in Patiala (sandy loam) and Derabassi (silt loam) showed relatively stable profile in chickpea-rice-chickpea cropping pattern as compared to decreasing trend in wheat-rice-wheat cropping pattern. However, cropping patterns observed at Rajpura (silt loam) and Samana (sandy loam) showed increase in total microbial population during chickpea-rice-chickpea as compared to

decrease in wheat-rice-wheat cropping pattern. In the present study, although there was limited or no definite trend in phosphate solubilizers or cellulose degraders with reference to a cropping pattern, a observation on detailed microbial diversity could not be carried out at this juncture. The composition and density microbial population in a soil is however reported to be significantly dependent on the water content and soil organic carbon availability (Moody *et al*., 1994; Drenovsky *et al*., 2004). Observations in the present study indicated increase in organic carbon in chickpea-rice-chickpea system when compared to wheat-rice cropping pattern; and higher water input the wheat-rice system which might influence the overall diversities of the microbial population at each varying condition.

Organic phosphorus compounds in soil constitute 5 – 50 percent of total phosphorus and the assimilation of this phosphorus by plants and microorganisms is preceded by soil enzymes. Several enzymes are involved in the decomposition of organic phosphorus compounds (Jennings, 1995). Soil phosphatases, then, play a major role in the mineralization processes (dephosphorylation) of organic phosphorus substrates. Phosphatase activity can also be influenced by soil erosion. In our study, a positive correlation was observed between available phosphorus and phosphatase activity similar to the observations reported by Harrison (1983) wherein such positive relationship was observed. In addition, the above research group (Harrison and Pearce, 1979; McCallister *et al.,* 2002) also reported that the variations in phosphatase activity, to a notable extent, is attributable to seasonal climatic variations as well as crop growth intensity.

In our studies at Patiala (sandy loam) the maximum phosphate solubilisers population as well as soil phosphatase activity was during chickpea sowing under chickpea-rice-chickpea cropping as compared to wheat sowing in wheat-rice-wheat

cropping. Similarly available phosphorus increased during chickpea-rice-chickpea as compared to wheat-rice-wheat cropping. At Rajpura (silt loam) also the maximum phosphate solubilisers population, soil phosphatase activity and available phosphorus was observed during chickpea harvest under chickpea-rice-chickpea cropping as compared to fall in wheat-rice-wheat cropping. Similar differences in phosphatase activity between grain-legume rotation and grain-monocultures systems were observed by Khan (1970), with significantly higher activity in former system over the latter. It is also clearly evident that the presence of plants has significant effect on enzyme activities, including phosphatase (Gavrilova *et al.,* 1973; Kiss *et al.,* 1974; McLachlan, 1980; Juma and Tabatabai, 1988; Tadano *et al.,* 1993). The effect of plants can be direct when the roots secrete phosphatase and indirect when it relates to changes in soil organic matter content and microbial populations (rhizosphere effect).

A greenhouse study by Reddy *et al.* (1987) showed that enzyme activity was higher in rhizosphere soils than in before planting and non – rhizosphere soils. According to Hedley *et al.* (1983) in unamended soil, rhizosphere phosphatase activity increased with an increase in P deficiency caused by increased root density and decreased soluble inorganic P levels. Plant P uptake and yield correlated with the phosphatase activity in the rhizosphere (Tarafdar and Rao, 1990). In the present study, at Derabassi (silt loam) the phosphate solubilisers population was not different under chickpea-rice-chickpea and wheat-rice-wheat cropping although the soil phosphatase activity increased during chickpea-rice-chickpea as compared to no change in wheat-rice-wheat cropping. Similarly, there was increase in available phosphorus during wheat-rice-wheat as compared to that in chickpea-rice-chickpea cropping. But at Samana (sandy loam) decrease in phosphate solubilisers population was observed during wheat-rice-wheat cropping as compared to no significant change in chickpea-

rice-chickpea cropping. Contrary to this the soil phosphatase activity increased during chickpea-rice-chickpea as compared to decrease in wheat-rice-wheat cropping. Similarly available phosphorus increased during wheat-rice-wheat cropping as compared to not much change during chickpea-rice-chickpea cropping. Thus these results do not indicate a uniform pattern in correlation between soil phosphatase activity, phosphorus solubilising microbes and available phosphorus in all types of soil. The correlation is greatly influenced by the soil type (Šarapatka and Kršková, 1997) and presence of plants as has also been observed by other investigators (Gavrilova *et al.,* 1973; Kiss *et al.,* 1974; McLachlan, 1980; Juma and Tabatabai, 1988; Tadano *et al.,* 1993).

At Patiala (sandy loam) and Rajpura (silt loam) maximum cellulose degraders population was observed during chickpea-rice-chickpea cropping as compared to steep decrease during wheat-rice-wheat cropping. This correlated positively with the soil cellulose activity as well as the organic carbon content during chickpea-rice-chickpea as compared to wheat-rice-wheat cropping. However at Derabassi (silt loam) the cellulose degraders population was observed to increase during chickpea-rice-chickpea cropping as compared to steep decrease during wheat-rice-wheat cropping but both soil cellulase activity and organic carbon decreased during both chickpea-rice-chickpea and wheat-rice-wheat cropping.

During the above comparative field experiments at Rajpura (silt loam), the overall scenario depicts a positive correlation with r-value of 0.766 among the soil cellulase activity and cellulose degraders population during the chickpea-rice cropping, as compared to wheat-rice and sunflower-rice with r-value of 0.655 and 0.354 respectively (Fig 5.30, 5.31 and 5.32). On the other hand, no correlation was observed among the soil phosphatase and phosphate solubilisers population in all

three cropping pattern of chickpea-rice, wheat-rice and sunflower-rice with r-value of 0.038, 0.007 and 0.002 respectively (Fig 5.33, 5.34 and 5.35). Aon and Colaneri (2001) described strong relationships between organic carbon and total nitrogen with enzymatic activities including acid and alkaline phosphatases. Alkaline phosphatase, protease, and cellulase were most sensitive to changes due to tillage management. Correlation between soil phosphatase activity with clay content, humic and fulvic acids ratio, available and total phosphorus, and pH were described by Šarapatka and Kršková, (1997) at locations in the Czech Republic with different soil types.

Marinari *et al.* (2000) described positive correlations between soil porosity, enzymatic activity and CO_2 production in organic and mineral treatment. Organic treatment stimulated soil biological activity probably due to an enrichment of soil organic matter, mineral fertilizer enhanced soil porosity by increasing regular and irregular pores and caused a priming effect of native soil organic matter. Organic carbon content was found to correlate with total nitrogen content. The effect of cultivation induced significant changes in the quality, chemical composition and molecular size of organic matter which in turn influenced the activities of enzymes involved in the carbon, nitrogen and phoshorus cycles.

In present study, 31 bacterial isolates showed cellulolytic activity in which 3 isolates such as MSK1, MSK13 and MSK24 were selected further based on their high cellulolytic potential and were identified as *Serratia* sp., *Pseudomonas* sp. and *Serratia* sp. respectively by using 16S rDNA analysis. Sindhu *et al.* (2001) reported both aerobic cellulolytic organisms such as *Pseudomonas* and actinomycetes, facultative anaerobes such as *Bacillus* and *Cellulomonas*, and also strict anaerobes such as *Clostridium* while *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35

have been shown to possess cellulolytic activity in the presence of crop residues (Hameeda *et al.*, 2006).

The identification of all isolates was confirmed by genetic analysis, thus, molecular finger printing of DNA of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 was analyzed for detection of genetic similarity among the three isolates. The successful result of agarose gel electrophoresis of PCR products (Fig. 6.1a) revealed that, *Serratia* sp. MSK1 amplified thirteen fragments were obtained, while the lowest number of synthesized DNA fragments (nine bands) was observed with *Pseudomonas* sp. MSK13. Thirteen fragments with DNA amplicons were obtained in case of ERIC-PCR of *Serratia* sp. MSK24. Four fragments were similar or identical in both *Serratia* sp.MSK1 and *Serratia* sp. MSK24 isolates, but no similarity was observed among the amplicons of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24. In conclusion, although there is genetic diversity between the three isolates demonstrate a high degree of genetic heterogeneity. A similar comparison of the diversity was observed in a study on biocontrol property of *Pseudomonas fluorescen.* and *Serratia marcescens* in Egypt (Mohamed *et al.*, 2009).

We determined the survival studies of the introduced bacteria under the chickpea crop plot (2X2 m) trails with survival of 40.2, 34.4 and 56.8 % of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 respectively. Results revealed that, a significantly enhancement of cellulose degraders population which was positively correlated (R^2=0.906) to the higher soil cellulase activity. The plot experiment results showed an increased organic carbon in the treated plots, which was observed as a positive correlation (R^2=0.916) among the soil cellulase activity and soil organic carbon. The similar mass inoculations were performed with sunflower (*Helianthus annuus*) estimated the survival of 52.3, 30.6 and 31.4 % of *Serratia* sp.

MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 respectively and results of plot trails resulted in a correlation which is positive (R^2=0.723) among cellulose degraders population and soil cellulase activity. But observed a decrease in organic carbon in the treated plots, as a negative correlation (R^2=0.145) among the soil cellulase activity and soil organic carbon, during the growth of sunflower crop in plot.

According to the aforementioned results obtained in this study, we concluded that, the local isolates of both *Serratia* sp. and *Pseudomonas* sp. could be mass produced as a biological agent towards magnification of microbial diversity and soil organic carbon content during the plot experiments. Consequently, the introduction of such bacteria in chickpea soils, or cultural practices aimed to increase the activity of native strains of these bacteria could greatly contribute to the efficiency of soil organic matter. The study investigated the cellulolytic potentials of soil bacterial communities as major contributors towards sustainable agriculture. As reported by Sivaramaiah *et al.* (2007) the efficacy of *Bacillus* sp. to enhance nodulation, plant dry matter and grain yield on co-inoculation with *Rhizobia* has also been reported for other legumes. Bacterial strains isolated from the maize rhizosphere including *Bacillus, Pseudomonas* and *Serratia* were reported to improve the yield by 9–14% (Lalande *et. al.*, 1989). Significant increase in the root dry mass of rapeseed was observed due to inoculation with *Proteus*, *Klebsiella* and *Bacillus*. The data from these studies along with earlier reported work of Sturz and Nowak (2000) indicated that rhizobacteria from the local rhizosphere soil could be exploited for use as microbial inoculants to improve nodulation (in legumes) and crop productivity of both cereals as well as legumes. Although potential clearly exists for developing such inoculants, their widespread application remains limited by a poor understanding of

microbial ecology and population dynamics in soil, and by inconsistent performance over a range of environments.

The expected variations in microbial diversity might be due to the changes in lignin-cellulosic index in environment during different cropping systems. The present findings indicate necessity of detailed investigation on the biological and chemical variations in soil matrices during each of cropping systems that influence the microbial community profile.

Further, in order to ensure food security in developing countries, there is an urgent need for the sustainable intensification of agricultural production systems towards supporting productivity grains and income generation.

According to us, important observation during the course of the study was that the database for the dynamics of the microbial population and activities was extremely poor. There is dire need to regularly monitor these along with nutritional status in the state. There is necessity to make recommendation and suggestions in package of practices existing in the agricultural extensions on the basis of which agro-advisories are issued for the entire state to consider the role of microbes. It is, indeed, surprising that though agriculture is the mainstay of the state's economy, adequate effort has not gone into building up of a reliable database. More shocking is the fact that the policies have been laid down based on inadequate and, often, misplaced information. The entire issue needs to be reviewed afresh.

Summary

SUMMARY

1. The theme for sustainability in agriculture integrates three main goals: environmental health, economic profitability, and social and economic equity. Sustainability rests on the principle that we must meet the needs of the present without compromising the ability of future generations to meet their own needs. Making the transition to sustainable agriculture is a process. For farmers, the transition to sustainable agriculture normally requires a series of small, realistic steps. The key to moving forward is the will to take the next step with good economic returns.
2. In the state of Punjab there has been a major emphasis on two crops (Wheat and Rice) due to the concept of minimum support price and the subsidy on farm products. This has lead to virtual monoculture practices, which is leading to degradation of nutritional status of the soils and thus the productivity. Diversification of crops is the need of the hour for the nutritional status maintenance of the soil. An alternative to rice-wheat cropping cycle had been demonstrated to convince the farmer of the viability of the alternative system in District Patiala (Punjab – India).
3. The diversification of wheat (*Triticum* spp.) crop could be with alternative crop of chickpea (*Cicer arietinum* L.) and/or sunflower (*Helianthus annuus*). Chickpea (*Cicer arietinum* L.) or Sunflower (*Helianthus annuus*) was grown as an alternative to wheat (*Triticum* spp.) during rabi season and followed by rice (*Oryza sativa*) during kharif season to study the microbial activities, population dynamics and nutritional status in following cropping pattern. Thus three different cropping patterns of chickpea-rice, wheat-rice and sunflower-rice were studied in field conditions using prescribed package of practices.

4. The study area was divided into 4 different blocks (District: Patiala) which were represented by selecting 8 fields as follows: Patiala (PT-1 & PT-1W), Rajpura (RJ-1 & RJ-1W), Derabassi (DB-1 & DB-1W), Samana (SM-5 & SM-5W). One test field of chickpea was sown along with adjacent control plot of wheat.
5. The major benefit apart from the increased returns is water conservation. Chickpea crop requires two irrigations as compared to five in case of wheat cropping thus reducing the consumption of water.
6. The overall trend in three different cropping patterns of chickpea-rice, sunflower-rice and wheat-rice showed significant increase in total microbial population in chickpea-rice cropping pattern as compared to decrease in sunflower-rice and wheat-rice cropping.
7. The overall trend showed no change or increase in cellulose degraders population during chickpea- rice -chickpea cropping in all locations except in Derabassi fields while during wheat- rice -wheat cropping the cellulose degraders population always decreased.
8. The results showed the increase in phosphate solubilizers population in chickpea-rice as well as sunflower-rice cropping pattern as compared to decrease in wheat cropping pattern. The overall trend in different cropping patterns did not show any significant differences in the phosphate solubilizers population at any stage of crop growth.
9. Among the three cropping patterns both chickpea-rice and wheat-rice showed increase in soil phosphatase activity only with rice crop while with sunflower-rice crops maximum soil phosphatase activity was at flowering in both sunflower and rice.

10. In all the three different cropping patterns an increase in soil cellulase activity was observed with not much significant difference between various stages of growth.
11. The elemental analysis of the soil in all four blocks indicated that during chickpea-rice cropping organic carbon, total nitrogen as well as available phosphorus increased as compared to wheat-rice cropping.
12. During the above comparative field experiments, the results showed a positive correlation with r-value of 0.766 among the soil cellulase activity and cellulose degraders population during the chickpea-rice cropping, as compared to wheat-rice and sunflower-rice with r-value of 0.655 and 0.354 respectively. On the other hand no correlation was observed among the soil phosphatase and phosphate solubilisers population in all three cropping pattern of chickpea-rice, wheat-rice and sunflower-rice with r-value of 0.038, 0.007 and 0.002 respectively.
13. During the course of the present study various bacterial isolates were screened from agricultural soils for good cellulase activity. Three isolates MSK1, MSK13 and MSK24 based on their high efficacy to degrade carboxy methyl cellulose (CMC) were chosen and were identified as *Serratia* sp., *Pseudomonas* sp. and *Serratia* sp. respectively by using 16S rDNA.
14. These cellulolytic bacteria *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 were resistant to ampicillin, thus ampicillin was selected as marker for population survival along with ERIC-PCR method. Plot studies were conducted by mass inoculation of the above bacterial inoculums in plots (2X2m) sown with chickpea (*Cicer arietinum L.*) and sunflower (*Helianthus annuus*).

15. During chickpea plot experiments, a significantly high value of soil cellulase activity as well as organic carbon was seen in *Serratia* sp. MSK24 inoculated plots which resulted in a significantly enhancement of cellulose degraders population which could be positively correlated (R^2=0.906) to the higher soil cellulase activity. The results showed an increased organic carbon in the treated plots, which was showed a positive correlation (R^2=0.916) between the soil cellulase activity and soil organic carbon during the chickpea plot trails. A positive correlation (R^2=0.853) was also observed among soil phosphatase activity and phosphate solubilisers population, which was also positively correlated (R^2=0.730) to available phosphorus.
16. In contrary to the above in sunflower plot experiments, a significantly high value of soil cellulase activity as well as organic carbon was seen in *Serratia* sp. MSK24 inoculated plots and resulted in a significantly enhancement of cellulose degraders population which could be mild positively correlated (R^2=0.723) to the higher soil cellulase activity. The experiment results showed a decreased organic carbon in the treated plots, which was observed as a negative correlation (R^2=0.145) between the soil cellulase activity and soil organic carbon. A negative correlation (R^2=0.280) was also among soil phosphatase activity and phosphate solubilisers population, which was also negatively correlated (R^2=0.570) to available phosphorus.
17. The introduction of these bacteria in chickpea soils, or cultural practices aimed to increase the activity of native strains of these bacteria could greatly contribute to the efficiency of soil organic matter with *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24.

18. The average yield of chickpea was 4 to 11 Q/acre as compared to 15 to 19 Q/acre of wheat. The cost of input for chickpea was 22% higher as compared to wheat mainly due to higher seed cost. However the net returns are approx. ₹ 5000 more per acre over wheat.
19. Sunflower sowing was done in Rajpura block only. The overall average yield of sunflower was 10 Q/acre. The costs of inputs for sunflower were 13% higher as compared to wheat. However due to higher selling price the net returns were 31% higher ie. ₹ 5000 more per acre over wheat.
20. The results further indicate that chickpea-rice cropping system gave the highest profit with benefit to cost ratio of 4.22 in year 2003-04 and similar trend was observed in current year 2008-09 with 5.50. This cropping pattern was found to be more sustainable in terms of yield, water saving, organic matter, microbial activities, economic analysis and prosperity of farmers. From the agricultural and ecological viewpoints, the aims will be achieved towards sustainable soil productivity, while maintaining environmental quality with low stress on water table.

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Annexure

ANNEXURE I

Soil extract media

Soil extract was made in 1L of tap water mixed with 400 grams of agricultural field soil and 1 gram sodium carbonate, kept at shaker (120 rpm) overnight and then steam sterilized in autoclave for 1 hour, filtered to separate water soluble part.

Ingredients	**Quantity**
Soil extract	50 mL
Distilled water	950 mL
Yeast extract	2.0 g
Peptone	5.0 g
Agar	15.0 g

pH 7 adjusted with 5N NaOH. Sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.

Pikovskaya's agar

Ingredients	**Quantity (g/L)**
Agar	15.0
Ammonium sulphate	0.5
Calcium phosphate	5.0
Dextrose	10.0
Ferrous sulphate	0.0001
Magnesium sulphate	0.1
Manganese sulphate	0.0001
Potassium chloride	0.2
Yeast extract	0.5

Suspended 31.3 g in 1000 ml distilled water. Boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.

Luria-broth (LB) medium

Ingredients	**Quantity (g/L)**
Nacl	10.0
Yeast extract	5.0
Tryptone	10.0
Agar	10.0

pH 7 adjusted with 5N NaOH. Sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min. (Added filter sterilized ampicillin 50 µg/ml to prepare LB - Ampicillin plates)

Bushnell Haas – broth/agar* (BHB/ BHA) medium with Carboxymethyl cellulose (CMC) for cellulose degraders studies

Ingredients	Quantity (g/L)
Magnesium sulfate	0.2
Calcium chloride	0.02
Monopotassium phosphate	1.0
Diammonium hydrogen phosphate	1.0
Potassium nitrate	1.0
Ferric chloride	0.05
Carboxymethyl cellulose (CMC)	10
*Agar	15

pH 7 adjusted with 5N NaOH. Sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.

Nitrate agar

Ingredients	Quantity (g/L)
Agar	12.0
Beef extract	3.0
Peptic digest of animal tissue	5.0
Potassium nitrate	1.0

Suspended 21 g of nitrate agar in 1000 ml distilled water. Boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min.

Bray's P-1 extractant

Dissolved 1.110 g of AR grade ammonium fluoride in one liter of 0.025N HCl.

DNSA (3,5-Dinitrosalicylic acid) Reagent

Solution A	
Sodium-potassium tartarate	300 g
Distilled water	500 mL

Solution B	
di-nitro Salicyclic Acid	10 g
NaOH Solution	200 mL (2 M)

Mixed the solution A and B and made the volume to 1 litre

Modified universal buffer (5X)

Ingredients	Quantity
Tris (hydroxyl methyl) amino methane	3.025 g
Maleic acid	2.90 g
Citric acid	3.50 g
Boric acid	1.57 g
NaOH (1N)	122 ml
Water	up to 250 ml
pH	6.5

Phosphate buffer

Stock solution A
2 M monobasic sodium phosphate, monohydrate (276 g/L)

Stock solution B
2 M dibasic sodium phosphate (284 g/L).

Mixing an appropriate volume (ml) of A and B as shown in the table below and diluting to a total volume of 200 ml, a 1 M phosphate buffer of the required pH at room temperature.

A (mL)	B (mL)	pH
39.0	61.0	7.0
33.0	67.0	7.1
28.0	72.0	7.2
23.0	77.0	7.3
19.0	81.0	7.4
16.0	84.0	7.5

TBE buffer (10x)

Tris-HCl	0.09 M (pH 8)
Boric acid	0.9 M
EDTA	0.02 M (pH 8)

Agarose gel loading dye (6X)

Bromophenol blue	0.25%
Xylene cyanol FF	0.25%
Glycerol in water	30.0%

Primers

ERIC forward primer	5’- ATGTAAGCTCCTGGGGAATCAC -3’
ERIC reverse primer	5’- AAGTAAGTGACTGGGGTGAGCG - 3’
16S rDNA forward primer	5’- AGAGTTTGATCCTGGCTCAG -3’
16S rDNA reverse primer	5’- ACGGGCGGTGTGTTC -3’

ANNEXURE II

Photo 1: Chickpea (*Cicer arietinum L.*) crop was sown on ridges in field during experimental trails.

Photo 2: Chickpea (*Cicer arietinum L.*) crop was sown on ridges in field during experimental trails.

Photo 3: Chickpea (*Cicer arietinum L.*) crop after germination during early stage (4 weeks after sowing) at Samana (SM) block.

Photo 4: Chickpea (*Cicer arietinum L.*) crop during midtime stage (8 weeks after sowing) at Samana (SM) block.

Photo 5: Root nodulation in Chickpea (*Cicer arietinum L.*) crop was observed during 4 weeks after sowing at Derabassi (DB) block.

Photo 6: Farmer in Chickpea (*Cicer arietinum L.*) experimental field during midtime / flowering stage (8 weeks after sowing) at Rajpura (RJ) block.

Photo 7: Chickpea (*Cicer arietinum L.*) crop during midtime / flowering stage (8 weeks after sowing) at Samana (SM) block

Photo 8: Chickpea (*Cicer arietinum L.*) crop during fruit bearing / pod setting stage at Rajpura (RJ) block

Photo 9: Chickpea (*Cicer arietinum L.*) crop during fruit bearing / pod setting stage at Samana (SM) block

Photo 10: Chickpea (*Cicer arietinum L.*) crop during harvest at Patiala (PT) block.

Photo 11: Farmer yielded 11.50 quintal/ acre at Rajpura (RJ) block.

Photo 12: Chickpea (*Cicer arietinum L.*) crop during harvest at Rajpura (RJ) block.

Photo 13: Sunflower (*Helianthus annuus*) blooming at Rajpura (RJ) block.

Photo 14: Sunflower (*Helianthus annuus*) crop during flowering stage at Rajpura (RJ) block.

Photo 15: Farmer in Rice field during experimental trails at Patiala (PT) block.

Photo 16: Farmer in Rice field during experimental trails at Rajpura (RJ) block.

Photo 17: Farmer in Rice field during experimental trails at Samana (SM) block.

Photo 18: Rice variety (HBC-19) at maturity of crop in Patiala (PT) block.

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Influence of diversified cropping pattern on microbial activity and population dynamics in agricultural soils

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ABSTRACT

Agricultural practices that reduce soil degradation and improve agricultural sustainability are needed particularly for tropical/sub-tropical soils. Soil enzyme activities and microbial populations can provide information on how soil management is affecting the potential to perform the processes in soils such as decomposition and nutrient cycling. Soil enzyme activities and microbial population dynamics were investigated in an experiment on silt loam soil where three cropping patterns (chickpea/rice, wheat/rice and sunflower/rice) were studied under field conditions. The enzyme activities showed that among the three cropping patterns, chickpea/rice showed good correlation between the cellulase and phosphatase activity and the cellulose and phosphate degraders and also increase in nutrient availability due to these enzyme activities, while in the sunflower/rice both enzyme activities decreased during the sunflower cropping but recovered during the rice crop. Thus, among the three cropping patterns chickpea/rice appears to be more sustainable.

Keys words : Agricultural practices, cropping pattern, ecosystem, enzyme activities, microbial population, soil quality

INTRODUCTION

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. It is a critical resource not only for agricultural production and food security but also towards maintenance of most life processes. The functions of soil biota are central to decomposition processes and nutrient cycling (Tilak *et al.*, 2005).

Plant growth promoting bacteria promote growth directly e. g. by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators (Kloepper, 1997). Cellulose is the most abundant organic compound in the biosphere, comprising almost 50% of the biomass synthesised by photosynthetic fixation of CO_2 (Eriksson *et al.*, 1990). Growth and survival of micro-organisms important in most agricultural soils is influenced by carbon availability in the soils. Cellulases play an important role in carbon availability and so can be used to give preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies (Ndakidemi and Makoi, 2008). In soil ecosystems, phosphatases play a critical role in plant growth by enhancing the availability of phosphorus due to enhanced solubilisation and remobilisation of phosphate (Speir and Ross, 1978). Understanding the dynamics of enzyme activities in these systems is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth in biologically-managed systems because of a higher quantity of organic C found in those systems (Aon and Colaneri, 2001).

In the present study, total microbial population as well as cellulose and phosphate degraders were estimated alongwith soil cellulase and phosphatase activity in the varying cropping pattern of agricultural soil.

MATERIALS AND METHODS

Soil Sampling and Analysis

Soil samples used in the present study

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were collected from wheat (*Triticum* spp.), chickpea (*Cicer arietinum* L.), sunflower (*Helianthus annuus*) during **rabi** season and followed by rice (*Oryza sativa*) during **kharif** season in agricultural plots sown at Village : Gajju Khera, Block : Rajpura in District : Patiala (Punjab). Soil samples were collected randomly at single depth of 30 cm at nine different sites in one acre field. The three composite samples were made from nine collected samples. Soils were processed the same day for microbial and enzymatic activities using standard protocols. Air-dried and pulverized soil samples were analyzed for organic carbon, nitrogen, available phosphorus, potassium, moisture level and pH by standard method (Jackson, 1967). Field studies were conducted in silt loam (26.91% sand, 54.69% silt and 18.40% clay) agricultural soil with organic carbon, total nitrogen and available phosphorus of 0.50±0.02%, 0.014±0.005 % and 35.52±2.48 mg/kg.

Enumeration of Microorganisms from Soil Samples

Microbial populations were estimated by soil dilution technique using plate count method. The three different media used for enumeration and detection of viable microbes i. e. Soil Extract Media for total microbial count, Pikovskaya Agar for phosphate solubilizers count and Bushnell Haas Agar with 1% (w/v) carboxy-methyl cellulose (CMC) for cellulose degraders count. The plates were incubated in BOD incubator for 24-48 h at 37°C. All the samples were plated in triplicates in three different dilutions and the colonies counted were expressed as cfu/g dry weight of soil.

Assaying for Soil Enzyme Activities

Soil microbial activity was measured in terms of two important soil enzymes i. e. cellulases and phosphatases. Cellulase activity was determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959), through the estimation of the amount of reducing sugars liberated from carboxy-methyl cellulose (CMC) solubilised in 50 mM Tris-HCl buffer, pH 7.0 (Bailey *et al.*, 1992). Cellulase activity was determined by using a calibration curve for glucose and expressed as µM glucose/g/h. Phosphatase activity was estimated with the PNP method (Tabatabai and Bremner, 1969) using modified universal buffer (MUB). All determinations were made in triplicate and expressed on a dry weight basis as µM *p*-nitrophenol (PNP) /g/h.

Data Analysis

Statistical analysis was performed by ANOVA and special effects were tested by the Newman Keuls test with a critical range of 5% using COSTAT software. Data characterized by the same letter were not significantly different.

RESULTS AND DISCUSSION

Chickpea (*Cicer arietinum* L.), wheat (*Triticum* spp.), sunflower (*Helianthus annuus*) during **rabi** season and followed by rice (*Oryza sativa*) during **kharif** season were used to study the total microbial count and soil enzyme activities viz., cellulases, phosphatases. The three different cropping patterns of chickpea-rice, wheat-rice and sunflower-rice were studied in field conditions using prescribed package of practices. The above crops were grown at farmers' field in village Gajju Khera of Rajpura block in Patiala district (Punjab. India). Nutritional content of the soil was analyzed by quantifying the organic carbon, total nitrogen, available phosphorus and available potassium. The microbial population dynamics was quantified in terms of total microbial count, phosphate solubilizers and cellulose degraders using plate count as well as the cellulose enzyme responsible for the formation of organic matter and phosphatase for phosphate solubilization were assayed at the different stages of crop (sowing of crop followed by pod/ grain setting stage (midtime) and during time of crop harvest).

The total microbial population observed at the time of sowing of chickpea was 39×10^6 which increased to 1.49×10^8 at the harvest. During the sowing of rice in the same field the population was 58×10^6 which increased to 87×10^6 during rice harvest. With wheat at time of sowing, the total microbial population was 94×10^6 which decreased to 62×10^6 during the harvest of the crop. Sowing of rice in the same field increased the population to 117×10^6 which then decreased to 99×10^6 at harvest time of rice. During the time of sowing of

sunflower, the total microbial population was 102 x 10^6 which decreased to 70 x 10^6 during the harvest. Sowing of rice in the same field increased the population to 71 x 10^6 which was 73 x 10^6 at harvest time of rice. (Figs. 1, 2 and 3). The overall trend in different cropping patterns as observed showed significant increase in total microbial population in chickpea-rice cropping pattern as compared to decrease in sunflower-rice and wheat-rice cropping pattern.

Cellulose degrading population during the chickpea sowing was 4.6 x 10^6 which increased to 32 x 10^6 at the time of crop harvest. Sowing of rice in the same field showed an increase in cellulose population to 42 x 10^6 during the rice crop growth.. In wheat fields cellulose degrading population was 54 x 10^6 which decreased to 30 x 10^6 at harvest time and then increased to 45 x 10^6 when rice was grown in these fields. With sunflower, the cellulose degrading population at sowing was 17 x 10^6, and increased to 35 x 10^6 at harvest time. Sowing of rice in the same field decreased the population to 22 x 10^6 at rice harvest (Figs. 1, 2 and 3). The overall trend in different cropping patterns as observed showed an increase in cellulose degrading population in all the three chickpea-rice, sunflower-rice and wheat-rice cropping patterns but the increase was not significant at various stages of crop growth.

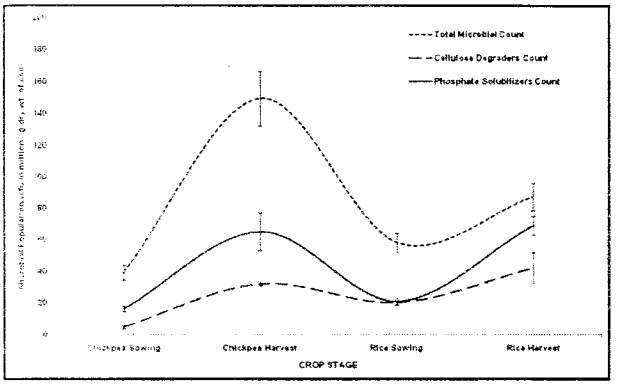

Fig. 1. Population dynamics in chickpea-rice cropping pattern at various stages of crop growth.

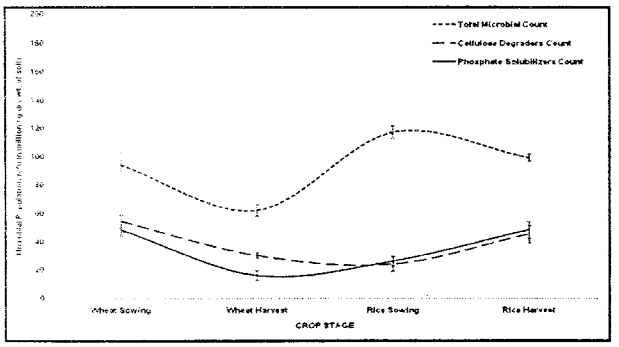

Fig. 2. Population dynamics in wheat-rice cropping pattern at various stages of crop growth.

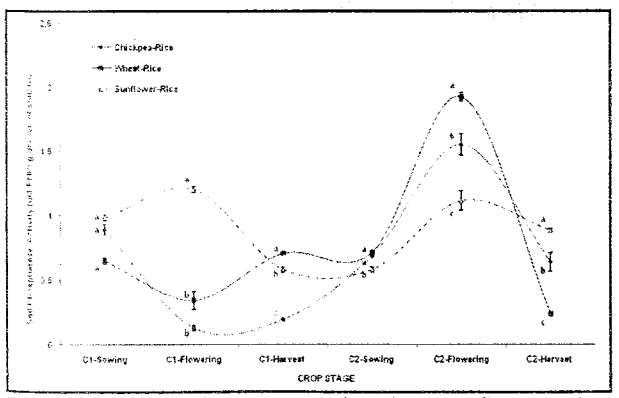

Fig. 3. Population dynamics in sunflower-rice cropping pattern at various stages of crop growth.

Phosphate solubilizers population showed an increase from 16 x 10^6 to 65 x 10^6 during the chickpea growth. Growth of rice in the same field did not increase the phosphate solubilizers population, it remaining at 69 x 10^6 at harvest of rice. In fields where wheat was grown phosphate solubilizers population decreased from 48 x 10^6 at the time of sowing to 16 x 10^6 at harvest. Growth of rice did not change the population much with an increase to 48 x 10^6 at rice harvesting. In sunflower-rice combination, phosphate solubilizers population decreased from 50 x 10^6 at the time of sowing of sunflower to 8 x 10^6 at sunflower harvest. Rice growth in the same field increased the population to 32 x 10^6 at rice harvest. (Figs. 1, 2 and 3). The results showed the increase in phosphate solubilizers population in chickpea-rice as well as sunflower-rice cropping pattern as compared to decrease in wheat cropping pattern. The overall trend in different cropping patterns observed to be no significant differences in the phosphate solubilizers population at each stage of crop growth.

Our results showed that total microbial count was promoted significantly in chickpea-rice grown field as compared to other treatments, which showed its dependence on its physical, chemical, microbiological and biochemical properties. The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content,

composition and activity of its living organisms as microbial metabolic activities and intensity of the biological processes (Stevenson, 1986).

The soil enzymes are constantly being synthesised, accumulated, inactivated and/or decomposed in the soil, hence, playing an important role in agriculture and particularly in nutrients cycling (Tabatabai, 1994; Dick *et al.*, 2000). The soil phosphatase activity during chickpea cropping was the highest at the time of sowing (0.891 µM PNP/g/h), decreased at flowering (0.129 µM PNP/g/h) and then increased slightly to 0.192 iM PNP/g/h first. Rice cropping in the same field stimulated phsophatase activity to 0.69 µM PNP/g/h during the sowing and which further increased to 1.55 µM PNP/g/h at flowering but decreased to 0.641 µM PNP/g/h of soil at harvest. In wheat crop, the soil phosphatase activity varied from 0.649 to 0.344 µM PNP/g/h at sowing and harvesting with the activity of 0.706 µM PNP/g/h during the flag-leaf or midtime stage of crop. When rice was grown in the same field after wheat the phosphatase activity increased to 0.716 µM PNP/g/h during the sowing and further increased to 1.92 µM PNP/g/h at midtime of crop but then decreased to 0.235 µM PNP/g/h of soil at rice harvest. With sunflower-rice cropping, the soil phosphatase activity increased with both sunflower and rice from 0.985 to 1.205 µM PNP/g/h at sowing and flowering of sunflower and then decreased to 0.584 µM PNP/g/h at harvest. With rice also the activity decreased to 0.584 µM PNP/g/h during the sowing and further increased to 1.11 µM PNP/g/h at flowering of crop, but decreased to 0.886 µM PNP/g/h of soil at harvest (Fig. 4). Among the three cropping patterns, both chickpea-rice and wheat-rice showed increase in phosphatase activity with rice crop, while with sunflower-rice crops maximum phosphatase activity was at flowering in both sunflower and rice. In soil ecosystems, phosphatase is believed to play critical roles in phosphorus cycle (Speir and Ross, 1978) as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Dick *et al.*, 2000). The amount of phosphatase exuded by plant roots has been shown to differ between crop species (Yadav and Tarafdar, 2001) and varieties (Izaguirre-Mayoral *et al.*, 2002), as well as crop management practices (Ndakidemi, 2006). To date, there have been few studies examining the influence of management options in the ecosystem on phosphatases activity in soil where most crops are grown. Understanding the dynamics of enzyme activities in these systems is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth.

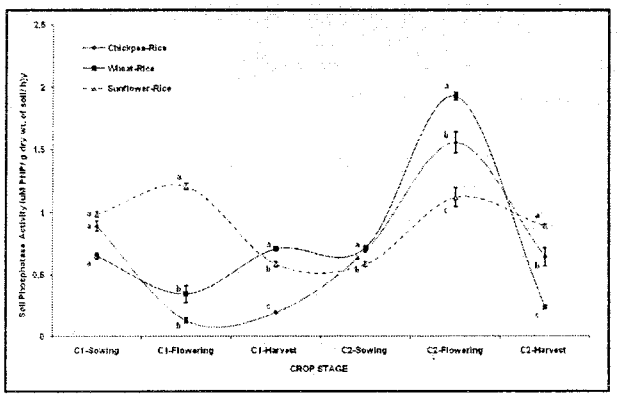

Fig. 4. Soil phosphatase activity in different cropping patterns at various stages of crop growth.

The activities of enzymes in soils undergo complex biochemical processes and in this regard, all soils contain a group of enzymes that determines soil metabolic processes (McLaren, 1975). Soil cellulase activity with chickpea-rice cropping showed an increase from sowing to harvest, increasing from 1.351 to 4.767 µM glucose/g/h at flowering and then to 6.612 µM glucose/g/h at harvest. When rice was sown after chickpea, cellulose activity decreased from 10.29 µM glucose/g/h at sowing to 7.24 µM glucose/g/h at flowering and then increased to 12.84 µM glucose/g/h at harvest. In wheat-rice cropping with wheat cellulose activity showed increase after sowing from 1.017 to 4.449 µM glucose/g/h at flowering and then to 5.389 µM glucose/g/h at harvest. With rice cellulose activity showed no increase from sowing to flowering (8.601 µM glucose/g/h to 8.307 µM glucose/g/h) and then decreased to 6.611 µM glucose/g/h at flowering. During the sunflower cropping, the soil cellulase activity varied from 7.16 to 5.749 µM glucose/g/h at sowing and maturity, respectively, with a transient increase at flowering (10.21 µM glucose/g/h) Rice crop after sunflower showed continuous increase from sowing to flowering and maturity (6.515 µM glucose/g/h to 8.354 to 10.76 µM glucose/

g/h). In all the three different cropping patterns, an increase in cellulose activity was observed with not much significant difference between various stages of growth (Fig. 5). Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme better so that it may be used more regularly as a predictive tool in our soil fertility programmes. More information on the role of this enzyme is needed since it is affected by different factors which may jeopardise its involvement in the decomposition of cellulolytic materials in the soil for microbial use and improve soil health in agricultural ecosystems. The maximum microbial population and activities increase was observed in chickpea-rice cropping pattern as compared to the in sunflower-rice and wheat-rice cropping pattern. In the present study, the increase in soil cellulase activity in all the three chickpea-rice, sunflower-rice and wheat-rice cropping pattern was observed. This suggests that the activities of cellulases in agricultural soils are affected by several factors. These include temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon, quality of organic matter/plant debris and soil mineral elements (Deng and Tabatabai,1994) and the trace elements (Arinze and Yubedee, 2000). All these findings suggest that activities of cellulases can be used to give preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies.

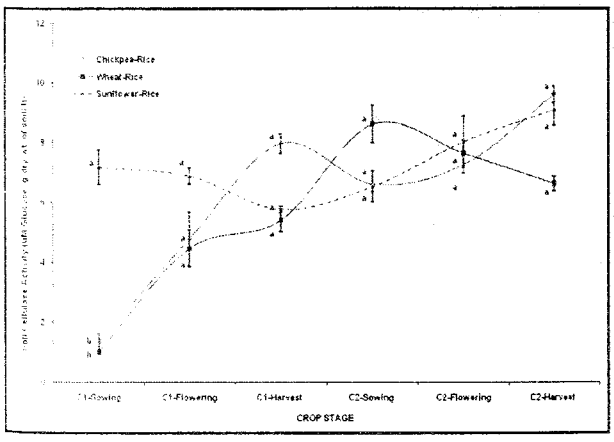

Fig. 5. Soil cellulase activity in different cropping patterns at various stages of crop growth.

In conclusion, differences in total microbial populations were found amongst the different cropping patterns and this confirmed the transitory nature of the soil biochemical reactions and interactions involved. The different crops growing in alternation with rice under the same soil and package of practices did not vary the microbial enzyme activities, but did result in different total microbial population present. Higher stability of soil after chickpea cropping was related to the higher microbial population and activities found.

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Fingerprinting of Cellulose Degrading Bacteria from Agricultural Soils

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Bacteria were isolated and screened for cellulase activities in agricultural soil of Patiala (India). Three most efficient cellulose degraders identified using 16S rDNA, as *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* showed cellulase activity of 3.83, 4.21 and 4.52 mM glucose ml^{-1} h^{-1} respectively. . Enterobacteriaceae Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was performed to obtain fingerprint and showed clear genetic variability among all three isolates. The bacteria were mass cultured and re-inoculated in soils to study the population dynamics under chickpea (*Cicer arietinum L.*) cultivation. The role of cellulose degrading bacteria was studied in sustainability of soil and organic carbon indicating good survival of 40.2, 34.4 and 56.8 % of the introduced strains respectively. The highly positive correlation (R^2=0.916) among the soil cellulase activity and soil organic carbon was observed during the chickpea plot trails towards inoculated bacterial strains.

Key words: Agricultural soils, Chickpea, Cellulase, *Pseudomonas*, *Serratia*, ERIC-PCR.

Intensification of agriculture and increases in population pressure has reduced the structural stability of soils. To cope with low productivity, inorganic fertilizers have been intensively used for the past two decades. This escalated the soil problems including structural degradation, reduction of organic matter and cellulose. Agricultural residues are a rich source of cellulose (Hameeda, 2006). This is the most abundant organic compound in the biosphere, comprising almost 50% of the biomass synthesised by photosynthetic fixation of CO_2 (Eriksson et al., 1990). Cellulose serves the most abundant carbon source for the growth and survival of agriculturally important microorganisms. However, cellulose in plant debris has to be degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulases enzymes (White, 1982). Cellulases are a group of enzymes that catalyse the degradation of cellulose, polysaccharides build up of α-1, 4 linked glucose units (Deng and Tabatabai, 1994). It has been reported that cellulases in soils are derived mainly from plant debris incorporated into the soil, and that a limited amount may also originate from fungi and bacteria in soils (Richmond, 1991). Studies have shown that activity of cellulases in agricultural soils are affected by several factors

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such as temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon (Rubidge, 1977; Gomah, 1980; Tabatabai, 1982; Klein, 1989; Alf and Nannipieri, 1995), quality of organic matter/plant debris and soil mineral elements (Hope and Burns, 1987; Sinsabaugh and Linkins, 1989;) and the trace elements (Arinze and Yubedee 2000; Atlas *et al.,* 1978). Several mechanisms have been proposed in the degradation of cellulose by cellulases (Rees, 1975; Wood, 1991). All these findings suggest that activities of cellulases can be used to give preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies. Research efforts now focused on discovering new enzymes from microbial diversity in the soil, the most appropriate practices that may positively influence their activities for improved plant growth as well as improving the biological environments in order to sustain other life types (Ndakidemi and Makoi, 2008).

The present study is on the isolation and screening of cellulose degrading bacteria from the agricultural fields of chickpea (*Cicer arietinum L.*) crop, which push us to identify and fingerprint new bacterial strains capable to utilize cellulose from local agricultural soils.

MATERIAL AND METHODS

Soil sampling and analysis

Soil samples used for the isolation of cellulose degrading bacteria were collected from different chickpea (*Cicer arietinum L.*) agricultural fields in four blocks of Patiala District, Punjab. The samples were drawn with a hollow pipe (4 cm in diameter) from 25cm in depth. Air-dried and pulverized soil samples were analyzed for organic carbon, total nitrogen, available phosphorus, moisture level and pH by standard method. (Jackson MH, 1967).

solation of bacterial strains for cellulose degradation

Soil (approximately 10 g) was enriched for cellulose degrading bacteria using carboxymethyl cellulose (CMC) as the only added source of carbon in Bushnell Haas Broth (BHB) medium having composition of magnesium sulphate-0.20 g/L; calcium chloride-0.02 g/L; monopotassium phosphate-1 g/L; di-potassium phosphate-1 g/L; ammonium nitrate-1 g/L; ferric chloride-0.05 g/L. Bacterial strains were then isolated by dilution technique on Bushnell Haas Agar (BHA) plates containing carboxymethyl cellulose (1%; w/v) and incubated at 37 °C for 3 days.

Enzyme assay for screening of bacterial isolates for cellulose degradation

Bacterial isolates were grown in 50ml Bushnell Haas Broth (BHB) with carboxymethyl cellulose (1% ; w/v) in 250-ml flask and incubated on rotary shaker at 37°C, 120 rpm. After 3 days, the cells were centrifuged (9000 xg, 10 min) and supernatant was collected for enzyme assay. Cellulase (CMCase) activity was measured by the DNS (3,5-dinitrosalicylic acid) method (Miller, G.L., 1959), through the determination of the amount of reducing sugars liberated from carboxymethyl cellulose (CMC) solubilised in 50 mM Tris–HCl buffer (pH 7.0) (Baily, M.J., *et al.,* 1992). Enzyme assay was carried out at 37 °C for 1hr and the reaction was stopped by the addition of DNS solution. Samples were then boiled for 10 min, cooled on ice for color stabilization, and the optical density was measured at 540 nm. Cellulase activity was determined by using a calibration curve for glucose and expressed as ìM glucose g^{-1} h^{-1}.

Molecular characterization of cellulose degrading bacterial isolates

Chromosomal DNA was extracted by a modified ROSE (rapid one step extraction) method (Steiner *et al.*, 1995). Bacterial isolates were identified by amplification of 16S rDNA gene sequence with the following primers: Forward primer 5' -AGA GTTTGATCCTGGCTCAG-3' and reverse primer 5'-ACGGGCGGTGTGTTC -3' (Weisberg et al. 1991). DNA amplification was performed with Genamp PCR system (Applied Biosystem, USA). Reaction mixture for the PCR contained 1X PCR buffer (Invitrogen, USA), each deoxynucleotide triphosphate at a concentation of 200µm, 1.5 mM $MgCl_2$, each primer at a concentration of 0.1 µm and 2.5U of Taq DNA polymerase (Invitrogen, USA) in a final volume of 100 µl. PCR conditions were as follows: Preheating at 92°C for 2min, 36 cycles of 92°C for 1min, 48°C 30sec and 72°C for 2min and final

extension 72°C for 6min. Amplified DNA was verified by electrophoresis of aliquots of PCR product (5µl) in 1.0% agarose gel in 1X TAE buffer. PCR product was cloned into the pDrive-cloning vector and transformed into *E. Coli* DH5á (Hanahan, 1983). 16S rDNA sequence of the bacterial isolates and similar sequences were aligned using the Clustal W and dendrograms were generated by the distance neighbour-joining method using MEGA3 software (Kumar, et al., 2004). Numbers are percent gap values after 500 bootstrap replications. Only values above 75% were considered.

Plot studies

Plot studies were conducted in sandy loam agricultural soil. Chemical composition of this soil was organic carbon, 0.78±0.03 %; total nitrogen, 0.0823±0.005 %; and available phosphorus 44.00±5.6 mg/kg. Randomized design was adopted to study the degradation of cellulose in the agriculture soil by inoculating the most efficient cellulose degraders in plots (2×2m) sown with chickpea (*Cicer arietinum L.*) crop. Cultures were grown in 10 litre shake flasks containing LB medium and incubated at (37°C, 120 rpm). After 36 h, the cells were harvested and washed with 10mM phosphate buffer (pH 6.8). Pellets were resuspended in buffer and inoculated to plots to achieve 2×10^8 cfu/g soil. Control plot received only BHB without any bacterial cells. The prescribed package of practices was followed for growth of the chickpea and monitored regularly. After regular intervals of time during different stages of crop growth, soil samples were collected from five different points, mixed together and then three samples were taken to evaluate the population dynamics and other soil parameters (microbial load, enzymatic activity, organic carbon, total nitrogen, available phosphorus and microelements). Equal amount of soil (1 g/ sample) was resuspended in 9 ml of sterilized saline water (0.8% NaCl; w/v) by vigorous vortexing and serially diluted and an aliquot of 100 ul was plated on Luria agar (LA) containing ampicillin (120 ug/ ml). Plates were incubated for 24–36 h at 37 °C. Individual colonies obtained after this incubation were patched on LA plates containing ampicillin (120 ug/ml). Colonies grown were scored and their DNA was isolated for DNA fingerprinting based on ERIC-PCR.

Development of DNA fingerprints and tracking the fate of introduced population

Enterobacteriaceae Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to obtain DNA fingerprints of the cellulose degraders. PCR primer sequences (Versalovic, *et al.,* 1991), ERIC – IR (5' – 3') – ATG TAA GCT CCT GGG GAA TCA C- and ERIC – 2 (5' – 3') - AAG TAA GTG ACT GGG GTG AGC G- were obtained from Life Technologies, USA. Single isolated colonies were picked at random from the LA plates, suspended in 50 µl water, and lysed by heating for 10 min at 95°C. Cell lysate was centrifuged (9000Xg, 3 min) at 4°C, and 2 µl of the supernatant was used in the reaction mixture. The reaction mixture (25 µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin (wt/vol), 0.25 mM each of dNTPs, 0.375 µM each of primers ERIC-1R and ERIC-2, and 2.0 units of Taq polymerase (Life Technologies, USA). The amplification was done on Genamp PCR system (Applied Biosystem, USA). The PCR conditions are as follows: initial denaturation at 94°C for 4 min followed by 41 cycles at 94°C for 1 min, at 50°C for 1 min and at 72°C for 2 min. The final extension was done at 72°C for 5 min. The reaction was terminated by using a loading dye (1 µl) containing 15% Ficoll, 0.25% bromophenol blue, and 0.25% xylene cyanol. Each PCR product was resolved by electrophoresis on a 1.5% agarose gel.

Data analysis

Statistical analysis was performed by ANOVA and special effects were tested by the Newman Keuls test with a critical range of 5% using COSTAT software. Data characterized by the same letter were not significantly different.

RESULTS AND DISCUSSION

Isolation and screening of cellulose degrading bacteria

Various bacterial isolates obtained from chickpea (*Cicer arietinum L.*) agricultural soils were screened for cellulase activity. Three isolates *MSK1, MSK13* and *MSK24* based on their high efficacy to degrade carboxy methyl cellulose (CMC) were chosen for further studies and were identified as *Serratia sp., Pseudomonas sp.* and

Serratia marcescens respectively by using 16S rDNA. *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* showed the cellulase activity of 3.83, 4.21 and 4.52 mM glucose ml^{-1} h^{-1} respectively after hrs (Fig: 1). Sindhu et al, (2001) reported both aerobic cellulolytic organisms such as *Pseudomonas* & actinomycetes, facultative anaerobes such as *Bacillus* and *Cellulomonas*, and also strict anaerobes such as *Clostridium* while *Serratia marcescens* EB 67 and *Pseudomonas sp.* CDB 35 have been shown to possess cellulolytic activity in the presence of crop residues (Hameeda *et al,* 2006).

Bacterial strains can be differentiated by genomic DNA fingerprints based on ERIC-PCR gel electrophoresis, as some of the standard strains are shown in Fig. 2 (Lane 11-13). DNA fingerprinting based on the ERIC-PCR (enterobacterial repetitive intergenic consensus) of these isolates showed 10 to 14 amplified bands with sizes ranging from 11,000 bp to 100 bp of varying intensity. Genotypic relationships among microorganisms have been determined by analyzing the genomic DNA with PCR-based methods (Pooler, 1996). In the present study, unique ERIC PCR genotypic fingerprints of different strains isolated from agricultural soil samples were standardized and used for the enumeration of the introduced cellulolytic bacterial isolates. ERIC-PCR has been used successfully to generate DNA fingerprints to distinguish between genetically unrelated isolates and closely related bacterial strains (Dombek, 2000). Although 16S rDNA sequence analysis showed that *MSK1* and *MSK24* to have close homology with *Serratia sp.*, but ERIC-PCR based fingerprinting analysis enabled us to differentiate between both the isolates.

Survival of bacterial strains inoculated in plot trails

The cellulolytic bacterial *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* were resistant to ampicillin, thus ampicillin was selected as marker for population survival along with ERIC-

Table 1. Survival of introduced population during the growth of chickpea crop in plots. Soil suspension diluted in physiological saline was plated on LA-ampicillin plate and genomic DNA of the ampicillin resistant bacteria was amplified by ERIC-PCR

		Colony forming units (X 10^7cfu/g of dry wt. soil)				
Time (weeks)		0	4	8	12	16
Control	Ampicillin count	2.2	1.8	1.5	1.7	1.3
	PCR count	0	0	0	0	0
Serratia sp.	Ampicillin count	15.0	13.0	10.0	8.5	8.0
	PCR count	11.7	10.4	7.2	5.8	4.7
Pseudomonas sp.	Ampicillin count	13.0	12.0	8.8	7.0	6.0
	PCR count	10.2	9.9	5.6	5.1	3.5
Serratia marcescens	Ampicillin count	15.0	14.0	11.5	10.5	9.0
	PCR count	13.4	12.0	9.3	8.7	7.6

Table 2. Cellulase activity and organic carbon in soils of chickpea plot. Cellulase activity and organic carbon were determined at 0 week (Chickpea sowing stage) and 16 weeks (Chickpea harvest stage) in soils

Treatmentplot	Cellulase activity (uM Glucose / g dry wt soil/ hr))		Organic carbon(%)	
	0 Week	16 Week	0 Week	16 Week
Control	0.784±0.048b	4.489±0.322d	0.340±0.030a	0.554±0.016c
Serratia sp.	1.404±0.102a	7.518±0.392c	0.388±0.030a	0.662±0.019b
Pseudomonas sp.	1.287±0.013a	9.026±0.233b	0.360±0.036a	0.711±0.041ab
Serratia marcescens	1.279±0.080a	10.251±0.391a	0.368±0.028a	0.836±0.045a

Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different in the rows. Above table represents Mean± Standard error of mean (SEM).

PCR method. In plot studies, ampicillin resistance plate count of *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* showed 15, 13 and15 (x10^7cfu g dry wt. $soil^{-1}$) respectively at the time of inoculation , The population decreased gradually to 10, 8.8 and 11.5 (x10^7cfu g dry wt. $soil^{-1}$) during 8^{th} week or midtime of crop growth for *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* respectively which further decreased to 8.0, 6.0 and 9.0 (x10^7cfu g dry wt. $soil^{-1}$) at the time of crop harvest (Table 1). Microbial population based on LA + ampicillin plates in control plot decreased

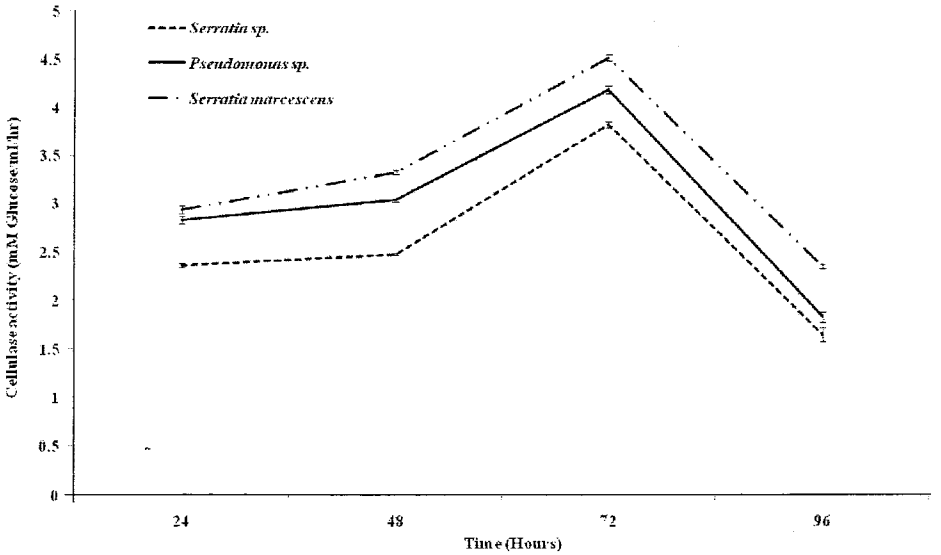

Fig. 1. Cellulase (CMCase) activity as a function of time of the three selected bacterial isolates (*Serratia sp., Pseudomonas sp.* and *Serratia marcescens*) in 1%CMC in BHB media by incubation at 37 for 120rpm.

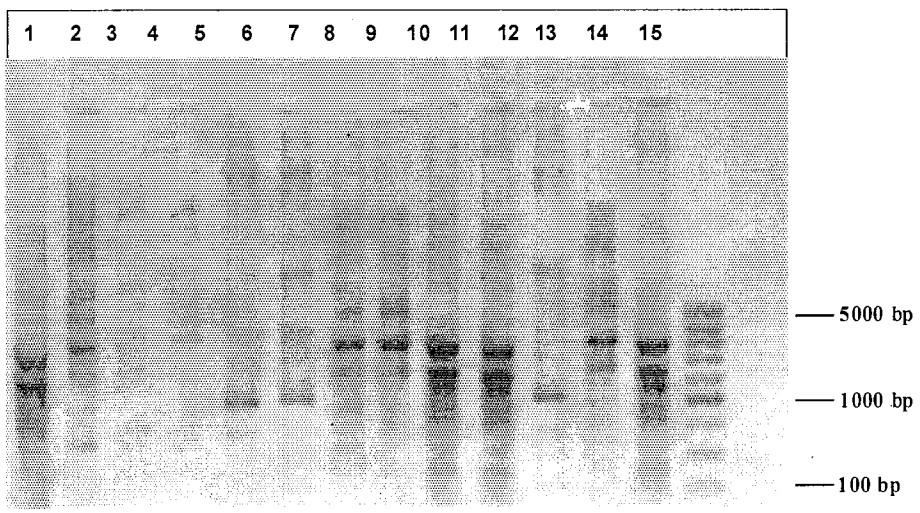

Fig. 2. ERIC-PCR fingerprint of unknown bacterial isolates on 1.5% agarose gel. Bacterial colonies were isolated from soil in the plots. Pure colonies were picked, heat lysed, and whole genomic DNA was subjected to ERIC-PCR to get the fingerprint. Lanes 1-10: fingerprints of different isolates obtained from selected plates containing 120ug/ml of Ampicillin, Lane 11: Standard bacterial strain of *Pseudomonas sp.*, Lane 12: Standard bacterial strain of *Serratia sp.*, Lane 13: Standard bacterial strain of *Serratia marcescens*, Lane 14: 500bp ladder and Lane 15: Negative Control.

from 2.2 to 1.3 (x10^7cfu g dry wt. soil^{-1}) after 16 week of chickpea harvest. Population dynamic studies based on ERIC-PCR showed that population of *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* were 11.7, 10.2 and 13.4 (x10^7cfu g dry wt. soil^{-1}) at the time of inoculation during germinating or 2-3 leaflet stage of chickpea, and then decreased to 4.7, 3.5 and 7.6 (x10^7cfu g dry wt. soil^{-1}) at the end of 16th week. (Table.1) thus showing a survival of 40.2, 34.4 and 56.8 % respectively after 16 weeks (Table. 1). *Serratia sp., Pseudomonas sp.*

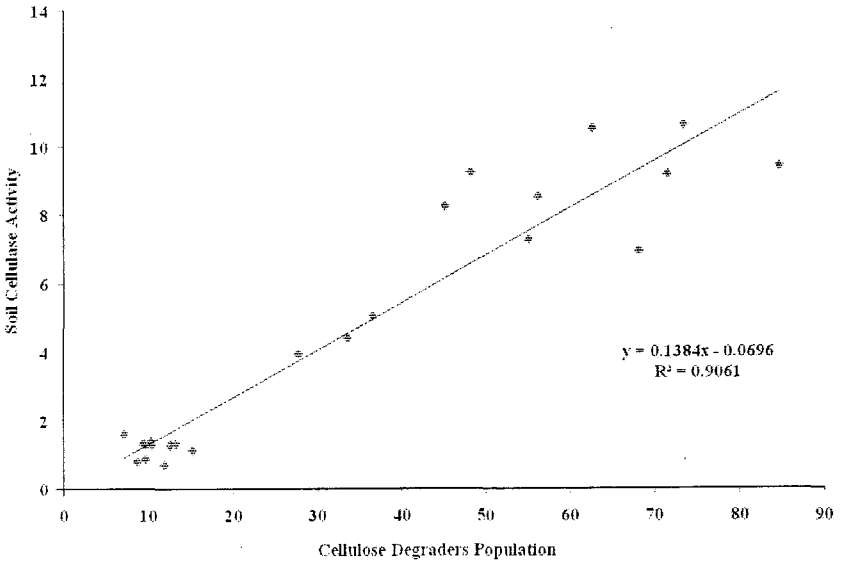

Fig. 3. Correlation between the cellulase activity & cellulose degraders population in soil during the chickpea plot experiments.

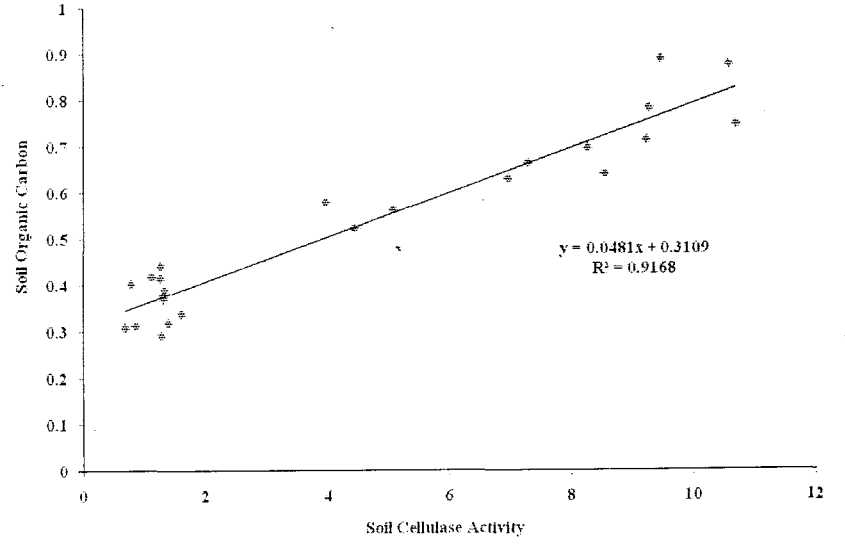

Fig. 4. Correlation between the cellulase activity & organic carbon in soil during the chickpea plot experiments.

and *Serratia marcescens* could not be detected in the soil at zero time (before application of the bacterial inoculums) and in control as well as test plots. These results clearly demonstrated that enumeration of the introduced cellulolytic bacterial isolates by antibiotic resistance method always shoed higher level of survival as compared to that by ERIC-PCR. Since ERIC-PCR enumerates the population of the introduced bacterial isolates without any interference from other bacteria, this method presents a very accurate estimation of the survival of the introduced bacteria.

Cellulase enzymes play a major role in degradation of carbohydrates in soils and the hydrolysis by these enzymes are believed to be important energy sources for the growth of soil microorganisms (Deng, 1996). The soil cellulase activity as well as organic carbon in the experimental plots was also observed and it found to be enhanced by the introduction of the inoculants in the plots in comparison to the control plot (Table.2). Soil cellulase activity in control plot increased from 0.784 (at the time of inoculation) to 4.489 μM glucose g dry wt. $soil^{-1}$ h^{-1}, 1.404 to 7.518 μM glucose g dry wt. $soil^{-1}$ h^{-1} in the *Serratia sp.* inoculated plot, 1.287 to 9.026 μM glucose g dry wt. $soil^{-1}$ h^{-1} in the *Pseudomonas sp.* inoculated plot and 1.279 to 10.251 μM glucose g dry wt. $soil^{-1}$ h^{-1} in the *Serratia marcescens* inoculated plot. Organic carbon also increased from 0.340 to 0.554 % in the control plot, 0.388 to 0.602 % in the *Serratia sp.* plot, 0.360 to 0.711% in the *Pseudomonas sp.* plot and 0.368 to 0.836% in the *Serratia marcescens* plot.

During plot experiments, a significantly high value of soil cellulase activity as well as organic carbon was shown in *Serratia marcescens* inoculated plots as compared to the plots inoculated with the other two isolates after 16 weeks of crop harvest (Table 2). Mass inoculations of bacterial culture in the plots resulted in a significantly enhancement of cellulose degraders population which could be positively correlated (R^2=0.906) to the higher soil cellulase activity (Fig.3). The overall scenario in the experiment results showed an inclined organic carbon in the treated plots (Table 2), which was observed as a positive correlation (R^2=0.916) among the soil cellulase activity and soil organic carbon during the chickpea plot trails (Fig. 4).

Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme as a predictive tool in soil fertility programmes. Understanding the dynamics of microbial populations and enzyme activities in these systems is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth. These bacterial populations or enzymes may have significant effects on soil biology, environmental management, growth and nutrient uptake in plants growing in ecosystems. Their activities may, however, be influenced by unknown cultural management practices.

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Influence of Cellulolytic Bacterial Augmentation on Organic Carbon and Available Phosphorus in Sandy Loam Soil under Cultivation

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Abstract

Microorganisms are major key players for sustaining the soil quality degraded by intensive use of synthetic chemicals for increasing crop production and therefore, use of them as inoculants or biofertilizers is an integral part of sustainable agriculture. An effort was, therefore, made to examine the effect of cellulose degrading bacterial isolates on legume (Chickpea) based cropping systems. No chemical/organic fertilizer was added during this study. The bacterial isolates viz., *Serratia* sp. (MSK1 and MSK24) and *Pseudomonas* sp. (MSK13) exhibiting cellulase activity of 3.83, 4.21 and 4.52 mM glucose ml^{-1} h^{-1} respectively were introduced as inoculants. The ERIC-PCR results showed the good survivability of introduced strains in soil, measured after crop harvest, respectively 40.2. 56.8 and 34.4 %. A significant enhancement in organic carbon and available phosphorus was observed in the inoculated plots over the control plot, indicating beneficial effect of the bioaugmentation of these inoculants.

Keywords: Agricultural soils, Phosphatase, Cellulase, Rhizobacteria, Survivability

1. Introduction

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem (Corstanje *et al*., 2007). It is a critical resource not only for agricultural production and food security but also towards maintenance of most life processes. The functions of soil biota are central to decomposition processes and nutrient cycling (Vikram *et al.,* 2007). However, soils under intensive crop production are prone to organic matter losses that may result in reduced enzyme activity and microbial biomass, which bring about deterioration of the physic-chemical and biological condition (Haynes and Tregurtha, 1999).

Agricultural residues are a rich source of cellulose (Hameeda, 2006). As the main component of plant fiber structures, cellulose is arranged in crystalline to amorphous forms and is a substrate to numerous species of both fungi and bacteria relying on extracellular enzymes. Up until now, the most studied group of cellulose-degrading microorganisms is the fungi, which are characterized by multicomponent, synergistic cellulolytic enzyme systems (Eriksson *et al.,* 1990; Berg and Laskowski, 2006). Although cellulose-decomposing bacteria are ubiquitous in soils, systematic studies on the structure and activities of cellulolytic communities are rare. Cellulases play an important role in carbon availability and so can be used to give a preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies (Ndakidemi and Makoi, 2008). In

soil ecosystems, phosphatases play a critical role in plant growth by enhancing the availability of phosphorus due to enhanced solubilisation and remobilisation of phosphate (Speir and Ross, 1978). Studies have shown that activity of these enzymes in agricultural soils are affected by several factors such as temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical characteristics of organic matter and its location in the soil profile horizon (Rubidge, 1977; Gomah, 1980; Tabatabai, 1982; Klein, 1989; Alf and Nannipieri, 1995). These findings suggest that activities of cellulases can be used to give a preliminary indication of some of the physical chemical properties of soil, thus, easing soil management strategies.

The present study, outlines: isolation, screening, characterization and identification of potential cellulose degrading bacteria and the use of their population dynamics as an index of organic fertility and nutritional status of soil.

2. Materials and Methods

2.1 Sampling and analysis of soil

Soil samples used for the isolation of cellulose degrading bacteria were collected from different chickpea (*Cicer arietinum L.*) agricultural fields of Patiala District, Punjab, India. Soil samples were collected randomly at from 0-30 cm of depth from nine different sites in one acre field by alcohol sterilized implements and stored in sterile containers. Three composites samples were prepared, air-dried and pulverized for organic carbon, nitrogen, available phosphorus, potassium, moisture level, texture and pH by following standard methods (Jackson, 1967).

2.2 Determination of Soil Enzyme Activities

The soil fertility was monitored by estimating two soil enzymes: cellulase (Bailey *et al.,* 1985) and phosphatase (Tabatabai and Bremner, 1969) in soil taken at planting (0 week) and harvest (16^{th} week) time. Soils were processed the same day for microbial and enzymatic activities using standard protocols and stored at 4 ^{o}C for soil analysis.

Estimation of soil phosphatase activity was carried out by taking 10g of soil in 4ml sterile universal buffer. 1ml of 0.115M disodium *p*-nitrophenyl phosphate was added and incubated in dark at 37^{0}C for 2h, followed by an addition of 5ml of 0.5N sodium hydroxide to stop the reaction. The reaction mixture was thoroughly mixed, filtered and filtrate was measured at 410nm. The phosphatase activity was expressed as *p*-nitrophenol (PNP) released per gram of soil i.e., 1μm of PNP/g dry wt. soi/h. Soils were processed the same day for microbial and enzymatic activities using standard protocols and stored at 4^{o}C for soil analysis. For estimating cellulose activity, 10gm of soil was suspended in 5ml of 0.05M phosphate buffer (pH 7.0). 0.1ml of filtered suspension was taken to which 0.9ml of 1.0% carboxymethyl cellulose (CMS) was added. The reaction mixture (1.0ml) was incubated at 37^{0}C for 1h, followed by addition of 2ml dinitro salicylic acid (DNSA) reagent and further incubation of 15min in boiling water bath. One unit of cellulase activity was defined as amount of enzyme necessary to release 1 μm of glucose equivalent/min.

2.3 Isolation and Enumeration of microbial population

For isolation and enumeration of cultivable bacteria, soil samples were diluted in saline (0.89% NaCl; w/v) solution and plated on soil extract agar for total microbial count, Pikovskaya agar (PA) for the isolation of phosphate solubilisers and Bushnell Hass Agar (BHA) supplemented with 1% carboxy methyl cellulose (w/v) as sole source of carbon for cellulose degraders. Plated triplicates of three different dilutions (10^{-5} to 10^{-7}) were incubated for 48 h at 37°C and colonies were counted (cfu g^{-1} dry wt. of soil).

2.4 Screening of Cellulolytic Isolates

Congo red was used as an indicator for the detection of cellulolytic activity on CM–cellulose–agar medium, as described by Teather and Wood (1982). Cellulolytic activity of isolates was evaluated according to the extent and intensity of the hydrolytic clearing zones. Bacterial isolates were grown in 50ml Bushnell Hass broth (BHB) with carboxymethyl cellulose (1% ; w/v) in 250-ml flask and incubated on rotary shaker at 37°C, 120 rpm. After 72 h, the cells were centrifuged (9000g, 10 min) and supernatant was collected for enzyme assay. Cellulase (CMCase) activity was measured by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959), through the determination of the amount of reducing sugars liberated from carboxymethyl cellulose (CMC) solubilised in 50 mM Tris–HCl buffer (pH 7.0) (Baily *et al.,* 1992). Enzyme assay was carried out at 37 °C for 1h and the reaction was stopped by the addition of DNS solution. Samples were then boiled for 10 min, cooled on ice for color stabilization, and the optical density was measured at 540 nm. Cellulase activity was determined by using a calibration curve for glucose and expressed as μM glucose g^{-1} h^{-1}.

2.5 ERIC-PCR based fingerprinting of isolates

Enterobacteriaceae Repetitive Intergenic Consensus - Polymerase Chain Reaction (ERIC-PCR) was carried out to obtain DNA fingerprints of the cellulose degraders. PCR primer sequences (Versalovic *et al.,* 1991), ERIC – IR (5' – 3') – ATGTAAGCTCCTGGGGAATCAC- and ERIC – 2 (5' – 3') - AAGTAAGTGACTGGGGTGAGCG- were obtained from Life Technologies, USA. Single isolated colonies were picked at random from the LA plates, suspended in 50 µl water, and lysed by heating for 10 min at 95°C. Cell lysate was centrifuged (9000 x g, 3 min) at 4°C, and 2 µl of the supernatant was used in the reaction mixture. The reaction mixture (25 µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin (wt/vol), 0.25 mM each of dNTPs, 0.375 µM each of primers ERIC-1R and ERIC-2, and 2.0 units of Taq polymerase (Life Technologies, USA). The amplification was done on Genamp PCR system (Applied Biosystem, USA). The PCR conditions were as follows: initial denaturation at 94°C (4 min) followed by 41 cycles at 94°C (1 min) then at 50°C (1 min) and finally at 72°C (2 min). The ultimate extension was done at 72°C (5 min). The reaction was terminated by using a loading dye (1 µl) containing Ficoll 15%, bromophenol blue 0.25%, and xylene cyanol 0.25%. Each PCR product was resolved by electrophoresis on a 1.5% agarose gel.

2.6 Plot studies

Plot studies were conducted under sandy loam agricultural soil. Chemical composition of this soil was organic carbon, 0.78±0.03 %; total nitrogen, 0.0823±0.005 %; and available phosphorus 44.00±5.6 mg/kg. Randomized design was adopted to study the degradation of cellulose in the agriculture soil by inoculating the most efficient cellulose degraders in plots ($4m^2$) sown with chickpea (*Cicer arietinum L.*) crop. Cultures were grown in 10 litre shake flasks containing Luria broth (LB) medium and incubated at 37°C with rotary shaking set at120 rpm. After 36 h, the cells were harvested and washed with 10mM phosphate buffer (pH 6.8). Pellets were resuspended in buffer and inoculated in plots at cell concentration of approximately 2 x 10^8 cfu/g soil. Control plot received only BHB without any bacterial cells. The prescribed package of practices (PAU, 2005) was followed and monitored during the cultivation of the chickpea. At 0 week (planting and inoculation time) and 16^{th} week after sowing (harvest time) soil samples were collected from different points, composite samples were prepared and taken in triplicate to evaluate the population dynamics and other soil parameters. One gram of soil was suspended in 9 ml of sterilized saline water (0.8% NaCl; w/v) by vigorous vortexing followed by serial dilution. An aliquot of 100 µl was plated on LA containing ampicillin (120 µg/ml). Plates were incubated for 36 h at 37 °C. Individual colonies obtained after this incubation were restreaked on LA plates containing ampicillin (120 µg/ml). Colonies grown were scored and their DNA was isolated for fingerprinting protocol based on ERIC-PCR.

2.7 Data analysis

Variance (ANOVA), regression and multiple comparison analysis were carried out at $p \leq 0.05$, using COSTAT software.

3. Results and Discussion

Thirty-one cellulose degrading bacterial isolates were isolated from agricultural soils cultivated with chickpea (Figure 1). Three isolates, namely MSK1, MSK13 and MSK24 showed their high efficacy to degrade carboxy methyl cellulose (CMC) in plate assay. The values were: 3.83, 4.21 and 4.52 mM glucose ml^{-1} h^{-1} respectively. These 3 strains were chosen for further morphological and biochemical characterisation (Table 1). MSK1, MSK13 and MSK24 were Gram negative, non-spore forming and positive to extracellular CMCase and xylanase activity. The optimum growth conditions for these isolates were 25 – 37 ^oC at pH 6.0-8.0. MSK1 and MSK24 showed catalase positive, oxidase and nitrate reduction negative reaction, but MSK13 was catalase negative, oxidase positive and nitrate reduction positive. Antibiotic screening showed that MSK1, MSK13 and MSK24 were sensitive to norfloxacin, gentamicin, chloramphenicol, cefuroxime, ciprofloxacin, resistant to ampicillin. These isolates were identified by partial sequencing of 16S rRNA, so homology search revealed that MSK1, MSK13 and MSK24 were 99% similar to *Serratia* sp., *Pseudomonas* sp. and *Serratia marcescens* respectively (data not shown).

Investigations by other researchers showed that aerobic bacteria belonging to species of *Pseudomonas*, *Bacillus* and *Cellulomonas*, and anaerobe such as *Clostridium* have cellulolytic potential (Sindhu *et al.,* 2001). *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 have been shown to possess cellulolytic activity in the presence of crop residues (Hameeda *et al.,* 2006). The residing microflora maintains the soil health and affects the agronomic parameters of the crops planted on those plots. Thus, farming management trials along with the inoculants enhance the growth and yield of the crops (Dickey *et al.,* 1994). Cellulolytic soil bacteria had been studied in various soils under different land use systems with respect to the effect of environmental conditions on the abundance and decomposing activity (Hiroki and Watanabe, 1996; Dilly *et al.,* 2001). All of these studies

were mostly based on the estimates of population densities using agar plate techniques; with population dynamics and characterisation being rarely analyzed (Ulrich *et al.,* 2008)

Survival of the microorganisms in the soil after their application is a deciding factor in the rate of degradation of carbon source (Ramos *et al.,* 1991) which is observably assessed with confidence mainly with PCR based molecular techniques (Versalovic *et al.,* 1991; Thiem *et al.,* 1994; Weidmann-Al-Ahmad *et al.,* 1994; Giovanni *et al.,* 1999). Repetitive DNA sequences have been characterized primarily from *Escherichia coli* and *Salmonella typhimurium*. These sequences, referred to as enterobacterial repetitive intergenic consensus sequence (ERIC) or intergenic repeat units (IRUs), are 126-bp elements containing a highly conserved central inverted repeat and are located in extragenic regions (Versalovic *et al.,* 1991). The analysis of this element by PCR-synthesized oligonucleotide has provided unique DNA fingerprints. It is reported that ERIC probes hybridized preferentially to genomic DNA from Gramnegative bacteria (Giovanni *et al.* 1999). In the present study, survival of the microorganisms was tracked with the ERIC-PCR based genomic DNA fingerprinting method. ERIC-PCR of the isolates in the present study showed 10 to 14 distinctly amplified bands with sizes ranging from 11,000 bp to 100 bp (Figure 2). Although 16S rDNA sequence analysis showed that *MSK1* and *MSK24* to have close homology with *Serratia* sp., ERIC-PCR enabled to differentiate between these isolates. The populations of *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 showing a survival of 40.2, 34.4 and 56.8 % respectively after 16 weeks (Figure 3). The strains of *Serratia* sp. and *Pseudomonas* sp. were found to be stable at the end of 16 weeks in the treated plots which is presumed to be due to their adaptability to local soil environment from where these were isolated. Among all the three treatments tested, significantly high values of microbial count and soil enzyme activities were observed in plots inoculated with *Serratia* sp. compared to control (Table 2). It was observed that the conventional plate counting method resulted in lower rates of survival of these isolates (41%) as compared to the survival rate obtained from PCR fingerprinting (58%). In absence of proper control, mere decrease in antibiotic counts cannot be related to the survival of specific inoculated bacterial isolate in a complex environment like agricultural soil. This is expected since in natural environment several microorganisms have no specific-drug resistance (Nakaune *et al.* 1998; Mathew *et al.* 1999) and therefore are selected on ERIC-PCR fingerprinting. The results of investigation also support the hypothesis that indigenous isolates will results in greater adaptation to the system as well promote the microbial population and activities in the soil.

The modulations in the soil enzyme activity and nutritional properties influenced by the microbial inoculation were studied by examining the cellulase activity, phosphatase activity, organic carbon, total nitrogen content and available phosphorus in the experimental plots. Most of these parameters were observed and found to be enhanced with augmentation of inoculants in the plots in comparison to the control plot. The present study also indicated increase in availability of carbon and other nutrients assumed to be facilitated through enhanced enzyme activity. The increased phosphate activity in the soil drawn from the plots amended with *Pseudomonas* sp. MSK 13 could be related to the metabolic versatility of this microbial group that facilitates increasing activity of phosphatases when P is a limiting nutrient (Tadano *et al.,* 1993). The plots treated with *Serratia* sp. MSK1, *Pseudomonas* sp. *MSK13* and *Serratia* sp., *MSK24* showed significantly enhanced soil cellulase activity of 7.51, 9.02 and 10.25 (μM glucose g^{-1} dry wt. soil h^{-1}) respectively, in comparison to control plots (Table 2), which also correlated with the increase in population of the cellulose degrading inoculants (r^2 : 0.730). Similarly, an increase in soil phosphatase activity was also observed ranging from 0.858 μM PNP g^{-1} dry wt. soil h^{-1} in plots inoculated with *Serratia* sp. (MSK13) to 1.497 μM PNP g^{-1} dry wt. soil h^{-1} in plots augmented with *Serratia* sp. (MSK24) (Table 4) correlating with the levels of available phosphorus (Table 5). A significantly high value of total nitrogen was observed in the plots inoculated with *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 (0.234 %, 0.245 % and 0.272% respectively) when compared to control (Table 3). The increase in organic carbon also correlated with increase in cellulase activity (r^2: 0.916). The increased population of phosphate solubilisers showed the increased availability of available phosphorus significantly high as compared to the control plot, to 98.66 mg/kg, 96.66 mg/kg and 99.66 mg/kg in the *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 inoculated plots respectively. Similar investigations have earlier shown that legumes secrete more phosphatise enzymes than cereal (Yadav and Tarafdar, 2001). This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. The maximum available potassium of 115.0 mg/kg and significant enhanced nitrogen of 0.27 % was observed in the *Serratia* sp. MSK24 plot (Table 4). As reported by Sivaramaiah *et al.* (2007) the efficacy of *Bacillus* sp. to enhance nodulation, plant dry matter and grain yield on co-inoculation with rhizobia has also been reported for other legumes Bacterial strains isolated from the maize rhizosphere including *Bacillus, Pseudomonas* and *Serratia* were reported to improve the yield by 9–14% (Lalande *et. al.,* 1989). Significant increase in the root dry mass of rapeseed was observed due to inoculation with *Proteus*, *Klebsiella* and *Bacillus* (Bertrand *et. al.,* 2001). Considering the results

of this study in addition to the previous observation, it may be suggested that mass inoculation of aerobic cellulolytic bacteria used in present investigation can be a useful tool to maintain soil fertility by increasing microbial activity to decompose organic matter in leguminous ecosystem.

4. Conclusion and Recommendations

- Thirty-one strains were isolated from chickpea cultivated soils.
- Three of the isolates: MSK1, MSK13 and MSK24, showed higher efficacy to degrade carboxy methyl cellulose in plates.
- 16S rDNA sequence analysis and fingerprint method (ERIC-PCR) allowed identifying three species: *Serratia* sp. (MSK1), *Pseudomonas* sp. (MSK13) and *Serratia* sp. (MSK24), 99% close to *Serratia marcescens*.
- A significant enrichment in organic carbon and available phosphorus was observed in the inoculated plots over the control plot indicating beneficial effect of the bioaugmentation of these inoculants.

The local isolates of both *Serratia* sp. and *Pseudomonas* sp. could be mass produced as a biological agent towards magnification of microbial diversity and soil organic carbon. Moreover, the introduction of such bacteria in chickpea soils, or cultural practices aims to increase the activity of native strains of these bacteria and they could greatly contribute to the efficiency of soil organic matter and the cellulolytic potential of soil bacterial communities as major contributors towards sustainable agriculture. Also, the rhizobacteria from the local rhizosphere soil could be exploited for use as microbial inoculants to improve nodulation (in legumes) and crop productivity of both cereals as well as legumes. Although potential clearly exists for developing such inoculants, their widespread application remains limited by a poor understanding of microbial ecology and population dynamics in soil; these studies are needed.

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Table 1. Biochemical characterization of the screened bacterial isolates from agricultural soils

Character	MSK1	MSK13	MSK24
Colony morphology	White	Creamy Yellow	Pinkish White
Gram stain	-	-	-
Spore	-	-	-
Catalase	+	-	+
Oxidase	-	+	-
Nitrate reduction	-	+	-
Growth at 25-37^{o}C	+	+	+
Growth at pH 6-8	+	+	+
CMCase	+	+	+
Xylanase	+	+	+
Ampicillin	R	R	R
Tetracyclin	S	S	S
Gentamycin	S	S	S
Kanamycin	S	S	S

+: positive reaction; -: negative reaction; R: Resistant at 120µg; S: Sensitive

Table 2. Microbial populations and soil enzyme activities in soils of chickpea plot. Cellulose degraders count, Phosphate solublisers count, Cellulase and phosphatase activity were determined at 0 week (Chickpea sowing stage) and 16 weeks (Chickpea harvest stage) in soils

Treatment	cellulose degraders (cfu x10^6 / g dry wt soil)		phosphate solubilisers (cfu x10^6 / g dry wt soil)		cellulase activity (um glucose / g dry wt soil/ hr)		phosphatase activity (um pnp / g dry wt soil/ hr)	
	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK
Control	10.04±0.93a	32.61±2.57b	13.00±0.96a	26.76±1.43b	0.784±0.048b	4.489±0.322d	0.167±0.004a	0.554±0.016c
Serratia sp. MSK1	9.64±1.56a	56.11±6.64a	17.09±1.28a	40.37±5.41ab	1.404±0.102a	7.518±0.392c	0.171±0.004a	0.662±0.019b
Pseudomonas sp MSK13	10.88±0.88a	58.64±6.83a	14.09±2.10a	44.99±4.38ab	1.287±0.013a	9.026±0.233b	0.161±0.013a	0.711±0.041ab
Serratia sp. MSK24	12.87±1.41a	73.58±6.35a	14.37±0.71a	53.14±6.28b	1.279±0.080a	10.251±0.391a	0.157±0.009a	0.836±0.045a

Data characterized by the same letter are not significantly different ($p \leq 0.05$) in the rows. Above table represents Mean± Standard error of mean (SEM).

Table 3. Soil nutritional status of chickpea plot. Organic carbon (OC), Nitrogen (N), Carbon/Nitrogen ratio (C/N), Available phosphorus (P) and Available potassium (K) were determined at 0 week (Chickpea sowing stage) and 16 weeks (Chickpea harvest stage) in soils

Treatment	OC (%)		N (%)		C/N ratio		P (mg/kg)		K (mg/kg)	
	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK
Control	0.340±0.030a	0.554±0.016c	0.0823±0.0056a	0.1695±0.0089b	4.13±0.8	3.26±0.4	44.0±5.68a	70.3±6.11a	52.3±3.17a	86.7±7.21
Serratia sp. MSK1	0.388±0.030a	0.662±0.019b	0.0818±0.0063a	0.2340±0.0204ab	4.74±1.2	2.82±0.4	47.0±6.24a	98.7±7.05a	50.7±8.98a	96.7±6.48
Pseudomonas sp. MSK13	0.360±0.036a	0.711±0.041ab	0.0851±0.0059a	0.2456±0.0219ab	4.23±0.2	2.89±0.5	48.7±4.97a	96.7±14.31a	54.3±7.62a	94.0±4.93
Serratia sp. MSK24	0.368±0.028a	0.836±0.045a	0.0867±0.0078a	0.2728±0.0237a	4.24±0.4	3.07±0.8	48.0±8.50a	99.8±11.89a	54.3±9.26a	115.0±11.37

Data characterized by the same letter are not significantly different in the rows. Above table represents Mean± Standard error of mean (SEM)

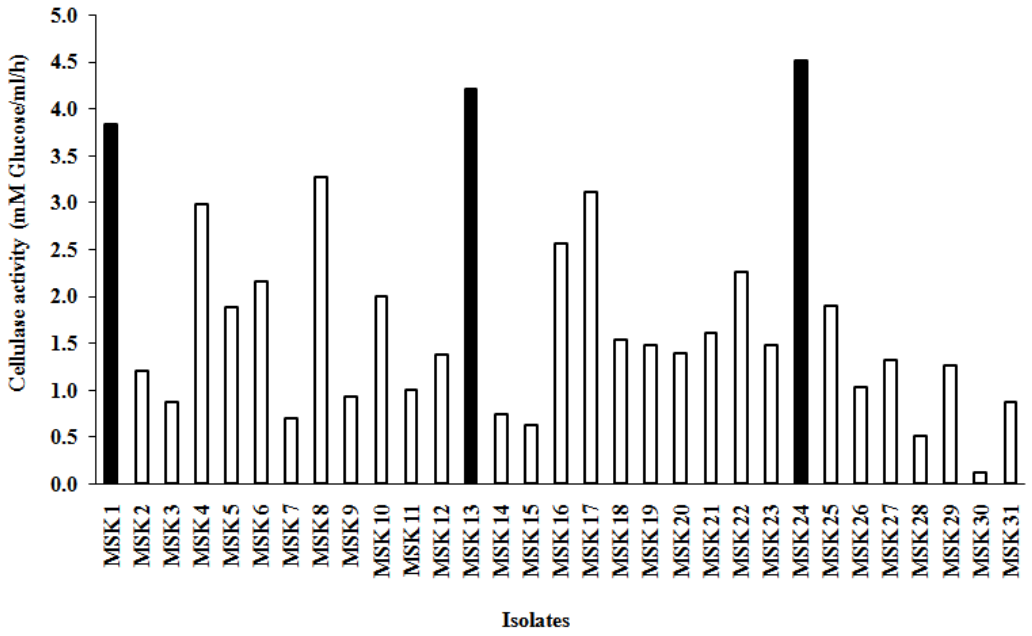

Figure 1. Screening of isolates for Cellulase (CMCase) activity from agricultural soils

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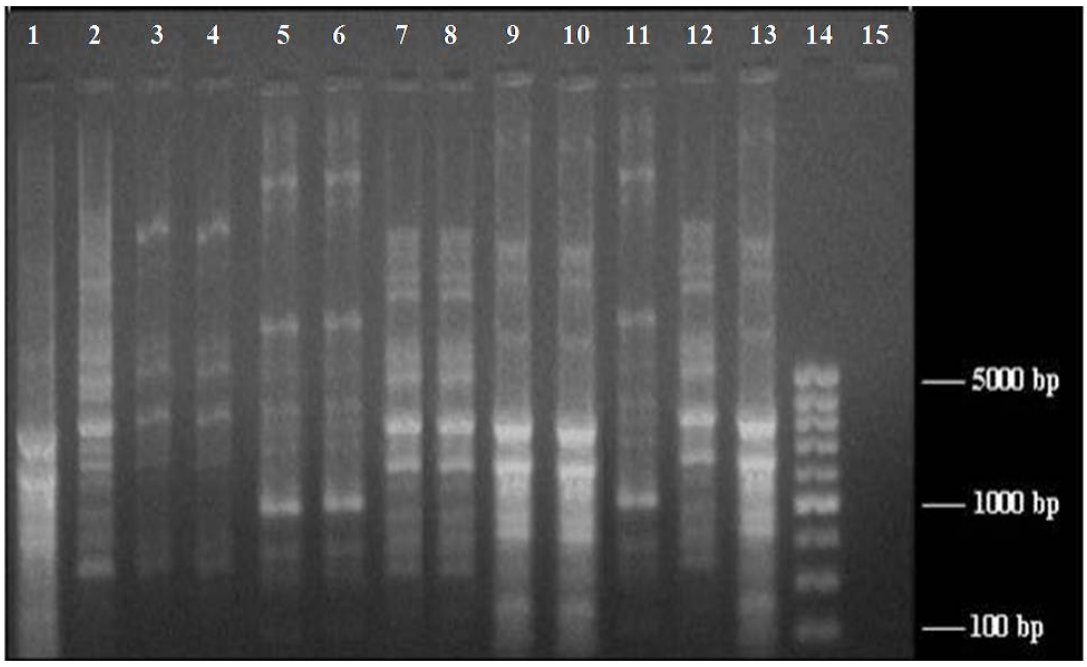

Figure 2. ERIC-PCR fingerprint of unknown bacterial isolates on 1.5% agarose gel. Bacterial colonies were isolated from soil in the plots. Pure colonies were picked, heat lysed, and whole genomic DNA was subjected to ERIC-PCR to get the fingerprint.

Lanes 1-10: fingerprints of different isolates obtained from selected plates containing 120µg/ml of Ampicillin
Lane 11: Standard bacterial strain of *Pseudomonas* sp. MSK13
Lane 12: Standard bacterial strain of *Serratia* sp. MSK1
Lane 13: Standard bacterial strain of *Serratia* sp. MSK24
Lane 14: 500bp ladder
Lane 15: Negative Control.

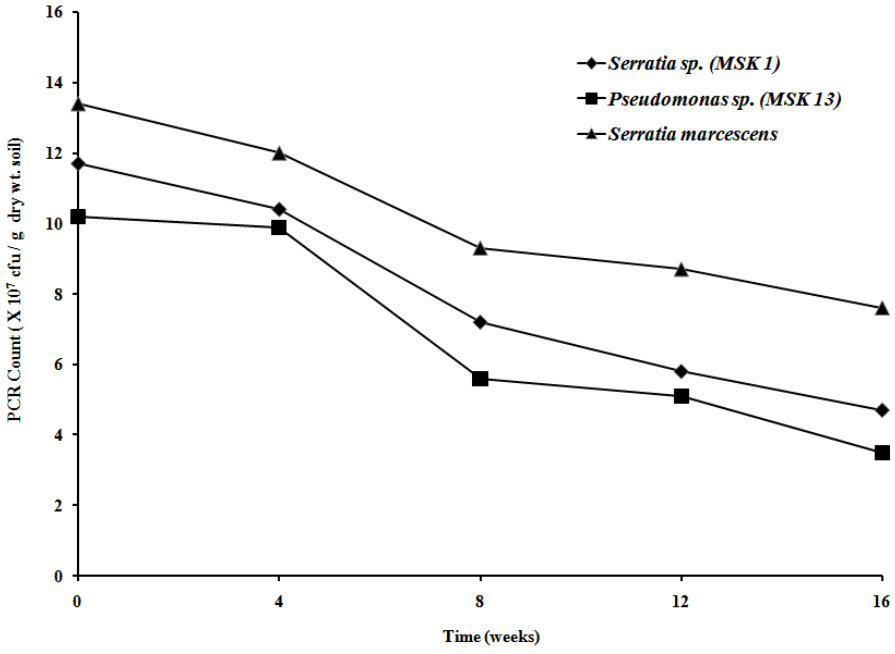

Figure 3. Survival of introduced bacterial strains during growing chickpea crop in plots

CHARACTERISATION OF PHOSPHATE SOLUBILISING BACTERIA IN SANDY LOAM SOIL UNDER CHICKPEA CROPPING SYSTEM

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ABSTRACT

With the aim to explore the possible role of phosphate-solubilizing bacteria (PSB) in phosphorus (P) cycling in agricultural soils, we isolated PSB inhabiting naturally in the sandy loam soils under chickpea cropping of Patiala (Punjab State). A total of 31 bacterial isolates showing solubilizing activities were isolated on Pikovskaya agar plates. The potent phosphate solubilizers were selected for further characterization. These isolates were shown to belong to the genera *Pseudomonas* and *Serratia* by partial sequencing analysis of their respective 16S rDNA genes. ERIC-PCR based fingerprinting was done for tracking the survival of introduced populations of the PSB during mass inoculation of these strains under chickpea plots. The results showed positive correlation (r^2=0.853) among soil phosphatase activity and phosphate solubilisers population, which was also positively correlated (r^2=0.730) to available phosphorus. Identification and characterization of soil PSB for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field application.

Keys words: Agricultural soils, Chickpea, Phosphatase, Phosphorus, *Pseudomonas, Serratia*

1. INTRODUCTION

In natural environments, i.e. the rhizosphere of different plant species, phosphate-solubilizing bacteria (PSB) are considered to play an important ecophysiological role: indeed, PSB mobilize insoluble inorganic phosphates from their mineral matrix to the bulk soil where they can be absorbed by plant roots. In turn, the plants supply rootborne C compounds, mainly sugars, which can be metabolized for bacterial growth [1, 2]. Furthermore, the organisms possessing a phosphate-solubilizing ability can also convert the insoluble phosphatic compounds into soluble forms [3, 4] in soil and make them available to the crops. The crop microbial ecosystem can thus be energized in sustainable agriculture with considerable ecological stability and environmental quality. The PSB increase the availability of soluble phosphate and can enhance plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc., and by production of plant growth-promoting regulators [5, 6]. The discovery of this mutual relationship between plants and PSB encouraged the development of new technologies, such as the use of PSM for biofertilization to improve crop yield [7, 8, 9]. This work reports the efficacy of Phosphate Solubilising Bacteria (PSB) in improving the organic fertility and nutritional status of sandy loam soil under chickpea cropping during plot studies.

2. MATERIALS AND METHODS

2.1 Isolation and Screening of PSB

Soil samples were collected from chickpea (*Cicer arietinum L.*) agricultural fields of Patiala District (Punjab, India). All samples were collected randomly at from 0-30 cm of depth from

nine different sites and three composites samples were prepared, air-dried and pulverized for soil physic-chemical parameters by standard methods [10]. The soil biochemical parameter was monitored by estimating soil phosphatase [11] taken at the initial time (0 week) and after the harvest of crop (16^{th} week). Soils were processed the same day for microbial and enzymatic activities and stored at 4 ^{o}C for soil analysis. For isolation of PSB, serial soil dilutions were spread plated on modified Pikovskaya agar [12] and the colonies were counted (cfu g^{-1} dry wt. of soil). Phosphate-solubilization by each PSB isolate was screened in Pikovskaya liquid media. The flasks were incubated at 37 ± 2ºC for 72 h and the contents were centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed for soluble P-content using the method as described by Jackson (1967). The pH of the supernatant was also recorded. The culture broth having approximately 10^{8}cfu/ml was spotted on Pikovskaya plates for determination of the P solubilization efficiency. The plates were incubated at 37± 2ºC for 48 h and the diameter of the colony as well as the halo zone was measured. P-solubilizing index (PSI) was calculated as:

PSI (%) = (Z–C)/C × 100; where, Z = Halo zone diameter, C = Colony diameter

2.2 Molecular studies

The isolation of chromosomal DNA, PCR amplification, and digestion of 16S rDNA gene fragments were performed as described. Bacterial isolates were identified by amplification of 16S rDNA gene sequence with the following primers: Forward 5’ –AGA GTTTGATCCTGGCTCAG – 3’ and reverse 5’ – ACGGGCGGTGTGTTC – 3’ [13]. DNA amplification was performed with Genamp PCR system (Applied Biosystem, USA). Reaction mixture for the PCR contained 1X PCR buffer (Invitrogen, USA), each deoxynucleotide

triphosphate at a concentation of 200µm, 1.5 mM magnesium chloride, 0.1 µm of each primer and 2.5U of Taq DNA polymerase (Invitrogen, USA) in a final volume of 100 µl. PCR reaction sequence was as follows: Preheating at 92^{o}C for 2min, 36 cycles of 92^{o}C for 1min, 48^{o}C 30sec and 72^{o}C for 2min and final extension 72^{o}C for 6min. Amplified DNA was verified by electrophoresis of aliquots of PCR product (5µl) in 1.0% agarose gel in 1X TAE buffer. The product was cloned into the pDrive-cloning vector and transformed into *E. Coli* DH5α [14]. The PCR products were digested with the restriction enzymes *Eco*RI, *Hinf*I, *Rsa*I and *Mbo*I (New England Biolabs) and analysed on 1.5% agarose gel and stained with ethidium bromide. The restriction profiles were scored and documented using a Biorad Gel Documentation system. Partial 16S rDNA sequences similarities of soil isolates were calculated and compared with those available in GenBank database using BLAST program [15] and SEQMATCH of RDP-II [16]. The sequences of closely related bacteria were retrieved from RDP-II and aligned using multiple alignments CLUSTALW program [17]. The evolutionary distance was calculated by Kimura 2 parameter and phylogenetic dendograms were constructed by neighbor-joining using MEGA3.0 software [18]. For analysis, 500 bootstrap replicates were performed to assess the statistical support for the tree. Enterobacteriaceae Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to obtain DNA fingerprints of the PSB. PCR primer sequences [19], ERIC – IR (5’ – 3’) – ATGTAAGCTCCTGGGGAATCAC- and ERIC – 2 (5’ – 3’) - AAGTAAGTGACTGGGGTGAGCG- were obtained from Life Technologies, USA. Single isolated colonies were picked at random from the LA plates, suspended in 50 µl water, and lysed by heating for 10 min at 95°C. Cell lysate was centrifuged (9000 x g, 3 min) at 4°C, and 2 µl of the supernatant was used in the reaction mixture. The reaction mixture (25 µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin (wt/vol), 0.25 mM each of

dNTPs, 0.375 µM each of primers ERIC-1R and ERIC-2, and 2.0 units of Taq polymerase (Life Technologies, USA). The amplification was done on Genamp PCR system (Applied Biosystem, USA). The PCR conditions were as follows: initial denaturation at 94°C for 4 min followed by 41 cycles at 94°C for 1 min, at 50°C for 1 min and at 72°C for 2 min. The final extension was done at 72°C for 5 min. The reaction was terminated by using a loading dye (1 µl) containing 15% Ficoll, 0.25% bromophenol blue, and 0.25% xylene cyanol. Each PCR product was resolved by electrophoresis on a 1.5% agarose gel.

2.3 Evaluation of PSB activities under plot studies

Plot studies were conducted under sandy loam agricultural soil. Randomized block design was used for layout of the plots which included one control and three plots inoculated with MSK1, MSK13 and MSK24 respectively. Chemical composition of this soil was organic carbon, 0.78±0.03 %; total nitrogen, 0.0823±0.005 %; and available phosphorus 44.00±5.6 mg/kg. Randomized block design was adopted to study the efficacy of inoculated PSB strains in the plots ($4m^2$) sown with chickpea (*Cicer arietinum L.*) crop. Cultures were grown in 10 litre shake flasks containing LB medium and incubated at 37°C at120 rpm. After 36 h, the cells were harvested and washed with 10mM phosphate buffer (pH 6.8). Pellets were re-suspended in buffer and inoculated in plots at cell concentration of approximately 2×10^8 cfu/g soil. The prescribed package of practices [20] was followed and monitored during the cultivation of the chickpea. At regular intervals of time during 0, 4, 8, 12 and 18 week soil samples were processed to evaluate the population dynamics and other soil parameters. PSB were isolated by plating serial soil dilutions plated on Luria agar (LA) containing ampicillin (120 ug/ml). Colonies grown were scored and their DNA was isolated for fingerprinting protocol based on ERIC-PCR.

Simultaneously, along with the monitoring of PSB colonization over the duration of the study, the available P was also measured following ascorbic acid method [21].

2.4 Data analysis

Statistical analysis was performed by ANOVA and were tested by the Newman Keuls test with a critical range of 5% using COSTAT software. All values are the means of three replicates.

3. RESULTS AND DISCUSSION

3.1 Biochemical characterisation and Screening of Phosphate solubilising bacterial isolates

In the present study, 167 isolates of phosphate solubilising bacteria were isolated by plating soil dilutions on Pikovskaya agar, of which 31 isolates showed the development of sharp phosphate solubilization zones, ranging from 5 to 29 mm on modified Pikovskaya agar with phosphate solubilisation index. The maximum solubilization index was observed with the isolate *MSK13* and the minimum solubilization with the isolate *MSK2*. Nine isolates showed the phosphate solubilisation index greater than 400 were selected for quantification of phosphate solubilization in liquid media. The results of phosphate solubilization were significantly higher among nine isolates namely *MSK1, MSK2, MSK9, MSK13, MSK14, MSK19, MSK23* and *MSK24* (Figure 1). The TCP solubilization by these isolates ranged from 125 to 413 ug/mL compared with 450 from the alkaline soils of tropical India [22]. Johri *et al.* [23] have reported 510 ug/mL TCP solubilization by the best bacterial strain from the alkaline soils. These isolates appear to be among the most efficient phosphate-solubilizing bacterial strains in comparison with the earlier reports. Similarly, TCP solubilization ranged from 96 to 139 lg/mL by the 13 best isolates clustered under the genera Enterobacter, Pantoea, and Klebsiella from Korean soils [24]. A significant decline in the pH of medium was recorded during the solubilization of TCP, which

suggested secretion of organic acids by the bacterial isolates [25, 26]. The release of soluble phosphates is not necessarily correlated with soil pH [27]. In the present study the quantity of phosphate solubilization and decline in pH during solubilization of TCP was observed which is reported in earlier findings on the solubilization of rock phosphates by some efficient microbial strains. Bardiya and Gaur [28] investigated that phosphate solubilization varies greatly with the nature of phosphate substrates available and the organisms. These three best phosphate solubilisers were chosen for further morphological and biochemical characterisation. *MSK1, MSK13* and *MSK24* were Gram negative, non-spore forming, and positive to extracellular CMCase and xylanase activity. *MSK1* and *MSK24* showed catalase positive, oxidase and nitrate reduction negative reaction, but *MSK13* was catalase negative, oxidase positive and nitrate reduction positive. Antibiotic screening showed that *MSK1, MSK13* and *MSK24* were sensitive to norfloxacin, gentamicin, chloramphenicol, cefuroxime, ciprofloxacin, resistant to ampicillin. These bacterial isolates were subjected to 16S rDNA amplification using universal primers, and 1.5 kb amplicon was observed. These isolates were identified by partial sequencing of 16S rDNA. Homology search of 16S rDNA revealed that *MSK1, MSK13* and *MSK24* were 99% similar to *Serratia sp., Pseudomonas sp.* and *Serratia marcescens* respectively.

3.2 ERIC fingerprinting and Survival Efficacy of bacterial strains

Genetic variability among the phosphate solubilising bacterial strains assessed using ERIC-PCR fingerprints showed high level of polymorphism in the banding profile varied from ten to fourteen and their size varied 0.1 to 11 kb for these strains (Figure 2). Although 16S rDNA sequence analysis showed that *MSK1* and *MSK24* to have close homology with *Serratia sp.*, ERIC-PCR enabled us to differentiate between these isolates. ERIC-PCR showed results population of *Serratia sp MSK1, Pseudomonas sp. MSK13* and *Serratia* sp. *MSK24* showing a

survival of 40.2, 34.4 and 56.8 % respectively after 16 weeks (Table 1). The strains of *Serratia sp.* and *Pseudomonas sp.* were found to be stable at the end of 16 weeks in the treated plots which is presumed to be due to their adaptability to local soil environment from where these were isolated. Among all the three treatments tested, significantly high values of total microbial count and soil phosphatase activities were observed in plots inoculated with *Serratia sp. MSK24* compared to control (Table 2). It was observed that the conventional plate counting method resulted in lower rates of survival of these isolates (41%) as compared to the survival rate obtained from PCR fingerprinting (58%). In absence of proper control, mere decrease in antibiotic counts cannot be related to the survival of specific inoculated bacterial isolate in a complex environment like agricultural soil. Since in natural environment several microorganisms have no specific-drug resistance [29, 30] and therefore are selected on ERIC-PCR fingerprinting. The results of investigation also support the hypothesis that indigenous isolates will results in greater adaptation to the system as well promote the microbial population and activities in the soil sustainablity.

3.3 Soil Nutrient and Enzyme Activities

The present study also indicated increase in availability of phosphorus and organic carbon assumed to be facilitated through enhanced enzyme activity. The increased phosphate activity in the soil drawn from the plots amended with *Pseudomonas sp. MSK 13* could be related to the metabolic versatility of this microbial group that facilitates increasing activity of phosphatases when P is a limiting nutrient [31]. The plots treated with *Serratia sp. MSK1, Pseudomonas sp. MSK13* and *Serratia* sp., *MSK24* showed significantly enhanced soil phosphatase activity ranging from 0.858 μM PNP g^{-1} dry wt. soil h^{-1} in plots inoculated with *Serratia sp.* (*MSK13*) to 1.497 μM PNP g^{-1} dry wt. soil h^{-1} in plots augumented with *Serratia* sp. (*MSK24*) (Table 2). The

increased population of phosphate solubilisers showed the increased availability of available phosphorus significantly high as compared to the control plot, to 98.66 mg/kg, 96.66 mg/kg and 99.66 mg/kg in the *Serratia sp. MSK1, Pseudomonas sp. MSK13* and *Serratia sp. MSK24* inoculated plots respectively. A positive correlation (r^2=0.853) was also observed among soil phosphatase activity and phosphate solubilisers population (Figure 3), which was also positively correlated (r^2=0.730) to available phosphorus (Figure 4). Similar investigations have earlier shown that legumes secrete more phosphatase enzymes than cereal [32]. This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. The reports on the efficacy of *Bacillus* sp. showed enhance nodulation, plant dry matter and grain yield on co-inoculation with *Rhizobia* in other legumes [33]. Bacterial strains isolated from the maize rhizosphere including *Bacillus, Pseudomonas* and *Serratia* were reported to improve the yield by 9–14% [34]. In addition to these traits, phosphate solubilising bacterial strains were found to competent, able to survive and colonize in the sandy loam soil. Fortunately, the interaction between PSB and chickpea plants was observed to be much stable even after 16 weeks of crop. The good results obtained produced under field conditions. It is expected that inoculation with other rhizobacteria containing plant growth promoting characteristics consequently promote root and shoot growth as well as nodulation. Further evaluation of the isolates exhibiting multiple plant growth promoting traits on soil–plant system is needed to uncover their efficacy as effective bioinoculants.

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Figure

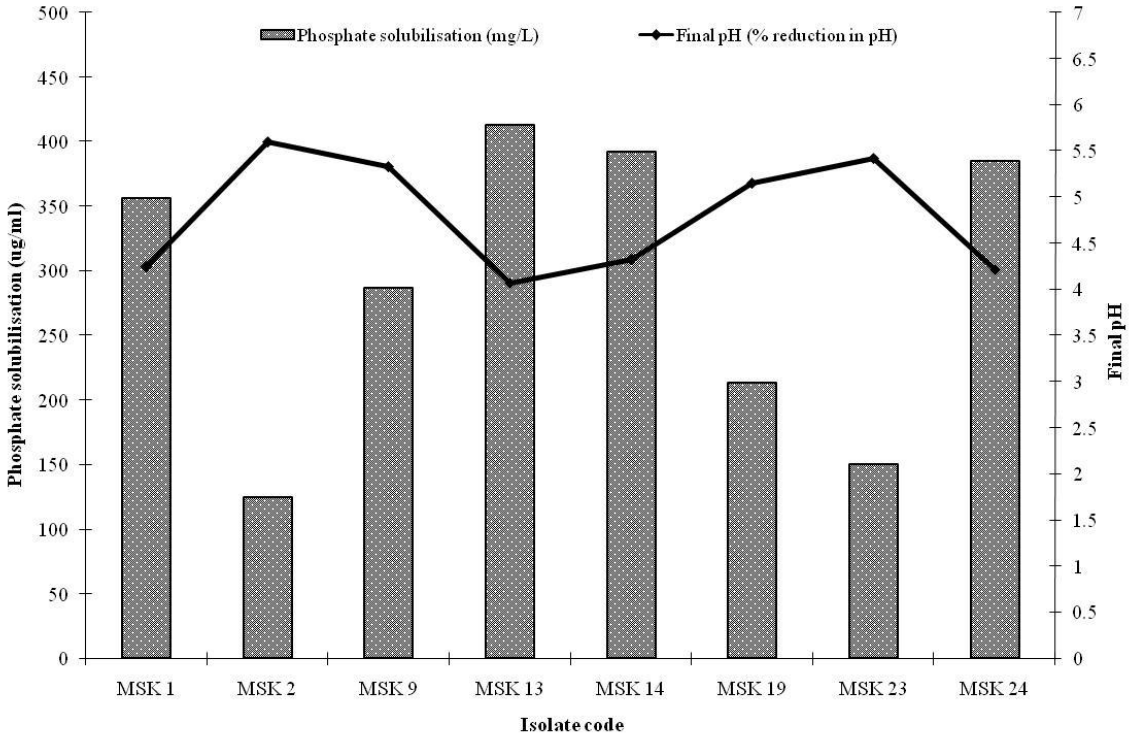

Figure 1: Secondary screening of PSB isolates in liquid Pikovskaya broth for Phosphate solubilising activity and reduction in media pH.

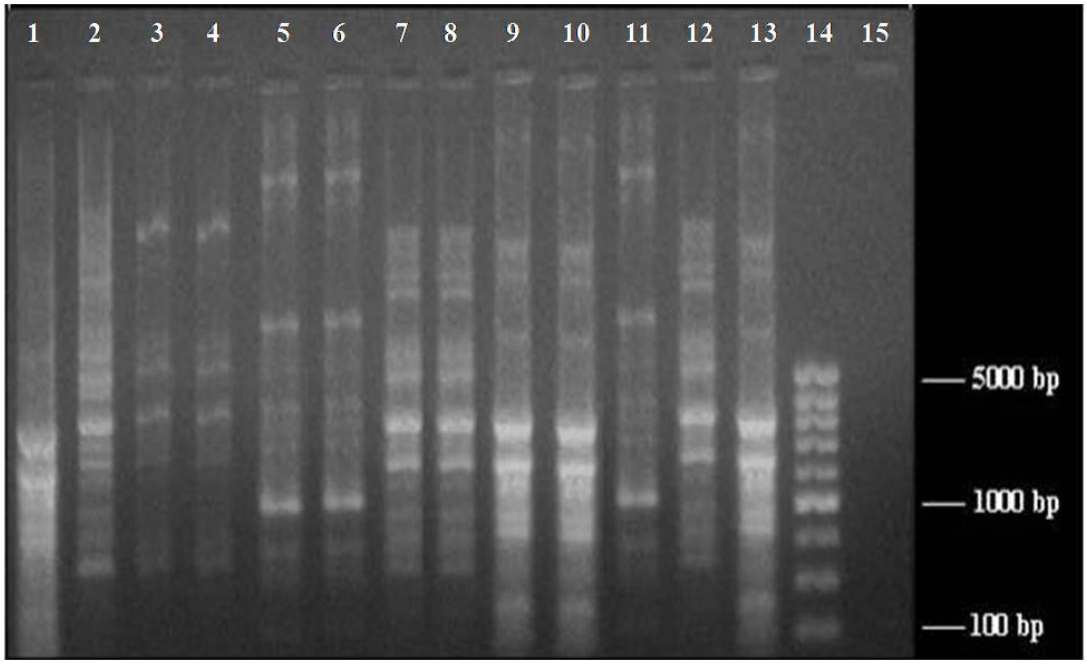

Figure 2. ERIC-PCR fingerprint of unknown bacterial isolates on 1.5% agarose gel. Bacterial colonies were isolated from soil in the plots. Pure colonies were picked, heat lysed, and whole genomic DNA was subjected to ERIC-PCR to get the fingerprint. **Lanes 1-10:** fingerprints of different isolates obtained from selected plates containing 120ug/ml of Ampicillin, **Lane 11:** Standard bacterial strain of *Pseudomonas* sp MSK13., **Lane 12:** Standard bacterial strain of *Serratia* sp MSK1., **Lane 13:** Standard bacterial strain of *Serratia* sp.MSK24, **Lane 14:** 500bp ladder and **Lane 15:** Negative Control.

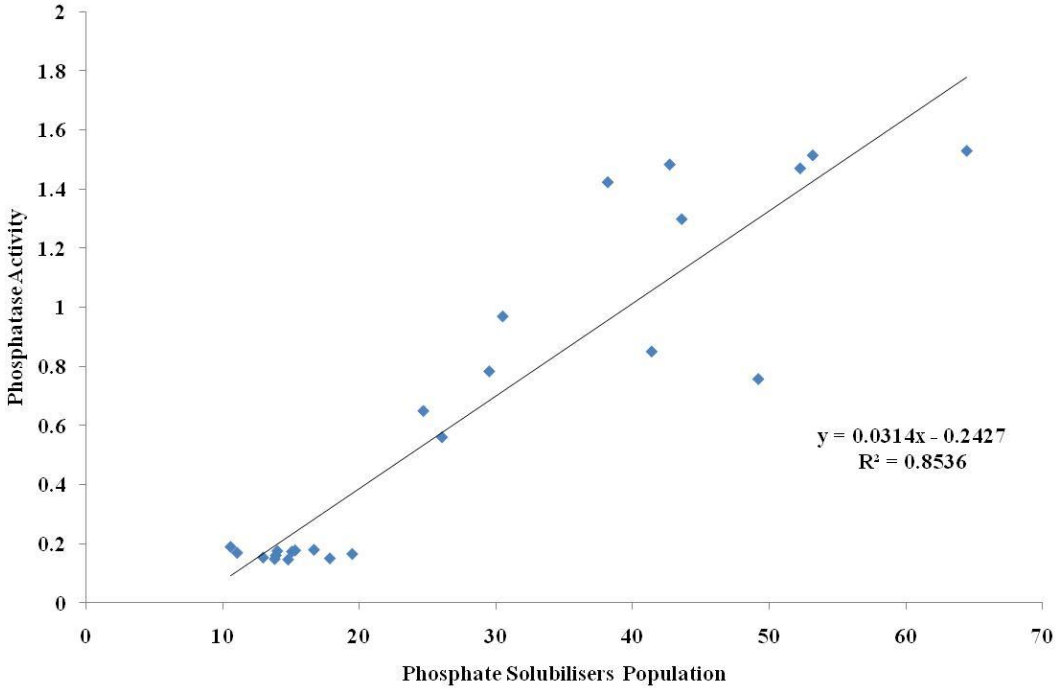

Figure 3: Correlation between the phosphatase activity & phosphate solubilisers population in soil during the chickpea plot experiments.

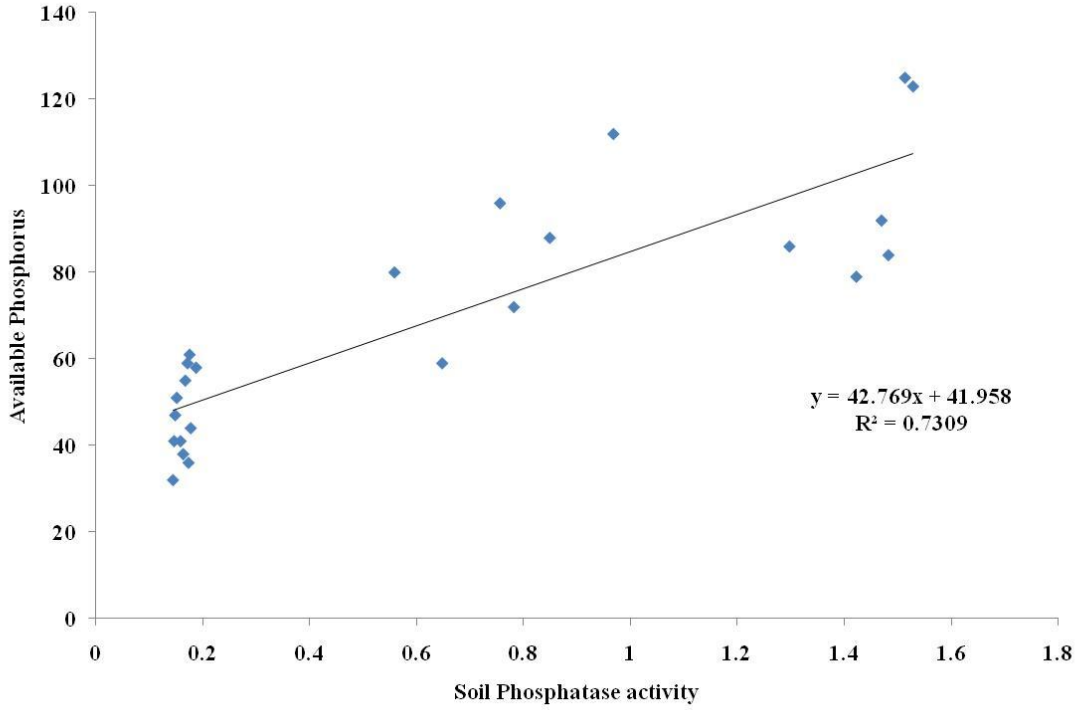

Figure 4: Correlation between the phosphatase activity & available phosphorus in soil during the chickpea plot experiments.

Table

Table 1:Survival of introduced phosphate solubilisers population during growing chickpea crop in plots. Soil suspension diluted in physiological saline was plated on LA-ampicillin plate and genomic DNA was amplified by ERIC-PCR.

Colony forming units (X 10^7cfu/g of dry wt. soil)						
Time (weeks)		**0**	**4**	**8**	**12**	**16**
Control	Ampicillin count	2.2	1.8	1.5	1.7	1.3
	PCR count	0	0	0	0	0
***Serratia* sp. MSK1**	Ampicillin count	15.0	13.0	10.0	8.5	8.0
	PCR count	11.7	10.4	7.2	5.8	4.7
***Pseudomonas* sp. MSK13**	Ampicillin count	13.0	12.0	8.8	7.0	6.0
	PCR count	10.2	9.9	5.6	5.1	3.5
***Serratia marcescens* MSK24**	Ampicillin count	15.0	14.0	11.5	10.5	9.0
	PCR count	13.4	12.0	9.3	8.7	7.6

Table 2: Microbiological and Bio analytical results in soils of chickpea plot. Total microbial count, phosphate solublisers count, soil phosphatise activity, available phosphorus and organic carbon were determined at 0 week (Chickpea sowing stage) and 16 weeks (Chickpea harvest stage) in plot soils.

TREATMENT	TOTAL COUNT (CFU x10^6 / g dry wt. soil)		PHOSPHATE SOLUBILISERS (CFU x10^6 / g dry wt. soil)		SOIL PHOSPHATASE ACTIVITY (uM PNP / g dry wt. soil/ hr)		AVAILABLE PHOSPHORUS (mg/kg)		ORGANIC CARBON (%)	
	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK	0 WEEK	16 WEEK
Control	84.91±8.16a	115.10±3.74b	13.00±0.96a	26.76±1.43b	0.167±0.004a	0.554±0.016c	44.00±5.68a	70.33±6.11a	0.340±0.030a	0.554±0.016c
***Serratia* sp.MSK1**	86.06±5.18a	192.73±11.91a	17.09±1.28a	40.37±5.41ab	0.171±0.004a	0.662±0.019b	47.00±6.24a	98.66±7.05a	0.388±0.030a	0.662±0.019b
***Pseudomonas* spMSK13**	87.10±4.43a	193.10±8.36a	14.09±2.10a	44.99±4.38ab	0.161±0.013a	0.711±0.041ab	48.66±4.97a	96.66±14.31a	0.360±0.036a	0.711±0.041ab
***Serratia* sp. MSK24**	87.80±7.20a	232.66±19.19a	14.37±0.71a	53.14±6.28b	0.157±0.009a	0.836±0.045a	48.00±8.50a	99.66±11.89a	0.368±0.028a	0.836±0.045a

Tested by the Student Newman Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents Mean± Standard error of mean (SEM)