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EFFECT OF INTEGRATED PLANT NUTRIENT SYSTEM (IPNS) ON THE SOIL BIOLOGICAL REGIMES IN RED LOAM SOIL

by

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(2011-11-120)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Effect of integrated plant nutrient system (IPNS) on the soil biological regimes in red loam soil"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Dedicated to

My Family and Teachers

who lit my path.

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LIST OF ABBREVIATIONS

%	percent
@	at the rate of
µg	microgram
^{0} C	Degrees Celsius
B	Boron
B:C	Benefit: Cost
C	Carbon
CD	Critical difference
cm	centimetre
Cu	Copper
DAS	days after sowing
dS	deci Siemens
DTPA	Diethylene Triamine Penta Acetic acid
et al.	And others
Fe	Iron
Fig.	Figure
FYM	Farm Yard Manure
g	gram
h	hour
ha^{-1}	per hectare
i.e.	that is
K	Potassium
KAU	Kerala Agricultural University
kg	kilogram

m	Metre
mg	Milligram
ml	Millilitre
Mn	Manganese
MSL	Mean Sea Level
N	Nitrogen
nm	nanometre
P	Phosphorous
PDI	Percentage Disease Incidence
PGPR	plant growth promoting rhizobacteria
POP	Package of Practices
ppm	parts per million
PSB	Phosphorous Solubizing Bacteria
RDF	recommended dose of fertilizers
s	seconds
t	tonnes
TPF	Triphenyl Formazone
TTC	Triphenyltetrazolium chloride
Var.	Variety
Zn	Zinc

Introduction

1. INTRODUCTION

India has the onerous task of feeding almost 17 percent of global human population and11 percent of the livestock population with only 2.3 percent of the world's land. The entire burden of producing enough depends upon the first few inches of the earth's crust-SOIL. Increasing crop productivity, to meet food requirements of teaming millions in our country, possesses a greater challenge. Hence the situation calls for efforts to intensify the production in both time and space.

There has been a spectacular increase in the use of chemical fertilizers over the past three decades coupled with intensive cropping systems, as the high yielding varieties are extremely fertilizer responsive. During the green revolution era from 1965-1995, fertilizers have been responsible for increasing 55% of yield in developing countries (FAO, 1995). FAO study (2000) reveals that the fertilizer consumption in the world is expected to increase from 134 million tons in 1995-97 to 182 million tons in 2030 at an annual growth rate of 0.9 percent

Replacement of organic manures by inorganic fertilizers is depleting the soil organic matter content. Depletion of organic matter in soil discourages activity of soil micro flora responsible for decomposition of organic matter to enrich soil fertility. Inherent fertility of soil is thus seriously imbalanced due to the dependence on chemical fertilizers. Further, negative nutrient imbalance between crop removal and fertilizer addition has been around 8-10 million t $year^{-1}$. Presently alternate farming strategies viz. organic farming, low input farming is being increasingly studied to sustain the soil resources base even while meeting the needs and concerns.

The balanced fertilization of major elements (NPK) for plant nutrient could be beneficial for the growth of plant above ground parts and roots. However farmers are often forced to make decision about their fertilization strategy that reflects economic rather than agronomic pressure. When economic pressure is lifted, nitrogen and to a lesser extent, phosphorous are the nutrients of choice and

the need for potassium is either under estimated or ignored. As a result imbalanced fertilization is still widespread. Hence the concept of Integrated Plant Nutrient System (IPNS) encompassing adequate and balanced use of nutrients in an integrated manner employing chemical, organic and biofertilizers is the most ideal system of nutrient management. IPNS is a concept and farm management strategy which embraces and transcends from single crop fertilization effects to planning and management of plant nutrients in crop rotation and farming systems on long term basis for enhanced productivity, profitability and sustainability. The system enhances nutrient-use efficiency, maintains soil health, enhances crop yields and reduces cost of cultivation. The primary goal of integrated nutrient management is to combine old and new methods of nutrient management into ecologically sound and economically viable farming systems that utilize available organic and inorganic sources of nutrients in a judicious and efficient way. Integrated nutrient management optimizes all aspects of nutrient cycling. It attempts to achieve tight nutrient cycling with synchrony between nutrient demanded by the crop and nutrient release in the soil, while minimizing losses through leaching, runoff, volatilization and immobilization.

The basic concept underlying the principle of IPNS is to maintain or adjust plant nutrient supply to achieve a given level of crop production by optimizing the benefits from all possible sources of plant nutrients and to reduce the inorganic fertilizer requirement, to restore organic matter in soil, to enhance nutrient use efficiency and to maintain soil quality in terms of physical, chemical and biological properties. Bulky organic manures may not be able to supply adequate amount of nutrients, nevertheless their role become important in meeting the above objectives. The development of IPNS to suit different farming system is a major challenge for all stake holders in agriculture to ensure sustainable food security.

To avoid the side effects of fertilizers and to provide socioeconomic and ecological benefits, biofertilizers are generally recommended. Biofertilizers contains living micro organisms and it is expected that their activities will

influence the soil ecosystem and produce supplementary substance for the plants. The region and crop- specific consortia of biofertilizers (combining *Azotobacter, Azospirillum,* Phosphate solubilising bacteria, *Rhizobium* and Plant Growth Promoting Rhizobacteria) should be developed. Though there are a number of government and private agencies engaged in production and sale of microbial inoculants, the use of these inoculants by users is very limited compared to total acreage under agricultural crops. There is a thus dire need to popularize the technology of integrating these bioinoculants with inorganics by the way of substitution which can be achieved through IPNS.

Thus IPNS approach aims to enhance soil and crop productivity through a balanced use of mineral fertilizers combined with organic and biological sources of plant nutrients to ensure sustainability of the production systems. The present project investigates the effect of biofertilizers in combination with chemical fertilizers on nutrient management in red loam soil with a test crop bhindi. Bhindi or Ladies finger which is also known as okra is grown throughout the tropical-subtropical regions and also in the warmer parts of temperate regions. It is one of the most important vegetable cultivated in India. In Kerala, it is cultivated in 1128 ha and production is about 11100 tonnes.

Hence with the aforesaid points in mind the present project is envisaged with the following objectives.

- to assess the conjugal effect of manures and chemical fertilizers on dynamics of major agriculturally significant soil enzymes , available nutrient status of the soil , its relation with the activities of major soil enzymes, soil microflora, yield and yield attributes of the test crop (Bhindi)
- to compute Soil Fertility Index through Enzyme Activity Number

Review of literature

2. REVIEW OF LITERATURE

Soil is an important component of all terrestrial ecosystems, as well as a main source of production in agriculture and forestry. Its function is essential for maintenance of the global biogeochemical cycles for all important nutrients, and thus, the processes in soils affect many other components of ecosystems, both biotic and abiotic. Processes performed by soil microorganisms and other members of the soil biota provide life to the soil. These soil microorganisms, processes are greatly influenced by the activities of human beings so called anthropogenic effects like management, fertilization etc. In order to sustain soil health and agriculture, Integrated Plant Nutrient System (IPNS) has been developed. In this chapter an attempt is made to present a selective overview of literature on the effects of Integrated Plant Nutrient System on soil enzymes, Soil Fertility Indices and Enzyme activity number.

2.1. Integrated nutrient management

2.1.1. INM and Soil health

Generally three terminologies are used for conveying the same meaning namely Integrated Plant Nutrient System (IPNS). Integrated Plant Nutrient Supply System (IPNSS) and Integrated Nutrient Management (INM). Although all these three kinds of terminologies appear to be different, they convey the same meaning (Acharya *et al.*, 1998).

The high cost of fertilizers and unstable crop production call for substituting part of the inorganic fertilizers by locally available low cost organic sources viz., manures, green manures, biofertilizers etc. in an integrated manner for sustainable production and to maintain soil health (Acharya, 2002).

Tolanur and Badanur (2003) reported that available soil nutrients like N, P and K increased significantly with the application of various organic sources of nutrients in combination with the fertilizers than with the application of inorganics alone.

Baskar (2003) opined that the continuous use of organics along with the inorganic fertilizers increased nutrient uptake and nutrient use efficiency of major nutrients than the inorganic fertilizers alone.

Organics and fertilizers are not only complementary but also synergistic since organic inputs have beneficial effects beyond their nutritional components and enhance the efficiency of the applied mineral fertilizers (Laxminarayana, 2006).

Integrated use of organic manures along with optimum doses of chemical fertilizers not only produced highest and sustainable crop yields but also enhanced the efficiency of added fertilizers as well as fertility status of the soil (Laxminarayana and Patiram, 2006).

2.1.2. Bioinoculants

Biofertilizers are low-cost and ecofriendly input have tremendous potential for supplying nutrients which can reduce the chemical fertilizer dose by 25-50% (Vance, 1997).

The beneficial effect of *Azospirillum* can be accrued from its nitrogen fixation and stimulating effect on root development (Wua *et al.*, 2004 ; Noshin *et al.*, 2008).

El- Komy (2004) observed that *Azospirillum* spp. can exert a positive effect on plant growth is probably composed of multiple effects including synthesis of phyto- hormones, N_2-fixation, nitrate reductase activity and enhancing minerals uptake.

Noshin, *et al.*, (2008) reported that *Azospirillum* plant association is accompanied by biochemical changes in roots, which in turn promote plant growth and tolerance to low soil moisture. The bacteria stimulate plant-growth even in the presence of several stresses such as drought.

Jen-Hshuan (2006) reported that phosphorous solubilising bacteria culture increased yield up to 200-500 kg ha^{-1} thus saving nearly 50% of dose of P.

Javed *et al.*, (2009) observed that production of biologically active substances or plant growth regulators (PGRs) is one of the major mechanisms through which PGPR influence the growth and development of plants.

2.1.3. Farm Yard Manure

Sarkar *et al.* (2000) opined that combined use of chemical fertilizers and FYM could obtain higher yields of soybean and wheat besides improvement of soil fertility.

Singh and Ram (2000) concluded that the addition of urea along with organic manures was advantageous as compared to the addition of urea alone. This addition will be helpful in improving physic-chemical properties of soil with positive effects on microbial population and their activities.

Farm Yard Manure which is a composted mixture of cowdung and farm waste, widely used as a nutrient source in the Indian subcontinent for various cropping systems is reported to have increased the activities of various soil enzymes and microbial biomass (Goyal *et al.*, 2003).

Zaller and Kopke (2004) found that plots with the application of FYM (30 t ha^{-1} $year^{-1}$) had significantly increased soil pH, P and K concentrations, microbial biomass, dehydrogenase activity, earthworm cast production and altered earthworm community composition than plots without FYM application.

The highest N, P and K uptake was associated with the conjunctive use of soil test based application of N, P, K and S, FYM and green manuring treatment (Singh *et al.*, 2006)

2.1.4. Organic amendments

Vermicompost is homogenous, with desirable aesthetics, plant growth hormones and high levels of soil enzymes, enhancing microbial populations and tends to hold more nutrients over longer periods without adverse impacts on the environment (Ndegwa and Thompson, 2001).

High and diverse populations of native microorganisms favour biochemical reactions and Vermicompost enriched with rock phosphate showed high P bioavailability and led to enhanced yield and uptake of nutrients in cowpea (Kumari and Ushakumari, 2002).

Dahia *et al.,* (2003) reported that sugarcane trash enriched with Mussorie rock phosphate increased the ratoon yield of sugarcane.

Vermicomposting is the non-thermophilic biodegradation of organic material through the interaction between earthworms and microorganisms (Arancon *et al.*, 2004), and the final product, vermicompost, is enriched in humus and available P (Le Bayon and Binet, 2006).

Vermicompost is an efficient vehicle and support medium for growth of *Rhizobium*, and its supplementation with native diazotrophic bacteria and mycorrhizas resulted in enhancement of maize growth as reported by Gutierrez-Miceli *et al.,* (2008).

Masils *et al.,* (2009) reported that application of vermiwash along with enriched vermicompost increased the yield and quality of crops

Battikopad *et al.* (2009) reported that application of cattle dung enriched with rock phosphate along with Effective microorganisms (0.5 mL kg^{-1}) improved the microbial activities and enhanced the health and productivity of soil.

Aria *et al.*, (2010) observed that vermicompost inoculated with *Thiobacillus* had a positive effect on the conversion of hard rock phosphate into water-soluble phosphate (WSP).

2.1.5. INM and enzyme activities

Acid phosphomonoesterase is a good index of the quality and quantity of organic matter in the soil (Jordan *et al.*, 1995; Mullen *et al.*, 1998; Bergstrom *et al.*, 2000) and can be very high in arable soils as long as the levels of organic matter in the soil are maintained (Dick *et al.*, 1994).

Urease, the enzyme that catalyses the hydrolysis of urea, has also been widely used in the evaluation of changes in soil quality due to soil management. Its activity increases due to organic fertilisation (Pascual *et al.*, 1999; Chakrabarti *et al.*, 2000) and after the addition of cattle slurry to the soil (Kandeler and Eder, 1993) and decreases as a consequence of ploughing (Saviozzi *et al.*, 2001).

Singaram and Kamalakumari (2000) reported that farm yard manure addition stimulates soil enzyme activity while addition of graded doses of NPK had no marked detrimental effect on the enzyme dynamics of the soil.

Dinesh *et al.* (2000) reported that addition of organic manures increased microbial activity/diversity and C turnover, which subsequently led to greater enzyme synthesis and accumulation in the soil matrix

Additions of organic amendments stimulate microbial production of enzymes such as dehydrogenase and phosphatase enhanced organic matter decomposition and organic P mineralization (Garcia-Gil *et al.*, 2000; Takeda *et al.*, 2009).

Greater enzyme activities in the green manure amended soil was the result of not only due to a large microbial mass, but also higher amount of endoenzymes, greater enzyme production by microbial biomass and direct contribution of enzymes by green manure (Dinesh *et al.*, 2000) and higher degree

of stabilization of enzymes in humic substances and increased soil organic carbon concentration (Elfstrand *et al.*, 2007).

A long-term intensive monoculture usually supplies lower amounts and diversity of organic matter than crop rotation, thus suppressing microbial activities and consequently decreasing enzyme activities (Klose and Tabatabai, 2000).

Albiach *et al.* (2000) found increase in the selected enzymatic activities (dehydrogenase, alkaline phosphomonoesterase, phosphodiesterase, arylsulphatase, and urease) in soil after the use of organic amendments in a long-term field experiment.

The decomposition of soil carbon depends on the microbial production of exocellular enzymes that convert complex compounds into smaller products (Nannipieri *et al.*, 2002)

Srinivas and Saroja (2002) reported that the addition of organic manures as farm yard manure at 10 t ha^{-1} caused significant differences in dehydrogenase activity in submerged vertisol planted rice.

Sriramachandrasekharan (2002) found that the application of farmyard manure along with the recommended dose of fertilizer registered the highest dehydrogenase and urease activity as compared to the other treatments.

Parham *et al.* (2002) reported that the microbial biomass C and activities of alkaline phosphomonoesterase, phosphodiesterase, inorganic pyrophosphatase and dehydrogenase were significantly higher in the soils treated with cattle manure.

Integration of organic manures (vermicompost) with chemical fertilizers triggered the activities of major soil enzymes such as urease, phosphatase, protease, dehydrogenase and cellulase (Aparna and Rajendran, 2002).

Arancon *et al.* (2004) reported that organic fertilizers can increase crop growth and yield even in a single growing season when they are applied in small quantities suggesting the existence of some sort of short-term biological plant growth promoting mechanism.

Bhattacharyya *et al.* (2005) and Krishnamurthy *et al.* (2011) reported that addition of organic manures increased the urease activity over mineral N and control to the significant extent.

Krishnakumar *et al.* (2005) and Krishnamurthy *et al.*, (2011) reported higher phosphatase activity in organic manure amended rice soil.

Gianfreda and Ruggiero (2006) observed a typical increase in enzyme activity shortly after the addition of organic amendments to the soil.

Tejada *et al.* (2006) found an increase of urease, β-glucosidase, alkaline phosphatase and arylsulfatase activities after the application to the soil of diverse organic wastes such as cotton gin compost, beet vines composted with crushed cotton gin compost and poultry manure to the soil.

The negative effect of agricultural practices could be rectified by the correct utilization of manures within cropping system either alone or in combination with mineral fertilizer (Mandal *et al.*, 2007)

Balakrishnan *et al.* (2007) reported reduction in dehydrogenase activity in organic amended soil after 60 days of addition.

Gilani and Bahmanyar (2008) observed a positive correlation between soil enzyme activity and organic matter content of the soil, and with the water soluble soil organic C.

Gaind and Nain (2010) reported that poultry manure amended paddy straw compost improved soil microbial biomass, enzymatic activities and highest grain yield of rice in a study using rice as the test crop

Gao *et al.*, (2010) observed that the mixed input of organic and inorganic substrates increases the synthesis of soil hydrolytic enzymes.

2.2. Soil biological regimes – Enzymes

Soil enzyme activities have been suggested as suitable indicators of soil quality and mainly originate from microorganisms (Ladd, 1978; Zimmermann and Frey, 2002), animals and plants (Tabatabai, 1994) as well as from the decomposition of plants and animal residues (Shan *et al.*, 2008).

The enzymes play a key role in biochemical functions, in the overall process of organic matter decomposition (Sinsabaugh *et al.*, 1993) and for the maintenance of concentration of soil ions and climate (Jimenez *et al.*, 2002) in the soil system.

The activity of soil enzymes are influenced by the nature, age of crops and addition of fertilizers and manures (Singaram and Kamalakumari, 2000).

Soil enzyme activities are 'sensors' of soil degradation since they integrate information about microbial status and physico-chemical conditions of soil in relation to nutrients availability (Aon and Colaneri, 2001; Baum *et al.*, 2003).

According to Burns and Dick (2002) the decomposition of soil organic matter is mediated by extracellular enzymes that degrade the biopolymers contained within plant and microbial cell walls and reduce macromolecules to soluble substrates for microbial assimilation.

Beknazarov (2002) reported a lower activity of enzymes with increasing soil depth and a two fold increase with the addition of fertilizers.

Graham and Haynes (2005) noted that major indicators of microbial functional pool include microbial biomass carbon (MBC) and activity of exocellular enzymes involved in the transformations of carbon (i.e. amylase,

cellulase and invertase), nitrogen (i.e. protease) and phosphorus (i.e. phosphatases).

Enzymes play key roles in the cycling of nutrients in nature and their activity is sensitive to agricultural practices and considered as an index of soil fertility (Nannipieri *et al.,* 2002; Yao *et al.,* 2006).

Enzymes in the soil can give information on the different biochemical reactions, which often reflect natural or anthropogenic processes (Kremer and Li, 2003).

Heavy metals can affect soil microbial activities such as respiration, ammonification, nitrification and enzyme activities (Zhang *et al.,* 2006).

Ling *et al.,* (2010) opined that soil enzymes are potential indicators of soil quality due to their biological nature, simple measurement and rapid response to changes in soil management when compared to other biological properties.

Nannipieri *et al.,* (2011) reported that during decomposition processes, C, nitrogen (N), phosphorous (P), and sulphur (S) are transformed into products available for use by microorganisms and plants which are catalyzed by soil enzymes, making the measurement of enzyme activities an effective tool in gauging biogeochemical changes occurring in soils.

Enzymes are important for catalysing several vital reactions necessary for the life processes of microorganisms in soils and are also important for the stabilization of soil structure, formation of organic matter, nutrient cycling and decomposition of organic wastes, hence playing an important role in agriculture and agroforestry (Garcia and Nahas, 2012)

2.2.1. Urease

Roscoe *et al.,* (2000) also reported a high correlation (r¼0.97**) between urease activity and organic matter.

Saviozzi *et al.,* (2001) suggested that urease has been widely used to evaluate changes on soil quality related to management, since its activity increases with organic fertilization and decreases with soil tillage.

Low level of urease activity in fertilizer treated soil indicated that mineral N without sufficient amount of available organic substrate may not have impact on urease activity (Zaman *et al.,* 2002).

Yang *et al.,* (2006) opined that urease activity is used as a soil quality indicator because it is influenced by soil factors such as cropping history, organic matter content, soil depth, management practices, heavy metals and environmental factors like temperature and pH.

Makoi and Ndakidemi (2008) proposed that the understanding of urease activity provides better way to manage urea fertilizer, especially in warm high rainfall areas, flooded soils and irrigated conditions.

Wang *et al.,* (2008) the results showed that long-term application of chemical fertilizers and organic manure increased the activities of urease, invertase and phosphatase in 0-20 cm and 20-40 cm soil layers in different degree and the combined application of them increased the activities of the three enzymes significantly, with an increment of 43.6%-113.2%, 25.9%-79.5% and 14.7%-134.4% in 0-20 cm soil layer and 56.1%-127.2%, 14.5%-113.8% and 16.2%-207.2% in 20-40 cm soil layer respectively.

Srinivas *et al.,* (2004) reported that urease is an important soil enzyme, which directly decides the hydrolysis of urea.

2.2.2. Phosphatase

Phosphatase enzyme plays a key role in the soil system apart from being good indicator of soil fertility (Dick *et al.,* 2000).

Dick *et al.* (2000)suggested that for cropping systems that rely heavily on natural biological processes to maintain productivity, measuring the alkaline phosphatase and acid phosphatase ratio may be preferable than the chemical approaches for evaluating effective soil pH and liming needs.

Phosphatase activity apart from indicating changes in the quantity and quality of soils' phosphorated substrates are also a good indicator of its biological state (Pascual *et al.*, 2002).

Turner and Haygarth (2005) evaluated phosphatase activity in temperate grassland, and found a strong correlation between phosphatase activity and soil properties such as pH, total N, organic P and clay content.

Chen *et al.,* (2009) opined that soil alkaline phosphatase activity was significantly positively correlated with soil microbial biomass and dehydrogenase and urease activities, but negatively correlated with soil pH.

2.2.3. Dehydrogenase

The activity of dehydrogenases basically depends on the metabolic activity state of the soil biota which significantly correlated with soil biomass carbon in organic amended soil (Garcia-Gill *et al.*, 2000)

Dehydrogenase activity (DA) is related to a group of enzymes which participate in the metabolic reactions producing energy in the form of ATP through the oxidation of organic matter, which is especially interesting in the composting process and has been studied in few works to monitor the biological activity of the composting process (Wong and Fang, 2000; Tiquia *et al.*, 2002).

Masciandaro *et al.* (2000) suggested the usage of dehydrogenase activity as an index of microbial activity.

As presence of dehydrogenases, which are intracellular to the microbial biomass, is common throughout microbial species and they are rapidly degraded following the cell death. The measurement of microbial dehydrogenase activity in soils and sediments has been used extensively and serve as indicators of soil quality (Obbard, 2001).

Dehydrogenase activity in soil depends on the content of soluble organic carbon and the increased organic matter in the surface soil enhanced the soil dehydrogenase activity as reported by (Zaman *et al.*, 2002, Kizilkaya and Hepsen, 2007; Kizilkaya, 2008).

Activities of dehydrogenase and phosphatases were significantly improved upon inoculation with *Azospirillum, Azotobacter* and *Glomus fasciculatum* (Aseri and Rao, 2005).

Benitez *et al.* (2005) opined that dehydrogenase activity has been correlated with some operational and biochemical parameters such as temperature, nitrogen content or other enzymatic activities.

Manjunatha *et al.* (2006) found a marked increase in dehydrogenase activity in the soils of organic farms than conventional farms in the selected major cropping systems viz. cotton, sugarcane, jowar and grapevine.

Furczak and Joniec (2007) reported that stimulation of dehydrogenase activity was accompanied by an increase in the number of the microbial groups and improvement in other living conditions such as aeration and moisture.

The activity of the dehydrogenase activity is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity because it is linked to viable cells (Kizilkaya and Hepsen, 2007).

Dehydrogenase activity is an indicator of microbial activity and has been used as a valued bio marker of soil quality under different agricultural management practices (Garcia-Ruiz *et al.*, 2008).

Barrena *et al.* (2008) opined that dehydrogenase activity is a useful method to describe the biological activity of the whole composting process and can be correlated with static respiration index (used as stability parameter) during the composting maturation stage.

2.2.4. Cellulase

Cellulase is characteristically useful as a soil quality indicator, and reflects past biological activity, the capacity of soil to stabilise the soil organic matter, and can be used to detect management effect on soils (Bandick and Dick, 1999; Ndiaye *et al.*, 2000).

Cellulase enzyme is very sensitive to changes in pH, and soil management practices (Acosta-Martinez and Tabatabai, 2000; Madejon *et al.*, 2001).

Cellulase plays an important role in energy availability in the soil which is directly related to labile C content and with the ability to stabilize soil organic matter, showing low seasonal variability (Knight and Dick, 2004).

Srinivasulu and Rangaswamy (2006) reported a significantly more stimulatory effect of cellulases in black soil than in red soil.

Extracellular enzymes mediate the degradation, transformation and mineralization of soil organic matter. The activities of cellulases, phosphatases and other hydrolases have been extensively studied and in many cases stoichiometric relationships and responses to disturbances are well established (Sinsabaugh *et al.*, 2008).

Cellulase activity, catalyzing the hydrolysis of cellobiose, has been shown to be sensitive to changes in soil as well as an early indicator of changes in soil organic carbon before these changes are reflected in changes in total organic C content (Stott *et al.,* 2010).

2.2.5. Protease

Asmar *et al.* (1992) found that protease activity was increased in soil samples amended with nutrients and glucose. In their study, protease activity was correlated with ATP content, respiration and bacterial biomass

According to the Narasimha *et al.*, (1999) discharged effluence from cotton ginning mill improved the soil protease activity.

Proteases are enzymes that break the peptide bonds between amino acids of proteins and production of free amino acids (Chang *et al.,* 2007)

Proteases catalyze the conversion of organic nitrogen into ammonia (NH_3) or ammonium (NH_4^+). Therefore, protease synthesis by soil microorganisms depends on the availability of N (Geisseler and William, 2008).

2.2.6. Soil fertility index

Specific enzymatic activities have been used to compare and discuss values of enzymatic activities in soils with different organic matter contents and could be considered as simple indexes of soil quality (Barriuso *et al.*, 1988).

Bentham *et al.*, (1992) used principal component analysis (PCA) to evaluate the degree of recovery of mine soils, based on three different biochemical indexes viz. dehydrogenase activity, ATP and ergosterol content.

Most widely used simple indices to assess the soil fertility are the metabolic quotient (qCO2), the death rate quotient (qD) and the ratio between biochemical properties and the total C and N soil content (specific activities if the

biochemical property used is an enzymatic activity, (Barriuso *et al.*, 1988) or the C-biomass content (Kandeler and Eder, 1993; Landi *et al.*, 2000).

Multiple-variable indicator kriging (MVIK) developed by Smith *et al.*, (1993), elegantly integrates different properties into a single, joint index with the aim of predicting the probability of an area satisfying a given quality standard.

Yakovchenko *et al.* (1996), employed probability maps using parameters deduced from the relation between the amount of N taken by the crop, and the mineralized nitrogen (microbial respiration plus net mineralized N) during the growth season to assess the soil quality.

Halvorson *et al.* (1996) combined six variables, transforming them with a threshold-based indicator variable transform, into one single variable (MVIT), indicating the probability of a soil being high or low quality.

Trasar-Cepeda *et al.* (1998) showed that in native soils under climax vegetation, and without human distortion, the biochemical equilibrium that is characteristic of a mature stable ecosystem can be expressed mathematically as a combination of several microbiological and biochemical properties which can be used as an index of soil quality.

Dilly and Blume (1998) suggested that the use of indices which combine data from a small number of biochemical properties could mask important microbial features in the soil ecosystem and suggested to prefer basic biochemical properties related to microbial biomass and microbial activity.

Nannipieri *et al.* (2002) proposed that the cascade of enzyme activities approach (i.e. the lignocellulosic factor) can be considered as one of the best among those using biochemical properties as indicators of soil quality due to its accurate and focused selection of enzyme activities.

2.3 Micro flora in rhizosphere and non-rhizosphere in soil.

The differing physical, chemical, and biological properties of the root-associated soil, compared with those of the root-free bulk soil, are responsible for changes in microbial diversity and for increased number and activity of micro-organisms in the rhizosphere micro-environment (Kennedy, 1998).

Singh and Ram (2000) concluded that the addition of urea along with organic manures was always advantageous as compared to the addition of urea alone as this will be helpful in improving physicochemical properties of soil with positive effects on microbial population and their activities.

Soil microbial communities in the rhizosphere are the most important functional component of soil biota playing a key role in energy flows and nutrient reactions (Tate, 2000).

PGPR are able to increase plant growth, accelerate seed germination, improve seedling emergence responses to external stress factors, protect plants from disease, and promote root growth (Lugtenberg *et al.*, 2002)

Plant association with AMF fungi are known to increase nutrient and water uptake due to an increase in the volume of soil explored by fungal hyphae in the rhizosphere (Jeffries *et al.,* 2003; Aroca and Ruiz-Lozano 2009).

Root-colonizing plant beneficial bacteria, commonly referred to as plant growth-promoting rhizobacteria (PGPR), are capable of stimulating plant growth when cultivated in association with a host plant (Vessey, 2003; Hayat *et al.*, 2010).

The degree of intimacy between PGPR and the host plant can vary depending on where and how the bacteria colonize the host plant whether rhizospheric or endophytic (Vessey, 2003).

The rhizosphere provides a rich source of energy and nutrients to the bacteria resulting in higher bacterial diversity and larger populations when compared with bulk soil (Gray and Smith, 2005).

The microbial inoculation had improved the soil fertility status resulting in a significant increase in the leaf area and biomass production of ber and aonla (Aseri and Rao, 2005).

Rhizobacteria also secrete a wide variety of metabolites into the rhizosphere that are utilized by plants (van Loon, 2007).

Microbial activity is a key mechanism for increasing soil P availability after the application of organic materials (Gichangi *et al.*, 2009; Khan and Joergensen, 2009).

Hayat *et al.* (2010) plant-microbe interactions in the rhizosphere are the determinants of plant health and soil fertility.

2.3.1. Micro flora and enzyme activities

Factors influencing soil microbial activity exert control over soil enzyme production and control on nutrient availability and soil fertility (Sinsabaugh *et al.*, 1993).

Addition of organic amendments may stimulate microbial production of enzymes such as dehydrogenase and phosphatase to enhance organic matter decomposition and organic P mineralization (Garcia-Gil *et al.*, 2000; Takeda *et al.*, 2009).

Use of plant growth promoting rhizobacteria (PGPR) has a potential role in developing sustainable systems for crop production (Sturz *et al.*, 2000).

Poll *et al.* (2003) reported that the xylanase activity as well as fungal biomass increased only gradually with diminishing particle size, whereas the relative abundance of fungi decreased with diminishing particle size.

Fiedler *et. al.*, 2004 reported that reducing conditions in the soil were associated with high $Fe2^{+}$ concentration in the soil solution and a significant increase of extra plasmatic Fe^{2+} in roots of maize due to intense stimulation of microbial growth and dehydrogenase activities in the ecosystem.

Soil dehydrogenase is considered as an indicator of overall microbial activity because it occur intracellularly in all living microbial cells and is linked with microbial oxydoreduction processes (Stepniewska and Wolinska, 2005).

Jezierska and Frac (2005) found that both the use of organic fertilizers and spring wheat influenced the number of microorganisms and enzymatic activities.

Many soil borne microorganisms have proved beneficial over the years and are now integrated into a wide variety of growing systems as part of integrated pest and productivity management practices (Antoun and Prevost, 2005).

Lalfakzuala *et al.* (2006) reported that fertilizers treatment increases microbial population numbers and microbial enzyme activities.

Application of Suaeda compost in combination with farmyard manure and phosphate solubilizing bacteria significantly increased the soil microflora such as bacteria, fungi and actinomycetes and soil enzyme activities such as dehydrogenase, alkaline phosphatase, cellulase and urease in soil cultivated with *Arachis hypogaea* as observed by Balakrishnan *et al.*, (2007)

Kondratowicz (2007) opined that fertilization with nitrogen and manure resulted in an increase of microbial populations and a higher enzymatic activity in soil.

Avis *et al.* (2008) reported that the increased use of microorganisms afforded by their multifaceted beneficial effects may further help in reducing problems associated with the use of synthetic chemicals in agriculture.

Dehydrogenase activity was higher with combined application of organics and fermented organics than their individual applications and RDF +FYM (Shwetha, 2008).

Organic amendments like farm yard manure (FYM), poultry litter (PL) and biogenic waste compost (BWC) increased cumulative CO_2 release, microbial biomass C, N and P and activity of dehydrogenase and alkaline phosphomonoesterase compared to unamended soils. (Malik *et al.*, 2013)

2.3.2. Soil respiratory activity.

Soil respiration reflects the intensity of the soil organic matter decomposition, mineralization and the incidence of the microorganisms in soil, and it is often used for the biomass determination (Anderson and Domsch , 1990).

Respiration activity was frequently used to evaluate soil quality, soil fertility or soil contamination with organic pollutants or heavy metals and for the evaluation of the effect of the change in land use (Kubat *et al.*, 2002).

Ruzek *et al.* (2005) reported close relationships between the soil respiration activity, microbial biomass C and total organic C content in most of the investigated soils.

Iovieno *et al.* (2009) reported that organic amendment increased soil respiration, fluorescein diacetate hydrolysis, phosphatase and arylsulphatase activities in Mediterranean soils.

2.4. Enzyme kinetics

2.4.1. Vmax and Km

Rao *et al.*, (1996), reported a decrease in K_m of acid phosphatase after immobilization on artificial mineral, organic, or organo-mineral complexes.

Lower V_{max} values tend to increase soil C concentrations and higher C substrate availability which makes the sensitivity of K_m to temperature less

important. An increase in K_m can counteract an increase in enzyme V_{max} under warming conditions, thereby reducing the temperature sensitivity of decomposition in soils (Davidson *et al.*, 2006).

K_m is the substrate concentration at half-maximal enzymatic velocity (V_{max}), and is indicative of the affinity an enzyme has for its substrate (German *et al.*, 2011)

Stone *et al.* (2012) also observed more variation in the K_m response to temperature than in the V_{max} response

2.4.2. Enzyme activity number

Stefanic *et al.* (1984) used a weighted average to calculate the biological index of fertility (BIF) using dehydrogenase and catalase activity, respectively, and a proportional coefficient.

Beck (1984) proposed enzyme activity number (EAN) as biological index based on five different enzymes given viz. dehydrogenase, catalase, phosphatase, cellulase and protease.

Stevenson (1986) opined that in case of EAN index, cellulase activity can be used instead of amylase because cellulose is more important than starch in plant residues.

Enzyme activity number tends to be decreasing with the intensive agricultural practices like tillage, cultivating virgin soils etc. as reported by Saviozzi *et al.*, 2001.

Riffaldi *et al.*, 2002 observed higher enzyme activity number in untilled management system than the tilled management system.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Effect of integrated plant nutrient system (IPNS) on the soil biological regimes in red loam soil "was carried out in the Dept. of Soil Science and Agrl. Chemistry at College of Agriculture, Vellayani during March-June 2012. The present study is envisaged to assess the conjugal effect of manures and chemical fertilizers on dynamics of major agriculturally significant soil enzymes, available nutrient status of the soil, its relation with the activities of major soil enzymes, soil microflora, yield and yield attributes of the test crop and computation of Biological Fertility Index through Enzyme Activity Number. The investigation pertaining to the study consists of two parts i. preparation of enriched vermicompost using various bioinoculants and organic amendments, ii. Field experiment using a test crop Bhindi with variety Varsha uphar. The materials and the methods adopted for the study are briefly discussed in this chapter.

3.1. Details of the experimental site

3.1.1. Location

The experiment was conducted in D block of the Instructional Farm at College of Agriculture, Vellayani. The site is situated at 8^0 30 N latitude and 76^0 54 E longitude and at an altitude of 29 m above MSL.

3.1.2. Season

The experiment was conducted during the period of March 2012 to June 2012.

3.1.3. Weather

Data on weekly average of temperature, evaporation, relative humidity and average rainfall during the cropping season was collected from Agro Meteorological Observatory attached to NARP, Southern Region, at College of

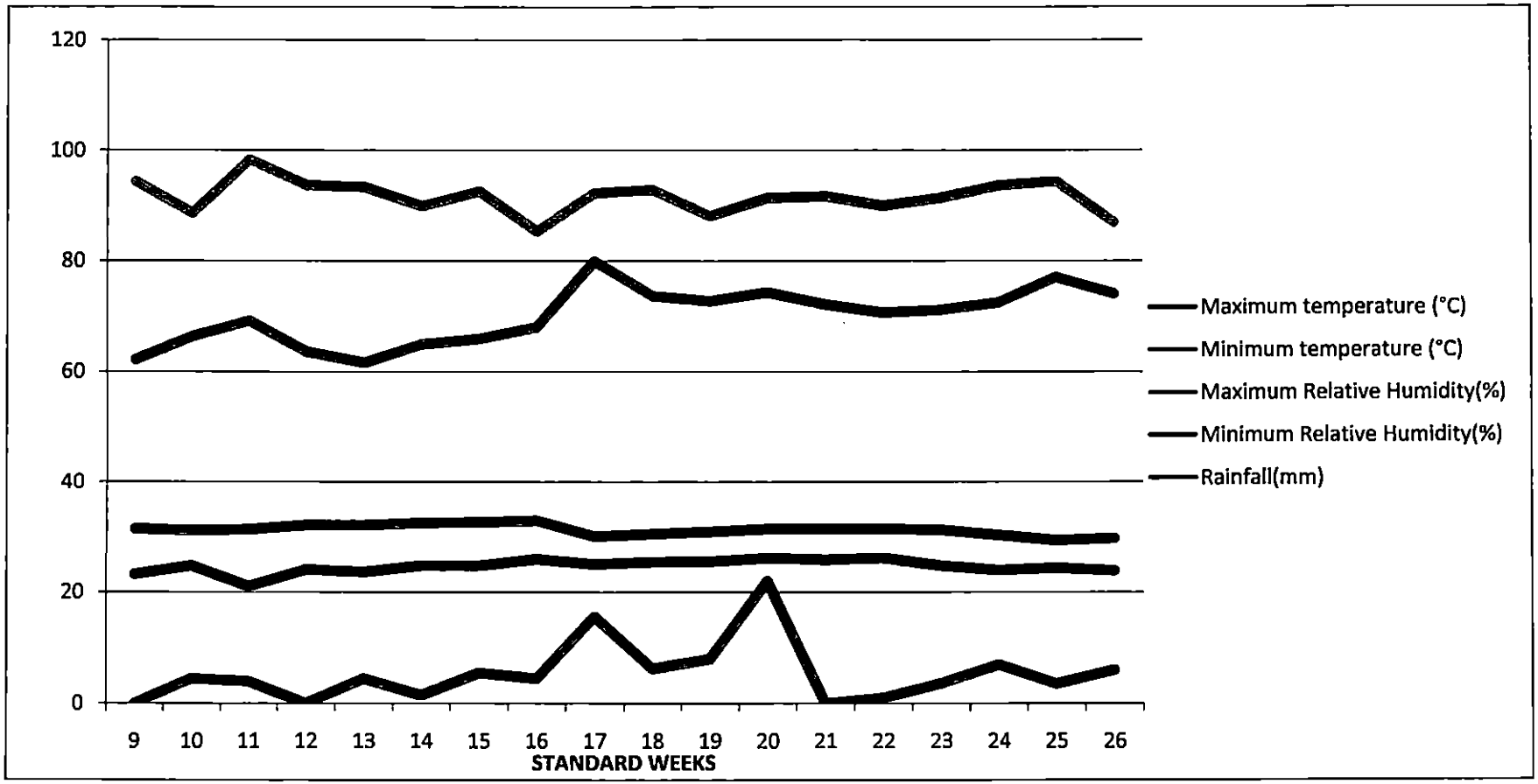
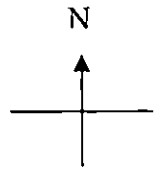


Fig. 1. Weather parameters during field experiment (March 2012- June 2012)



T_1	T_3	T_5
T_{15}	T_2	T_3
T_{12}	T_4	T_{11}
T_3	T_{14}	T_6
T_{13}	T_1	T_8
T_2	T_9	T_4
T_8	T_{12}	T_{14}
T_5	T_6	T_7
T_{11}	T_{10}	T_1
T_4	T_{15}	T_{13}
T_6	T_7	T_2
T_{10}	T_{13}	T_9
T_7	T_{11}	T_{12}
T_9	T_5	T_{10}
T_{14}	T_8	T_{15}

Fig. 2. Layout of the experimental field

Agriculture, Vellayani and are presented as Appendix 1 and graphically presented in Fig. 1.

3.1.4 Soil

The soil of the experimental site was sandy clay loam belonging to the family of Loamy Kaolinitic Isohypothermic Typic Haplustalf. The initial data on physical, chemical and biological properties of the soil where field experiment was conducted are given below in Table 1.

Table 1 Physical, chemical and biological properties of soil of the experiment site

Sl. No	Parameter	Content
Mechanical composition		
1.	Coarse sand	48.99%
2.	Fine sand	14.78%
3.	Silt	6.39%
4.	Clay	28.10%
5.	Texture	Sandy clay loam
Physical properties		
1.	Particle density	2.39g/cc
2.	Bulk density	1.24g/cc
3.	Porosity	48.11%
4.	Water Holding Capacity	23.40%
Chemical properties		
1.	pH	6.02
2.	Electrical conductivity	0.48 dSm^{-1}
3.	Available Nitrogen	205.6 kg ha^{-1}
4.	Available Phosphorus	53.31 kg ha^{-1}
5.	Available Potassium	60.97 kg ha^{-1}
6.	Organic carbon	0.49%
7.	Available Zn	2.581

	micronutrient Status (ppm)	Fe	21.25
		Cu	1.501
		Mn	10.59
		B	0.309
Biological properties			
1.	Urease activity(ppm of urea hydrolysed g^{-1} of soil hr^{-1})		135.92
2.	Phosphatase activity (µg p-nitrophenol released g^{-1} of soil hr^{-1})		15.32
3.	Protease activity(micro moles of amino nitrogen hydrolysed $g^{-1}$of soil)		81.72
4.	Dehydrogenase activity(µg of TPF hydrolysed g^{-1} of soil per 24 hrs)		67.89
5.	Cellulase activity(glucose hydrolysed g^{-1} of soil 24 hrs^{-1})		9.43
6.	Soil respiratory activity(µg of CO_2 evolved g^{-1} of soil hr^{-1})		2.8
7.	Microflora	Bacteria	42 x 10^6 CFU g^{-1} soil
		Fungi	2 x 10^4 CFU g^{-1} soil
		Actinomycetes	0

3.2. EXPERIMENTAL MATERIALS

3.2.1. Planting materials and variety

Seed of the Bhindi variety "Varsha Upahar" was obtained from Farming System Research Station, Sadanandapuram, Kottarakara. It is a green fruited variety suitable to southern Kerala having duration of 105 days.

Plate 1. A general view of the field experiment

3.2.2. Manures and Fertilizers

Recommendation for bhindi was FYM as basal dose @ 2 t ha^{-1} and Fertilizer dose of 110: 35: 70 N: P_2O_5: K_2O kg ha^{-1}. (KAU POP, 2011)

Fertilizers used were urea, rajphos and muriate of potash with the following analytical values.

Fertilizer	Nutrient content
Urea	46% N
Rajphos	20% P_2O_5
Muriate of Potash.	60% K_2O

3.3. Design and layout of the experiment

3.3.1. Experiment Details

Design : Randomized block Design (RBD)

Treatments : 15

Replications : 3

Plot size : 3 x 3 m

Spacing : 60 x 45 cm.

Crop : Bhindi

Variety : Varshauphar

3.3.2. Enrichment of vermicompost

Enrichment of vermicompost was carried out using *Azospirillum*, Phosphorus solubilizing bacteria, PGPR mix-1 at the rate of 2 %. Other source of enrichment used was Neemcake @ 5%. The nutrient content in the enriched composts were analysed and were applied to the crop to meet the nutrient requirement in specific doses. The rest of the crop requirement was supplemented

by the addition of inorganic fertilizers. Enriched vermicompost were analysed for major nutrients using standard analytical procedures and data are presented in Table 2.

Enriched Vermicompost	Rate	N (%)	P_2O_5 (%)	K_2O (%)
Neemcake	@ 5%	4.1	0.7	1.4
Azospirillum	@ 2%	4.7	0.7	0.5
PSB	@ 2%	1.5	1.8	0.5
PGPR mix-1	@ 2%	1.5	1.8	1.9

3.3.3. Treatment details (Table 3.)

T_1	Package of practice recommendation (KAU)
T_2	N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3	N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4	P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5	NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6	N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7	N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8	P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9	NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10}	N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11}	N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12}	P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13}	N,P, K,(75 %) as PGPR mix-1 enriched vermicompost + N P & K(25%)
T_{14}	N, P, K alone as inorganics
T_{15}	Absolute control

In the case of PGPR mix-1 enriched vermicompost nutrients were substituted on N- equivalent basis.

3.4. Details of operations during field experiment

3.4.1. Land Preparation

The experimental site was ploughed, thoroughly with power tiller. Weeds were removed. The field was laid out into blocks and plots according to the orientation of the land.

3.4.2. Manure and fertilizer application

The entire quantity of farm yard manure, rajphos and muriate of potash and half the quantity of urea were applied as basal dose. Enriched vermicompost was applied 15 days after sowing. The remaining quantity of urea was applied 30 days after sowing as first split application.

3.4.3. Sowing

Seeds were dibbled at the rate of three seeds per pit with the spacing of 60 x 45 cm.

3.4.4. After cultivation

Uniform germination was observed and gap filling was done 5 days after sowing. The crop was thinned to 1 plant per pit one week after emergence. Regular weeding was done throughout the cropping period. Irrigation was provided to the existing as and when required.

3.4.5. Plant protection

Yellow Vein Mosaic of bhindi was noticed in the field in the flowering stage. Rouging was done to prevent the spread of disease.

3.4.6. Harvesting

Harvest of harvestable mature fruits was done from 42 days after sowing. Green fruits were harvested on alternate days from all the treatments plots up to 95 days after sowing and fresh weight were recorded.

3.5. Observations recorded

3.5.1. Biometric observations

3.5.1. 1.Plant height at first harvest (cm)

Plant height was measured from the base of the plant to the terminal leaf at the time of first harvest.

3.5.1.2. Inter nodal length at final harvest (cm)

Vertical distance between two adjacent leaf axils is measured as the inter nodal length at the time of final harvest.

3.5.1.3. Number of branches at final flowering

Number of branches was noted at the time of final flowering.

3.5.1.4. Flowering stages days to 50% flowering

Number of days taken for fifty percent of the plant population to flower in each plot was recorded by visual observation and was recorded.

3.5.1.5. Number of fruits / plant

Number of fruits harvested from three observation plants from each plot was noted and the average was recorded.

3.5.1.6. Yield per plant (g)

Total weight of fruits from observation plants from each plot at each harvests were taken out and expressed as fruit yield per plant.

3.5.1.7. Yield per ha (t ha^{-1})

Total weight of fruits from each plot at each harvest were recorded and yield per ha was calculated.

3.5.1.8. Scoring of pest and diseases (%)

For the scoring of Yellow Vein Mosaic Virus Disease, Percentage Disease Incidence was calculated using the formula

$$PDI\ (\%) = \frac{\text{Number of affected plants}}{\text{Total number of plants}} \times 100$$

3.5.1.9. Shelf life (Keeping quality)

Sample fruits were collected treatment wise separately and the number of days taken from the harvest of fruits to the stage at which fruits become shrunken and lost firmness were recorded.

3.5.1.10. B: C ratio

B: C ratio was calculated for each treatment using the formula

$$\text{B: C ratio} = \frac{\text{Gross income}}{\text{Cost of cultivation}}$$

3.6. Methods

3.6.1. Collection of soil samples

Rhizosphere soils were collected by the method of destructive sampling of the plants. Plants were uprooted and the rhizosphere soils were collected in polythene bags. These soils were stored in deep freezers to ensure the viability of microorganisms.

Non rhizosphere soils were collected from the non-rhizosphere areas and stored as above. Soil for chemical analysis were collected, dried in shade, powdered with a wooden mallet, sieved through a 2 mm sieve and stored in polythene containers.

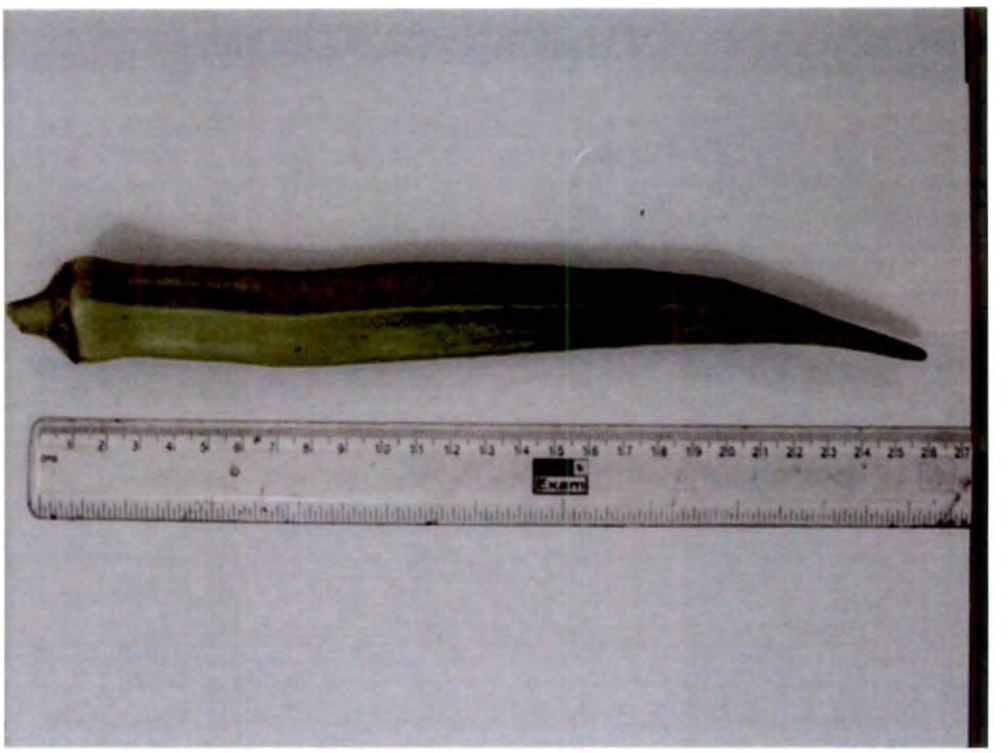


Plate 2. A view of the highest yielding treatment (T_{13})

3.6.2. Soil Analysis

3.6.2.1. pH

pH of the air dried soil were determined with a soil water ratio of 1:2.5 (Jackson, 1973) using a pH meter.

3.6.2.2. Electrical conductivity (dSm^{-1}).

Electrical Conductivity was determined with the same soil-water suspension used for the determination of pH with the help of a conductivity meter as per the procedure outlined by Jackson (1973).

3.6.2.3. Available Nitrogen (kg ha^{-1})

Available Nitrogen in the soil was determined as per the alkaline permanganate method (Subbiah and Asija, 1956).

3.6.2.4. Available Phosphurus (kg ha^{-1})

Available Phosphurus in the soil was estimated as per the Bray No.1 extraction and ascorbic acid reduced molybdo-phosphoric blue colour method (Bray and Kurtz, 1945).

3.6.2.5. Available Potassium (kg ha^{-1})

Ammonium acetate soil extract was collected for the determination of potassium using a flame photometer (Jackson, 1973).

3.6.2.6. Organic Carbon (%)

Organic carbon content of the soil samples were determined by the wet digestion method as defined by Walkley and Black (1934).

3.6.2.7. Available Micronutrient status

3.6.2.7.1. Available Fe, Cu, Mn and Zn (ppm)

Available micronutrient status (Fe, Cu, Mn and Zn) were estimated by the method of extraction using 0.1 N HCl and read in Atomic Absorption Spectrophotometer (Sims and Johnson, 1991).

3.6.2.7.2. Available B content (ppm)

Available B content in the post harvest soil was estimated by using Hot water extraction method (Gupta, 1967)

3.6.2.8. Urease activity (ppm of urea hydrolysed g^{-1} of soil hr^{-1})

The urease activity was determined by following the method described by Broadbent *et al.* (1964).

About 20 g was weighed into an Erlenmayer flask, to which 4 ml of urea substrate solution was added. Enough water was added to each flask to maintain a tension of 1/3 bar and incubated for 24 hours at 30^0C. Then the flasks were removed $CaSO_4$ solution was added to make up the volume to 100 ml. About 15 ml of the supernatant was taken and colour was developed by adding 10ml of p-dimethyl amino benzaldehyde which was then read in a Spectrophotometer at a wavelength of 420 nm. Standards were also prepared by using urea solutions of known concentrations. The results were expressed in terms of urea hydrolysed g^{-1} of soil hr^{-1} in ppm.

3.6.2.9. Phosphatase activity (µg of p- nitrophenol released g^{-1} of soil hr^{-1})

The phosphatase activity was determined by following a procedure described by Eivazi and Tabatabai (1977)

To 1 g soil in a 50 ml Erlen Meyer flask, 0.2 ml toluene, 4 ml modified universal buffer (pH-6.5) and 1ml p-nitrophenyl phosphate solution were added and incubated at 23^0C for one hour. After incubation, 0.5 ml $CaCl_2$ (1ml) and

0.05M NaOH (1ml were added). The contents were swirled and filtered through Whatman No.2 filter paper and the intensity of yellow colour developed was read in a spectrophotometer at a wavelength of 420 nm. One percent of p-nitropheyl phosphate was used for the preparation of standards. The results were expressed in terms of p-nitrophenol hydrolysed g^{-1} of soil hr^{-1} in micrograms.

3.6.2.10. Protease activity (μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1})

The protease activity was estimated as per the procedure defined by Nannipieri *et al.* (1978).

About 0.5g of the soil was weighed into a tissue culture tube to which 1.8 ml of 0.1M TRIS buffer and 2 ml of 0.002M phenyl alanine were added, and incubated for 60 minutes at 20°C. After the incubation period, the activity was arrested by adding 0.2 ml of 5M HCl and centrifuged. Supernatant was collected and 2 ml of Ninhydrin reagent was added. The violet colour developed was measured at 570nm using a Spectrophotometer. A series of standards were prepared in the same manner. The results were expressed as micromoles of amino nitrogen hydrolysed g^{-1} of soil hr^{-1}.

3.6.2. 11. Dehydrogenase Activity (μg of TPF hydrolysed g^{-1} of soil 24 hrs^{-1})

Dehydrogenase activity was estimated as per the procedure described by Casida *et al.*, 1964.

About 60 g of the air dried soil was weighed to a 250 ml Erlen Meyer flask. One ml of 3% Triphenyl Tetrazolium Chloride was added and incubated for 24 hrs at 27°C.After incubation, the soil was quantitavely transferred to a glass funnel and was given ethanol washings consecutively till the volume reached 100 ml. The colour intensity was then read in a Spectrophotometer at 485 nm. A series of standards were used for preparing the calibration curve. The results were expressed in terms of Triphenyl Formazon hydrolysed g^{-1} of soil 24 hrs^{-1} in micrograms.

Plate 3. A view of the dehydrogenase enzyme assay

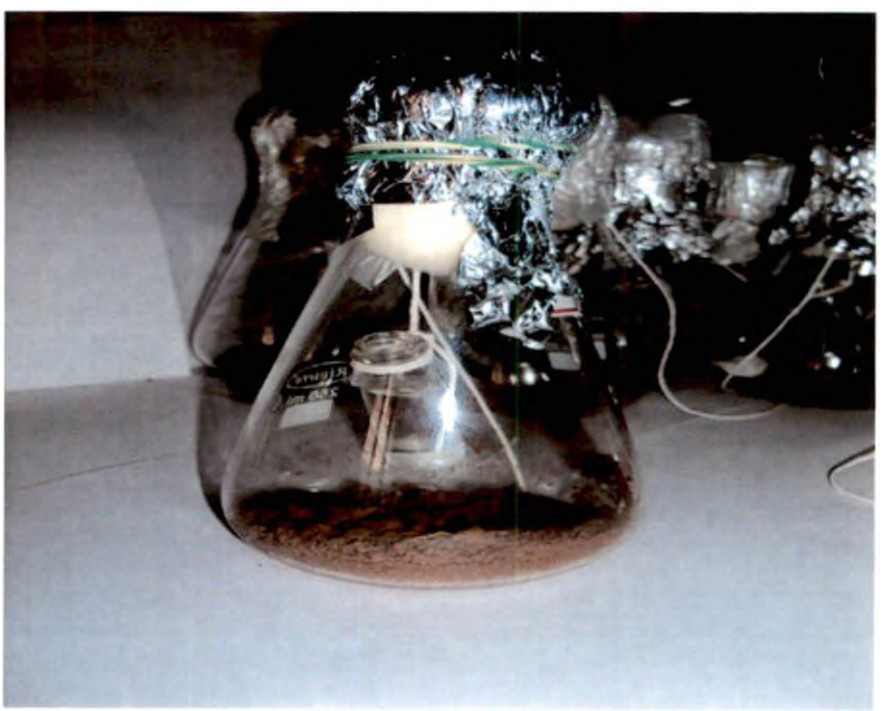


Plate 4. A view of the soil respiratory activity study

3.6.2.12. Cellulase activity (ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1})

Cellulase activity was estimated as per the method suggested by Pancholy and Rice (1973).

Five gram of air dried soil was taken in a 100 ml Erlen Meyer flask. Ten ml of acetate buffer and 1% carboxy methyl cellulose was added. Flasks were incubated for 24 hrs at 37°C and left undisturbed. After the incubation, 50ml of the filtrate was taken and 4ml of anthrone reagent was added. The intensity of the green colour developed was read in Spectrophotometer at 620 nm. Glucose was used as standard at different concentration for the preparation of standard calibration graph. The results were then expressed as the amount of glucose hydrolysed g^{-1} of soil 24 hrs^{-1} in ppm.

3.6.2.13. Soil Respiratory Activity (µg of CO_2 evolved g^{-1} of soil hr^{-1})

The respiratory activity of the soil samples were estimated using the method outlined by Jenkinson and Powlson (1976), where the CO_2 evolved from a fixed quantity of incubated soil was collected in an alkali and quantified.

3.6.2.14. Calculation of enzyme kinetics (V_{max} and K_m)

The kinetics parameters K_m and V_{max} of the five soil enzymes were evaluated based on the Line-Weaver-Burk plot. Varying substrate concentrations were employed for this study using soil as the enzyme source. Enzyme activity rates (V) were determined at these concentrations. According to the Line-Weaver-Burk equation, when 1/V is plotted against 1/S a straight line graph is obtained. The slope is K_m/V_{max}, the intercept on the ordinate is $1/V_{max}$ and the intercept on the abscissa is $-1/K_m$. K_m and V_{max} of the enzymes were calculated from the Line-Weaver-Burk plot prepared with 1/V against 1/S (Vaughan and Ord, 1991).

Six concentrations (0.005, 0.010, 0.015, 0.020, 0.035, 0.040 mol l^{-1}) of urea solution, six concentrations (0.0005, 0.001, 0.0025, 0.005, 0.015 and 0.050 mol l^{-1}) of p- nitrophenyl phosphate solution, six concentrations (0.005, 0.0075, 0.01, 0.015, 0.03 and 0.050 mol l^{-1}) of phenyl alanine, six concentrations (0.005,

0.0075, 0.01, 0.015, 0.03 and 0.050 mol l^{-1}) of CMC solution, six concentration(0.003, 0.007, 0.010, 0.020, 0.030 and 0.050 mol l^{-1}) of TTC solution were used as substrates for urease, phosphatase, protease, cellulase and dehydrogenase respectively and assays were carried out.

3.6.2.15. Computation of Biological Fertility Index through Enzyme Activity Number (EAN)

Biological Fertility Index, for the different combinations of treatments were computed based on the activity of five different enzymes as proposed by Beck, 1984 through enzyme activity Number. The Enzyme Activity Number for the different treatments was computed using the formula.

EAN= 0.2{TPF+ Catalase (%)/10 + phenol (µg)/40 + amino-N (µg)/40 + amylase (%)/20}.

3.6.2.15.1. Catalase assay

Catalase activity was determined by the method of Cohen *et al.* (1970) in which decomposed hydrogen peroxide is measured by reacting it with excess of $KMnO_4$ and residual $KMnO_4$ is measured spectrophotometrically at 480 nm.

One tenth ml of the supernatant was introduced into test tubes containing 0.5 ml of 2 mM H_2O and a blank containing 0.5 ml of distilled water. Enzymatic reactions were initiated by adding sequentially, at the same fixed interval, 1ml of 6 N H_2SO_4 to test tubes containing different concentrations of soil sample from 0.25 to 2% and to the blank sample. Also, 7 ml of 0.1N $KMnO_4$ was added within 30 s and thoroughly mixed. Spectrophotometer standard was prepared by adding 7 ml of 0.1 N $KMnO_4$ to a mixture of 5.5 ml of 0.05 N phosphate buffer, pH 7 and 1 ml of 6 N H_2SO_4. Results were expressed as ml O_2 g^{-1} dry soil

3.6.2.16. Comparison of micro flora between rhizosphere and non rhizosphere soils.

Microbial count in the soil was enumerated using serial dilution technique

proposed by Timonin (1940). Composition of the media was presented in Appendix 2.

Sl no.	Microflora	Medium
1	Actinomycetes	Ken knight's agar medium
2	Fungi	Martins' Rose Bengal agar
3	Bacteria	Nutrient agar

3.6.1.17. Statistical analysis

The data generated from these experiments were subjected to the analysis of variance as per the design and their significance was tested by the F test (Snedecor and Cochran, 1975). In the cases where the effects were found to be significant, CD was calculated using standard techniques.

Results

4. RESULTS

The present investigation was undertaken to study the effect of integrated plant nutrient system (IPNS) on the soil biological regimes in red loam soil. The study comprised of a field experiment to assess the conjugal effect of manures and chemical fertilizers on dynamics of major agriculturally significant soil enzymes, available nutrient status of the soil, its relation with the activities of major soil enzymes and soil microflora.

Results based on statistically analysed data pertaining to the experiment conducted during the course of investigation are presented in this chapter.

4.1. Chemical properties of soil

The data on soil chemical parameters viz. pH, EC, organic carbon, available N, P, K and available micronutrient status are presented below.

4.1.1. pH

Imposition of treatments had a significant effect on the pH of the post harvest soil. (Table 5) The mean values ranged from 5.37 to 6. Treatment T_{15} registered the highest mean value of 6.0, which was on par with T_{14} (5.97) and T_2 (5.93) while the lowest mean value was registered by the treatment T_{10} (5.37) with the application of N (75 %) as Neem cake enriched vermicompost + N (25%), P & K as inorganics which was on par with T_{12} (5.46), T_{11} (5.46), T_8 (5.4) and T_6 (5.53).

4.1.2. Electrical conductivity

Critical appraisal of the data (Table 5) revealed that treatment had influenced the electrical conductivity of the post harvest soil. The mean values ranged from 0.2 to 0.47 dSm^{-1}. Treatment T_8 (0.47 dSm^{-1}) with the application of P (50 %) as PSB enriched vermicompost + P (50%), N & K as inorganics recorded the highest value which was on par with T_7 (0.45 dSm^{-1}) and T_6 (0.44 dSm^{-1}). Treatment T_{15} (Absolute control) registered the lowest value of 0.20 dSm^{-1}, which was significantly lower to all other treatments. Treatment T_5 (0.42 dSm^{-1}) was found to

Table 4 Chemical properties of initial soil

Sl. no	Parameter		Content
1.	pH		6.02
2.	Electrical conductivity(dSm^{-1})		0.22
3.	Available nitrogen(kg ha^{-1})		205.6
4.	Available phosphorus(kg ha^{-1})		53.31
5.	Available potassium(kg ha^{-1})		60.97
6.	Organic carbon (%)		0.49
7.	Available micronutrient status	Zn (ppm)	2.581
		Fe (ppm)	21.25
		Cu (ppm)	1.501
		Mn (ppm)	10.59
		B (ppm)	0.309

Table 5 Effect of treatments on soil chemical properties- pH, EC, organic C

Treatments	pH	EC(dSm^{-1})	Organic Carbon(%)
T_1	5.76	0.28	0.78
T_2	5.93	0.34	0.90
T_3	5.73	0.42	0.97
T_4	5.63	0.35	0.76
T_5	5.60	0.42	0.77
T_6	5.53	0.44	0.96
T_7	5.73	0.45	0.85
T_8	5.40	0.47	0.82
T_9	5.80	0.35	0.86
T_{10}	5.37	0.37	0.69
T_{11}	5.46	0.36	0.87
T_{12}	5.46	0.34	0.71
T_{13}	5.53	0.41	0.90
T_{14}	5.97	0.24	0.66
T_{15}	6.00	0.20	0.63
CD (0.05)	0.158	0.324	0.023

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

be on par with T_3 (0.42 dSm^{-1}), T_{13} (0.41 dSm^{-1}), T_6 (0.44 dSm^{-1}) and T_7 (0.45 dSm^{-1}).

4.1.3. Organic carbon

The results revealed that the applied treatments had significant effect on the organic carbon content of the soil. (Table 5) The mean values ranged from 0.63 to 0.97 per cent. The highest value was recorded by T_3 (0.97%) followed by T_6 (0.96%) which were statistically on a par. Treatment T_5 (0.77%) was on par with T_4 (0.76%) and T_1 (0.78%). Treatment T_9 (0.86%) was on par with T_7 (0.85%) and T_{11} (0.87%). Lowest value was recorded by T_{15} (0.63%) the absolute control plot which was significantly lower than all other treatments.

4.1.4. Available Nitrogen

Various treatments had significantly influenced the available N content in the post harvest soil as observed from Table 6. The mean value ranged from 175.62 kg ha^{-1} to 255.06 kg ha^{-1}. Treatment T_{11} registered the highest mean value of 255.06 kg ha^{-1} which on par with T_5 (250.88 kg ha^{-1}), T_{13} (250.88 kg ha^{-1}) and T_9 (250.88 kg ha^{-1}). Treatment T_1 (238.34 kg ha^{-1}) was found to be on par with T_2 (238.34), T_3 (238.34 kg ha^{-1}), T_{12} (238.34 kg ha^{-1}), T_5 (250.88 kg ha^{-1}) and T_9 (250.88 kg ha^{-1}). Treatment T_{15} registered the lowest mean 175.62 kg ha^{-1} which was significantly inferior to all other treatments.

4.1.5. Available phosphorus

It is inferred from Table 6 that the mean values in the case of available P ranged from 26.60 to 71.71 kg ha^{-1}. Treatment T_{12} has recorded the highest mean the value of available P i.e. 71.71 kg ha^{-1} and was found to be on par with treatment T_8 (70.74 kg ha^{-1}). Lowest value of available P 26.60 kg ha^{-1} was recorded by the T_{15} absolute control. Treatment T_1 (55.36 kg ha^{-1}) and T_6 (52.49 kg ha^{-1}) were found to be on par with T_5 (54.22 kg ha^{-1}). Treatment T_3 (42.22 kg ha^{-1}) and T_2 (41.05 kg ha^{-1}) were also found to be on par.

Table 6 Effect of treatments on soil chemical properties- Available N, P, K (kg ha $^{-1}$)

Treatments	Available N	Available P	Available K
T_1	238.34	55.36	99.56
T_2	238.34	41.05	73.43
T_3	238.34	42.22	71.69
T_4	192.34	57.39	82.58
T_5	250.88	54.22	105.49
T_6	213.09	52.49	74.58
T_7	217.41	44.38	92.15
T_8	221.59	70.74	105.49
T_9	250.88	63.38	108.86
T_{10}	213.23	48.16	73.34
T_{11}	255.06	50.56	93.70
T_{12}	238.34	71.71	98.38
T_{13}	250.88	67.36	109.65
T_{14}	183.98	38.81	67.43
T_{15}	175.62	26.60	58.07
CD (0.05)	15.774	2.018	1.936

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

4.1.6. Available potassium

Various treatments significantly influenced the available potassium content of soil (Table 6). The mean values ranged from 58.07 to 109.65 kg ha^{-1}.The treatment T_{13} registered the highest mean (109.65 kg ha^{-1}) which is on par with T_9 (108.86) which had registered the second highest mean (108.86 kg ha^{-1}). Treatment T_8 (105.49 kg ha^{-1}) and T_5 (105.49 kg ha^{-1}) as well as T_7 (92.15 kg ha^{-1}) and T_{11} (93.70 kg ha^{-1}) were found to be on par. Treatment T_{10} (73.34 kg ha^{-1}) was on par with T_2 (73.43 kg ha^{-1}), T_3 (71.69 kg ha^{-1}), and T_6 (74.58 kg ha^{-1} kg ha^{-1}). Lowest value of 58.07 kg ha^{-1} was recorded by T_{15} (Absolute control) which was significantly inferior when compared to all other treatments.

4.1.7. Available Micronutrient status

The data on the micronutrient status of the post harvest soil are presented in Table 7.

4.1.7.1. Fe content

Table 7 reveals that there was significant difference due to treatments on Fe content in post harvest soil. Mean values ranged from 20.750 ppm to 36.796 ppm. Highest value of Fe was recorded by T_9 (36.796 ppm) and was significantly different from all other treatments. Second best treatment was T_2 which has recorded a value of 36.250 ppm. Lowest mean was recorded by the treatment T_{15} (20.750 ppm). None of the treatments were found to be on par.

4.1.7.2. Cu content

Copper concentration in post harvest soil was significantly influenced by different treatments. (Table 7) The mean values ranged from 1.652 to 2.844 ppm. The highest mean value of 2.844 ppm was recorded by T_9 with the application NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics and was significantly different from all other treatments. Second best treatment was T_7 with the application of N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K as inorganics with the mean value of 2.806 ppm. Lowest mean

Table 7 Effect of treatments on soil chemical properties Micronutrient content in ppm (Fe, Cu, Mn, Zn and B)

Treatments	Fe	Cu	Mn	Zn	B
T_1	21.267	1.637	16.667	4.667	0.328
T_2	36.250	1.652	18.010	3.140	0.332
T_3	25.370	1.915	12.320	3.725	0.334
T_4	23.346	2.175	17.263	3.421	0.334
T_5	22.193	2.379	17.353	5.345	0.335
T_6	29.400	1.656	12.990	3.302	0.337
T_7	28. 145	2.806	15.660	4.238	0.349
T_8	20.750	2.146	13.306	3.465	0.345
T_9	36.796	2.844	13.666	5.143	0.359
T_{10}	33.333	1.897	13.450	5.436	0.338
T_{11}	32.417	2.038	10.760	3.627	0.342
T_{12}	34.240	2.199	15.850	5.112	0.358
T_{13}	33.720	2.117	14.976	4.292	0.355
T_{14}	24.067	1.575	15.226	3.656	0.317
T_{15}	20.750	1.528	10.460	2.845	0.315
CD (0.05)	0.2915	0.0179	0.1902	.1601	0.0072

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

was recorded by the treatment T_{15} (Absolute control). Treatment T_2 (1.652 ppm) was observed to be on par with T_1 (1.637 ppm) and T_6 (1.656 ppm).

4.1.7.3. Mn content

Various treatments influenced Mn content of post-harvest soil significantly. (Table 7) The mean values ranged from 10.460 to 18.010 ppm. As per the data treatment T_2 (18.010 ppm) was found to be highly significant than the rest of the treatments. The absolute control registered the lowest value i.e. 10.460 ppm. T_8 (13.306 ppm) and T_{10} (13.450 ppm) as well as T_4 (17.263 ppm) and T_5 (17.353 ppm) were found to be on par with each other.

4.1.7.4. Zn content

Table 7 presents the Zn concentration in the post-harvest soil. The mean values ranged from 2.825 to 5.436 ppm. Highest mean value of 5.436 ppm of zinc was registered by T_{10} which is N (75 %) as Neem cake enriched vermicompost + N (25%), P & K and was significantly different from all other treatments. The lowest mean was observed in treatment T_{14} (2.845 ppm) which was inferior to all other treatments. Treatments T_9 (5.143 ppm) and T_{12} (5.112 ppm) were found to be on par.

4.1.7.5. B content

Perusal of data revealed that there was a significant difference in B content of post harvest soil. (Table 7) The mean values ranged from 0.315 to 0.358 ppm. Treatment T_9 recorded the highest value (0.359) which was on par with T_{12} (0.358 ppm) and T_{13} (0.355 ppm). Treatment T_{15} registered the least value (0.315 ppm) which was on par with T_{14} (0.317 ppm). Treatment T_2 (0.332 ppm) was found to be on par with T_4 (0.334 ppm), T_3 (0.334 ppm), T_5 (0.335 ppm), T_1 (0.328 ppm) and T_6 (0.337 ppm).

4.2. Biological properties of soil

Soil is a living system in which biological activities takes place with the help of enzymatic process. Soils are also considered as biological entity with complex biochemical reactions. Soil enzymatic assays act as potential indicators of ecosystem quality being operationally practical, sensitive, integrative described as "biological finger prints" of past and present soil management. Quantitative measurement of these enzyme activities can contribute to our understanding of transformations by allowing us to evaluate the microbes present in soil. Measurement of activity of extracellular enzymes provides information on the biological activities of microorganisms. The activities of various enzymes such as dehydrogenase, urease, phosphatase, protease and cellulase in both rhizosphere and non-rhizosphere soils were assayed and presented in Table 9 and Table 10 respectively.

4.2.1. Urease activity

Urease, the enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_3 is widely distributed in nature and has been detected in plants, animals and microorganisms. The activity of urease was expressed in terms of ppm of urea hydrolysed g^{-1} of soil hr^{-1}.

4.2.1.1. Rhizosphere soil

It is observed from the Table 9 that the treatments were found to impose significant effects with respect to urease activity. The mean values for urease activity ranged from 174.45 to 247.39 ppm of urea hydrolysed g^{-1} of soil hr^{-1}. The highest value was recorded for the treatment T_{11} (247.39 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) with the application of N 75% as *Azospirillum* enriched vermicompost +N (25%), P, K as inorganics which was found to be significantly superior to other treatments. However the absolute control plot recorded the lowest value for the urease activity 174.45 ppm of urea hydrolysed g^{-1} of soil hr^{-1} which was significantly lower than other treatments.

Table 8 Biological properties of initial soil sample

Sl. No	Parameter		Content
1	Urease (ppm of urea hydrolysed g^{-1} of soil hr^{-1})		135.92
2	Phosphatase (µg p-nitrophenol released g^{-1} of soil hr^{-1})		15.32
3	Protease (micro moles of amino nitrogen hydrolysed $g^{-1}$of soil)		81.72
4	Dehydrogenase (µg of TPF hydrolysed g^{-1} of soil per 24 hrs)		67.89
5	Cellulase (glucose hydrolysed g^{-1} of soil 24 hrs^{-1})		9.43
6	Soil respiratory activity (µg of CO_2 evolved g^{-1} of soil hr^{-1})		2.8
7	Micro flora	Bacteria	42×10^6 CFU g^{-1} soil
		Fungi	2×10^4 CFU g^{-1} soil
		Actinomycetes	0

Treatments	Urease (ppm of urea hydrolysed g^{-1} of soil hr^{-1})	Phosphatase(µg p-nitrophenol released g^{-1} of soil hr^{-1})	Protease (micro moles of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1})	Dehydrogenase (µg of TPF hydrolysed g^{-1} of soil per 24 hrs^{-1})	Cellulase(ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1})
T_1	189.52	47.53	140.75	158.51	29.24
T_2	204.14	48.83	148.31	162.82	33.08
T_3	212.78	49.93	154.44	165.46	35.31
T_4	199.08	63.71	157.74	203.86	38.30
T_5	210.51	59.46	149.5	185.09	40.35
T_6	236.97	62.12	163.13	183.01	42.21
T_7	245.49	62.22	163.73	188.49	43.59
T_8	227.46	79.67	175.49	188.32	44.13
T_9	221.37	75.33	181.13	227.79	49.83
T_{10}	236.97	51.70	160.76	205.32	38.81
T_{11}	247.39	53.54	170.82	208.83	42.84
T_{12}	239.57	68.56	173.53	218.63	43.37
T_{13}	242.08	67.57	176.38	220.38	45.87
T_{14}	180.81	40.46	141.08	132.44	26.79
T_{15}	174.45	39.46	101.45	127.18	25.79
CD (0.05)	1.283	1.101	1.223	16.133	1.379

Table 9 Activities of enzymes (Urease, phosphatase, protease,dehydrogenase and cellulase) at harvest stage in rhizosphere soil.

Treatments	Urease(ppm of urea hydrolysed g^{-1} of soil hr^{-1})	Phosphatase(µg p-nitrophenol released g^{-1} of soil hr^{-1}	Protease (micro moles of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}	Dehydrogenase (µg of TPF hydrolysed g^{-1} of soil 24 hrs^{-1}	Cellulase(ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}
T_1	158.28	20.64	106.75	98.84	14.68
T_2	159.61	21.42	112.63	110.64	15.16
T_3	161.81	22.68	116.60	111.59	15.62
T_4	164.06	24.49	118.59	122.39	15.00
T_5	160.09	26.63	120.74	114.82	16.21
T_6	159.40	26.69	116.62	108.61	16.53
T_7	168.20	24.75	120.61	112.69	14.29
T_8	158.28	29.27	122.70	114.57	15.52
T_9	187.58	26.63	124.43	126.57	17.23
T_{10}	174.51	29.46	117.84	136.87	14.30
T_{11}	172.58	27.87	118.63	119.55	16.29
T_{12}	168.61	28.44	106.49	120.46	15.24
T_{13}	176.83	25.41	112.65	118.32	18.40
T_{14}	152.53	21.41	88.64	110.68	13.22
T_{15}	141.92	17.81	85.42	92.66	11.14
CD (0.05)	0.418	0.503	0.387	0.425	5.672

Table 10 Activities of enzymes (Urease, phosphatase, protease, dehydrogenase and cellulase) at harvest stage in non- rhizosphere soil)

4.2.1.2. Non-Rhizosphere soil.

The activity assay of urease in non rhizosphere soils revealed a significant effect due to treatments. (Table 10) The mean values for the urease activity ranged from 141.92 to 187. 58 ppm of urea hydrolysed g^{-1} of soil hr^{-1}. The highest value for urease activity was noticed for T_9 (187.58 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) with the application of NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics and was significantly superior than all other treatments. Treatments T_{12} (168.61 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) and T_7 (168.20 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) as well as T_2 (159.61 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) and T_6 (159.40 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) were also found to be on par. The lowest value was recorded by T_{15} (141.92 ppm of urea hydrolysed g^{-1} of soil hr^{-1}) which was the absolute control.

4.2. 2. Phosphatase activity

Phosphatase is the enzyme that performs an important function in soil by transforming organic phosphorous to inorganic phosphate. The activity of enzyme phosphatase was expressed in terms of quantity of p-nitrophenyl phosphate released g^{-1} of soil hr^{-1} in micro grams.

4.2.2.1. Rhizosphere soil.

The mean values for the phosphatase ranged from 39.46 to 79.67 µg of p-nitrophenol released g^{-1} of soil hr^{-1} (Table 9). The highest value of phosphatase activity was noticed for the treatment T_8 (79.67 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) with the application of 50 % P as PSB enriched vermicompost + P (50%), N & K as inorganics and was found to be significantly superior to other treatments imposed while the lowest value of 39.46 µg of p- nitrophenol released g^{-1} of soil hr^{-1} was noticed with the treatment T_{15} (Absolute control) which was on par with the treatment T_{14} (40.46 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) with the application of NPK alone as inorganics. Treatments T_{13} (67.57 µg of p-nitrophenol released g^{-1} of soil hr^{-1}) and T_{12} (68.56 µg of p- nitrophenol released g^{-1} of soil hr^{-1}), T_6 (62.12 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) and T_7 (62.22

µg of p- nitrophenol released g^{-1} of soil hr^{-1}) were found to be on par. treatment T_3 (49.93 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) was found to be on par with T_1 (47.53 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) and T_2 (48.83 µg of p-nitrophenol released g^{-1} of soil hr^{-1})

4.2.2.2. Non- Rhizosphere soil

The mean value for phosphatase activity ranged from 17.81 to 29.46 micrograms of p-nitrophenol released g^{-1} of soil hr^{-1}. (Table 10) The treatments imposed a significant effect with respect to the phosphatase activity. The highest activity was recorded by T_{10} (29.46 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) which was on par with T_8 (29.27 µg of p- nitrophenol released g^{-1} of soil hr^{-1}). The treatment T_5 (26.63 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) and T_9 (26.63 µg of p-nitrophenol released g^{-1} of soil hr^{-1}) and T_6 (26.69 µg of p- nitrophenol released g^{-1} of soil hr^{-1}) were on par showing similar effect on phosphatase activity, but was significantly superior to the absolute control (T_{15}) which showed the least value (17.81 µg of p- nitrophenol released g^{-1} of soil hr^{-1}).

4.2.3. Protease activity

The activity of protease is expressed in micro moles of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}.

4.2.3.1. Rhizosphere soil.

The mean values of protease activity ranged from 101.45 to 181.13 micro moles amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}. (Table 9) The highest value for protease activity was noticed with treatment T_9 (181.13 µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) with the application of NPK(50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics which was found to be significantly superior over other treatments. The lowest value recorded for the absolute control was found to be significantly lower than the other treatments T_{15} (101.45µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}). Treatments T_6 (163.13µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) and T_7 (163.73µM of

amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) were found to be on par with each other. Treatment T_{14} (141.08μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) and T_1 (140.75 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) were also found to be on par with each other. Treatments T_{13} (176.38 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) andT_8 (175.49 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) were found to be on par with each other.

4.2.3.2. Non-Rhizosphere soil

Statistical analysis of data on protease activity indicated a highly significant effect due to treatments. (Table 10) The mean value ranged from 85.42 to 124.43 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}. With the highest value recorded for T_9 (124.43μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) which was significantly superior to all other treatment. T_8 (122.70 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) was found to be the second best treatment which was also significantly superior from others. Treatment T_4 (118.59μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) and T_4 (118.63μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) as well as T_2 (112.63μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) and T_{13} (112.65 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}) were found to be on par. Lowest value was registered by T_{15} (85.42 μM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}).

4.2.4. Dehydrogenase activity

Dehydrogenases are fundamental to the enzyme system of all microorganisms and thus play an essential role in initial stages of oxidation of soil organic matter by transferring electrons or hydrogen from substrates to acceptors. The activity of dehydrogenase serves as indicator of the microbial redox systems in soils and can be considered as a good measure of microbial oxidative activity.

4.2.4.1. Rhizosphere soil.

It is observed from Table 9 that the mean values of dehydrogenase activity ranged from 127.18 to 227.79 μg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$. The highest

value for dehydrogenase was noticed for the treatment T_9 (227.79 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) which was on par with T_{13} (220.38 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) and T_{12} (218.63 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$). Treatment T_{11} (208.83 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) was found to be on par with T_{13} (220.38 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$), T_{12} (218.63 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$), T_{10} (205.32 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) and T_4 (203.86 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$). Treatments T_8 (188.32 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$), T_7 (188.49 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$), T_6 (183.01 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) and T_5 (185.09 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) were found to be on par with each other. Similarly treatments T_3 (165.46 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$), T_2 (162.82 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) and T_1 (158.51 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) were found to be on par with each other.

4.2.4.2. Non-Rhizosphere soil

The activity of dehydrogenase expressed in non-rhizosphere soil showed highly significant different due to treatments. (Table: 10) Mean values of dehydrogenase activity ranged from 92.66 to 136.87µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$. The highest value was noticed for the treatment T_{10} (136.87 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) which was significantly superior from all other treatments. The second highest value was registered by T_9 (126.57 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$). The treatments T_5 (114.82 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) and T_8 (114.57 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$) were on par with each other. The lowest value was recorded in the absolute control plot T_{15} (92.66 µg of TPF hydrolysed g^{-1} of soil 24 hrs $^{-1}$), thus indicating a positive effect of treatments with respect to dehydrogenase activity.

4.2.5. Cellulase activity

Cellulases are a group of enzymes that catalyses the degradation of cellulose, a polysaccharide built of β -1, 4- linked glucose units. The activity is usually expressed as ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}.

4.2.5.1. Rhizosphere soil.

The treatment imposed significant difference with respect to cellulase activity. (Table 9) The mean values ranged between 25.79 to 49.83 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}. The highest mean value for treatments was noticed in T_9 (49.83 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) with application of NPK (50 %) with PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics and was significantly superior to other treatments. The lowest value for the treatments was observed in absolute control plot T_{15} (25.79 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) which was found to be on par with the treatment T_{14} (26.79 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}). Treatments T_4 (38.30 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) and T_{10} (38.81 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) as well as T_{11} (42.84 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) and T_6 (42.21 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) were found to be on par with each other. Treatments T_8 (44.13 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) and T_7 (43.59 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) were also on par with each other.

4.2.5.2. Non-Rhizosphere soil

Regarding the cellulase activity in the non-rhizosphere soils, application of treatments had significant effect when compared to the control. (Table 10) The mean values ranges from 11.14 to 18.4 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}. The highest mean value was recorded by T_{13} (18.4 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}) with the application of N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %) as inorganics which was on par with all other treatments except for treatment T_{15} (11.14 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1} which was the absolute control plot.

Table 11 Effect of treatments on soil respiratory activity of post harvest soil

Treatments	Soil respiratory activity(μg of CO_2 evolved g^{-1} of soil hr^{-1})
T_1	3.70
T_2	3.83
T_3	4.17
T_4	3.50
T_5	3.50
T_6	3.67
T_7	3.77
T_8	3.70
T_9	3.90
T_{10}	3.70
T_{11}	3.47
T_{12}	4.20
T_{13}	3.70
T_{14}	3.70
T_{15}	3.10
CD (0.05)	0.127

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

4.2.6. Soil respiratory activity

The respiratory activity of post harvest soil sample was determined in terms of amount of CO_2 evolved and the results pertaining to the analysis are presented in Table 11

Application of various treatments had significantly influenced soil respiratory activity. The mean values ranged from 3.10 to 4.20 µg of CO_2 evolved g^{-1} of soil hr^{-1}. Treatment T_{12} (4.2) registered the highest mean which was on par with T_3 (4.17 µg of CO_2 evolved g^{-1} of soil hr^{-1}) and significantly superior than all other treatments. Treatment T_9 (3.9 µg of CO_2 evolved g^{-1} of soil hr^{-1}) was found to be on par with T_2 (3.83 µg of CO_2 evolved g^{-1} of soil hr^{-1}). T_1 (3.70 µg of CO_2 evolved g^{-1} of soil hr^{-1}) was found to be on par with T_8 (3.70 µg of CO_2 evolved g^{-1} of soil hr^{-1}), T_{10} (3.70 µg of CO_2 evolved g^{-1} of soil hr^{-1}), T_{13} (3.70 µg of CO_2 evolved g^{-1} of soil hr^{-1}) and T_{14} (3.70 µg of CO_2 evolved g^{-1} of soil hr^{-1}) and T_7 (3.77 µg of CO_2 evolved g^{-1} of soil hr^{-1}). Lowest value was recorded by T_{15} (3.10 µg of CO_2 evolved g^{-1} of soil hr^{-1}) which was significantly inferior to all other treatments.

4.2.7. Calculation of enzyme kinetics (V_{max} and K_m) at fortnightly intervals.

Due to high microbial diversity and the presence of plant and animal cells, it is reasonable to suppose that many different enzymes catalysing the same reactions are present in soil. The primary function of enzymes is to enhance the rate of reactions so that they are compatible with needs of organisms. However, the kinetics of soil enzyme activities was determined by Michaelis-Menten constant.

The V_{max} and K_m values determined at fortnightly intervals for the enzymes urease, phosphatase, protease, dehydrogenase and cellulase using various substrate concentrations are presented in Table 12.

It is observed from the data presented in Table 12 the values for V_{max} with respect to urease activity ranged from 2.4×10^{-3} to 3.0×10^{-3} moles of urea hydrolysed g^{-1}

Period	V_{max}					K_m				
	Urease	Phosphatase	Protease	Dehydrogenase	Cellulase	Urease	Phosphatase	Protease	Dehydrogenase	Cellulase
2nd week	$2.6x10^{-3}$	$11.9 x10^{-3}$	$1.3 x10^{-3}$	$6.21 x10^{-3}$	2100	$0.92 x10^{-4}$	$3.8 x10^{-4}$	$1.15 x10^{-5}$	$1.2 x10^{-4}$	2.95
4th week	$2.4 x10^{-3}$	$12.9 x10^{-3}$	$1.6 x10^{-3}$	$6.00 x10^{-3}$	2800	$5.2 x10^{-4}$	$2.8 x10^{-4}$	$1.31 x10^{-5}$	$1.02 x10^{-4}$	3.20
6th week	$3.0 x10^{-3}$	$21.7 x10^{-3}$	$1.8 x10^{-3}$	$5.78 x10^{-3}$	3200	$0.99 x10^{-4}$	$4.1 x10^{-4}$	$1.18 x10^{-5}$	$0.98 x10^{-4}$	3.90
8th week	$2.77 x10^{-3}$	$16.4 x10^{-3}$	$1.9 x10^{-3}$	$5.21 x10^{-3}$	1400	$6.8 x10^{-4}$	$0.4 x10^{-4}$	$1.92 x10^{-5}$	$0.74 x10^{-4}$	0.9
10th week	$2.91 x10^{-3}$	$13.1 x10^{-3}$	$1.7 x10^{-3}$	$4.06 x10^{-3}$	2400	$0.94 x10^{-4}$	$0.3 x10^{-4}$	$1.3 x10^{-5}$	$0.96 x10^{-4}$	1.12
12th week	$2.9 x10^{-3}$	$9.4 x10^{-3}$	$1.85 x10^{-3}$	$3.56 x10^{-3}$	2500	$1.2 x10^{-4}$	$1.1 x10^{-4}$	$1.4 x10^{-5}$	$0.72 x10^{-4}$	1.74

Table 12 Calculation of enzyme kinetics (V_{max} and K_m) at fortnightly intervals.

Phosphatase : µg of p-nitrophenol released g^{-1} of soil hr^{-1}

Urease : ppm of urea hydrolysed g^{-1} of soil hr^{-1}.

Protease : µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}

Dehydrogenase : µg of TPF hydrolysed g^{-1} of soil per 24 hrs

Cellulase : ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}.

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of the soil hr^{-1}. The maximum values for V_{max} was noticed at the 6th week. K_m values ranged from 0.92x 10^{-4} to 6.8x 10^{-4} moles of urea hydrolysed g^{-1} of soil hr^{-1}.

With respect to phosphatase activity (Table 12) the values for kinetic parameters V_{max} and K_m ranged from 9.4x 10^{-3} to 21.7x10^{-3} µg of p-nitrophenol released g^{-1} of soil hr^{-1} and 0.3 x 10^{-4} to 3.8x 10^{-4}µg of p-nitrophenol released g^{-1} of soil hr^{-1} respectively. The maximum V_{max} and K_m was observed at the 6th week with values 21.7x10^{-3} µg of p-nitrophenol released g^{-1} of soil hr^{-1} and 4.1x 10^{-4} µg of p-nitrophenol released g^{-1} of soil hr^{-1} respectively

In the case of protease as observed from Table 12, the values for V_{max} ranged from 1.3x 10^{-3} to 1.9x10^{-3} µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1} with the highest value reported at the 8th week (1.9x10^{-3} µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}). K_m ranged from 1.15x 10^{-5} to 1.92x 10^{-5} µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}. The highest value K_m was reported at the 8th week (1.92x10^{-5} µM of amino nitrogen hydrolysed $g^{-1}$of soil hr^{-1}).

It is inferred from Table: 9 that the V_{max} values for dehydrogenase ranged from 3.56x10^{-3} to 6.21x 10^{-3} µg of TPF hydrolysed g^{-1} of soil 24 hrs^{-1}. The maximum V_{max} value is found to be highest at the 2nd week. The K_m values ranged from 0.72x 10^{-4} to 1.2x 10^{-4} µg of TPF hydrolysed g^{-1} of soil 24 hrs^{-1} with the highest value noticed at the 2nd week.

With regard to cellulase activity, the V_{max} ranged from 1400 ppm to 3200 ppm of of glucose hydrolysed g^{-1} of soil 24 hrs^{-1}, with the highest value noticed at the 6th week. The K_m values ranged from 0.90 to 3.90 ppm of glucose hydrolysed g^{-1} of soil 24 hrs^{-1} with the highest value noticed at the 6th week.

4.2.8. Computation of Biological Fertility Index through Enzyme Activity Number (EAN)

Biological fertility index for the treatments were calculated and were presented in Table 13. Treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%)) registered the highest value of 46.84 followed by the treatment T_{13}

Treatments	Catalase (ml O_2 g^{-1} dry soil)	Phosphate µg of p-nitrophenol released g^{-1} of soil hr^{-1} ()	Protease(µM of amino N-hydrolysed g^{-1} of soil hr^{-1})	Dehydrogenase (µg of TPF hydrolysed g^{-1} of soil per 24 hrs)	Cellulase (glucose hydrolysed g^{-1} of soil 24 hrs^{-1}.)	Enzyme activity number
T_1	2.47	47.53	140.75	158.51	29.24	32.64
T_2	3.15	48.83	148.31	162.82	33.08	33.54
T_3	3.47	49.93	154.44	165.46	35.31	34.11
T_4	7.00	63.71	157.74	203.86	38.30	41.88
T_5	7.01	59.46	149.5	185.09	40.35	38.06
T_6	6.14	62.12	163.13	183.01	42.21	37.73
T_7	6.37	62.22	163.73	188.49	43.59	38.79
T_8	6.91	79.67	175.49	188.32	44.13	38.97
T_9	8.27	75.33	181.13	227.79	49.83	46.84
T_{10}	7.21	51.7	160.76	205.32	38.81	42.12
T_{11}	7.12	53.54	170.82	208.83	42.84	42.89
T_{12}	7.30	68.56	173.53	218.63	43.37	44.93
T_{13}	8.13	67.57	176.38	220.38	45.87	45.29
T_{14}	2.47	40.46	141.08	132.44	26.79	27.39
T_{15}	1.57	39.46	101.45	127.18	25.79	26.14

Table 13 Computation of Biological Fertility Index through Enzyme Activity Number (EAN)

(N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %) as inorganics having a value of 45.29. The least value i.e. 26.14 was reported by the treatment T_{15} which was the absolute control plot.

4.2.9. Comparison of micro flora between rhizosphere and non rhizosphere soils

4.2.9.1. Rhizosphere soil

Table 14 shows the shift in microbial population in rhizosphere soil due to various treatments.

4.2.9.1.1. Bacteria

Bacterial count varied significantly with different treatments (Table 14). Mean value ranges from 66.67 x 10^6 to 184.67 x 10^6 CFU g^{-1} of soil. Highest value was recorded by T_8 (184.67x10^6 CFU g^{-1} of soil) which was on par with T_9 (169 x 10^6 CFU g^{-1} of soil) and T_5 (167x10^6 CFU g^{-1} of soil) .Lowest mean value of 66.67 x 10^6 CFU g^{-1} of soil was observed by the treatment T_{15} (Absolute control).

4.2.9.1.2. Fungi

Various treatments influenced the fungal population in rhizosphere soil. (Table 14) Mean values from 6 x 10^4 to 13.67 x10^4 CFU g^{-1} of soil. In the case of population of fungi in rhizosphere soil, T_9 recorded the highest mean value of 13.67 x10^4 CFU g^{-1} of soil which was on par with T_5 (13 x10^4 CFU g^{-1} of soil) and T_{13} (12.67 x10^4 CFU g^{-1} of soil). T_3 (11 x10^4 CFU g^{-1} of soil), T_4 (11.67 x10^4 CFU g^{-1} of soil), T_7 (11.67 x10^4 CFU g^{-1} of soil), T_8 (11.67 x10^4 CFU g^{-1} of soil) and T_{12} (12 x10^4 CFU g^{-1} of soil) were found to be on par. Lowest mean value of 6 x 10^4 CFU g^{-1} of soil was recorded by the treatment T_{15} (Absolute control)

4.2.9.1.3. Actinomycetes

Table 14 shows the treatments had an effect on the population of actinomycetes in rhizosphere soil. The mean value ranges from 2.33 x 10^4 to 9.67 x10^4 CFU g^{-1} of soil. Treatment T_{13} (9.67 x10^4 CFU g^{-1} of soil) recorded the highest mean value

Table 14 Effect of treatments on status of micro flora in rhizosphere soils

Treatments	Bacteria CFU g^{-1} soil(10^6 dilution)	Fungi CFU g^{-1} soil(10^4 dilution)	Actinomycetes CFU g^{-1} soil(10^6 dilution)
T_1	109.33	9.00	5.33
T_2	102.33	9.67	6.00
T_3	138.67	11.00	7.00
T_4	153.33	11.67	8.00
T_5	167.00	13.00	9.00
T_6	163.33	10.67	8.00
T_7	153.33	11.67	7.33
T_8	184.67	11.67	9.00
T_9	169.00	13.67	9.33
T_{10}	163.00	10.00	6.00
T_{11}	153.00	10.67	7.67
T_{12}	162.33	12.00	8.00
T_{13}	147.00	12.67	9.67
T_{14}	94.33	7.33	3.33
T_{15}	66.67	6.00	2.33
CD (0.05)	17.881	1.542	1.557

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *zospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

Table 15 Effect of treatments on status of micro flora in non-rhizosphere soils

Treatments	Bacteria CFU g^{-1} soil(10^6 dilution)	Fungi CFU g^{-1} soil(10^4 dilution)	Actinomycetes CFU g^{-1} soil(10^6 dilution)
T_1	90.00	6.00	3.33
T_2	101.67	6.67	5.00
T_3	120.33	7.00	5.67
T_4	115.67	7.67	7.33
T_5	118.33	8.33	6.33
T_6	115.67	6.67	6.33
T_7	116.00	6.67	6.33
T_8	119.67	6.00	6.67
T_9	121.00	8.67	8.00
T_{10}	110.67	7.33	5.33
T_{11}	126.33	7.67	6.33
T_{12}	126.67	6.33	6.00
T_{13}	121.67	8.67	7.67
T_{14}	84.00	3.33	2.33
T_{15}	41.33	2.67	2.33
CD (0.05)	20.045	1.444	0.997

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

which was on par with T_9 (9.33 x10^4 CFU g^{-1} of soil), T_8 (9 x10^4 CFU g^{-1} of soil) and T_5 (9 x10^4 CFU g^{-1} of soil). Lowest mean value of 2.33 CFU g^{-1} of soil was recorded by absolute control. (T_{15}).

4.2.9.2. Non- Rhizosphere soil

Perusal of data (Table 15) on microfora in non-rhizosphere soils revealed that different treatments significantly influenced the microbial population.

4.2.9.2.1. Bacteria

Treatments varied significantly with the bacterial count in non- rhizosphere soil. The mean values ranged from 41.33 x 10^6 to 126.67 x 10^6 CFU g^{-1} of soil. In the case of bacteria treatment T_{12} recorded the high mean value (126.67 x 10^6 CFU g^{-1} of soil) which was on par with T_{11} (126.33 x 10^6 CFU g^{-1} of soil), T_{13} (121.67 x 10^6 CFU g^{-1} of soil), T_9 (121 x 10^6 CFU g^{-1} of soil), T_3 (120.33 x 10^6 CFU g^{-1} of soil), T_8 (119.67 x 10^6 CFU g^{-1} of soil), T_5 (118.33 x 10^6 CFU g^{-1} of soil), T_7 (116 x 10^6 CFU g^{-1} of soil), T_4 (115.67 x 10^6 CFU g^{-1} of soil), T_6 (115.67 x 10^6 CFU g^{-1} of soil), T_{10} (110.67 x 10^6 CFU g^{-1} of soil). Lowest mean value of 41.33 x 10^6 CFU g^{-1} of soil was recorded by T_{15} (Absolute control).

4.2.9.2.2. Fungi

A significant different due to various treatments on population of fungi in non rhizosphere soils was noticed. The mean values ranged from 2.67 x10^4 to 8.67 x10^4 CFU g^{-1} of soil. The treatment T_9 and T_{13} registered the highest mean value i.e.: 8.67 x10^4 CFU g^{-1} of soil which was on par with treatments T_5 (8.33 x10^4 CFU g^{-1} of soil), T_4 (7.67 x10^4 CFU g^{-1} of soil), T_{11} (7.67 x10^4 CFU g^{-1} of soil) and T_{10} (7.33 x10^4 CFU g^{-1} of soil). Treatments T_2 (6.67 x10^4 CFU g^{-1} of soil), T_3 (7 x10^4 CFU g^{-1} of soil), T_6 (6.67 x10^4 CFU g^{-1} of soil), T_7 (6.67 x10^4 CFU g^{-1} of soil) and T_{12} (6.33 x10^4 CFU g^{-1} of soil) were found to be on par. Lowest mean value i.e. 2.67 x10^4 CFU g^{-1} of soil was registered by T_{15} (Absolute control).

4.2.9.2.3. Actinomycetes

Various treatments influenced the actinomycetes population in non rhizosphere soils (Table 15). The mean values ranged from 2.33 x 10^4 to 8 x10^4 CFU g^{-1} of soil Highest mean value was recorded by T_9 (8 x10^4 CFU g^{-1} of soil) with the application of NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics which was superior than all other treatments. Treatment T_{13} (7.67 x10^4 CFU g^{-1} of soil) was on par with T_4 (7.33 x10^4 CFU g^{-1} of soil). Lowest mean value i.e. 2.33 x 10^4 CFU g^{-1} of soil was registered by T_{15} and T_{14}.

4.3. Biometric observations

4.3.1. Plant height - at first harvest

The treatments varied significantly with respect to plant height as inferred from Table 16. The mean values ranged from 79.5 to 122.0 cm. The treatment T_9 with the application of NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics recorded the highest value of 122.0 cm and was found to be on par with the treatment T_7 (120.0 cm) with the application of N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K as inorganics and were highly significant than the other treatments. The least value for plant height i.e. 79.5 cm was observed in T_{15} (absolute control). Treatments T_8 (117.9 cm), T_3 (114.4 cm) and T_{10} (116.3cm) were found to be on par with each other.

4.3.2. Internodal length at final harvest

The internodal length of the crop was taken at the harvesting stage and is presented in Table 16. After statistically analysing the data, the internodal length revealed that the treatments varied significantly. The mean values ranged from 6.7 cm to 8.9 cm. The treatment T_1 [Package of practice recommendation (KAU)] recorded the highest value of 8.9 cm and was found to be on par with T_8 (8.5 cm) and T_{11} (8.5 cm). The lowest value for intermodal length was reported in treatment T_{15} (Absolute control) i.e. 6.7 cm which was significantly lower than all other treatments.

Table 16 Plant height - at first harvest(cm), internodal length at final harvest(cm), number of branches - at final flowering and days to 50 % flowering as affected by treatments on bhindi

Treatments	Plant height - at first harvest(cm)	Internodal length at final harvest(cm)	No of branches - at final flowering	Days to 50 % flowering
T_1	107.9	8.9	1.33	30.00
T_2	103.1	7.8	3.00	28.33
T_3	114.4	8.4	2.33	27.66
T_4	108.3	8.2	2.33	28.33
T_5	104.2	7.5	2.66	27.66
T_6	113.0	7.1	3.00	27.66
T_7	120.0	8.2	3.00	28.00
T_8	117.9	8.5	2.66	26.33
T_9	122	8.3	2.33	27.66
T_{10}	116.3	8.4	2.66	27.66
T_{11}	110.9	8.5	2.66	28.33
T_{12}	113.5	7.4	2.66	26.33
T_{13}	103.5	7.7	2.66	27.33
T_{14}	94.7	7.4	2.00	31.33
T_{15}	79.5	6.6	1.33	32.00
CD (0.05)	3.40	0.36	1.098	1.623

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

4.3.3. No of branches - at final flowering

Critical appraisal of the data presented in Table 16 revealed that the treatments did not vary significantly with respect to number of branches. The highest values were reported in the treatments T_2 (3.0), T_6 (3.0), T_7 (3.0) followed by treatments T_5 (2.66), T_8 (2.66), T_{10} (2.66), T_{11} (2.66), T_{12} (2.66), T_{13} (2.66) which were found to be on par with each other. However the treatments T_2 (3.0), T_6 (3.0) and T_7 (3.0) varied significantly over the control T_{15} (1.33) which was found to be on par with the treatment T_1 (POP recommendation).

4.3.4. Flowering stages - days to 50 % flowering

Treatment application significantly influenced the number of days to 50% of flowering as inferred from Table 16. The mean values ranged from 26.33 days to 32 days. The highest value of 32 days for 50% flowering was noticed with the treatment T_{15} (absolute control which was on par with T_{14} (31.33 days).The lowest value was noticed in the treatment T_8 (26.33 days) which was on par T_{12} (26.33days).

4.3.5. Fruit

4.3.5.1. No of fruits/ plant

The treatment imparted significant effect on the fruits per plant (Table 17). The mean values ranged between 13.0 and 27.33. The highest value was recorded for T_9 (27.33) with the application of N, P, K, (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50 %) as inorganics which was significantly superior than all other treatment. Treatments T_5 (23.33) was found to be on par with T_6 (21.33), T_4 (21.33), T_7 (21.33), T_8 (21.0), T_{10} (22), T_{12} (22.66) and T_{13} (24.33). Lowest value was recorded by the T_{15} (13) which is the absolute control.

4.3.5.2. Yield per plant

Imposition of treatments had significant influence in the yield per plant. (Table 17) The mean values ranged from 304.33 to 769.33 g. Treatment T_{13} with the

Table: 17 No of fruits/ plant, Yield per plant (g), Yield per ha (t/ha) and Disease incidence percentage as affected by treatments on bhindi

Treatments	No of fruits/ plant	Yield per plant (g)	Yield per ha(t/ha)	Disease incidence percentage
T_1	17.66	688.00	8.99	22.67
T_2	19.00	586.66	7.13	22.00
T_3	17.66	633.33	7.82	22.33
T_4	21.33	663.00	7.84	21.33
T_5	23.33	692.00	8.13	22.00
T_6	21.33	626.67	7.99	20.67
T_7	21.33	704.00	8.52	22.33
T_8	21.00	719.67	9.48	21.00
T_9	27.33	734.00	9.68	21.33
T_{10}	22.00	690.33	8.82	22.00
T_{11}	21.33	697.33	8.73	22.00
T_{12}	22.66	708.33	9.46	22.00
T_{13}	24.33	769.33	9.76	22.33
T_{14}	16.00	367.00	6.84	23.67
T_{15}	13.00	304.33	5.76	24.00
CD (0.05)	2.835	16.621	0.282	1.779

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

application of N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N; P & K (25 %) as inorganics had registered the highest value of 769.33g which was significantly superior than other treatments. Treatment T_{15} had registered the lowest value of 304.33, which was significantly inferior to all other treatments.

4.3.5.3. Yield per ha

The results of the analysis of biometric observations indicated an effect of treatments on the yield per ha (Table 17). The mean value ranged between 5.76 t ha^{-1} and 9.76 t ha^{-1}. The highest value was recorded by T_{13} with the application of N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %) as inorganics (9.76 t ha^{-1}) which was on par with T_9 (9.68 t ha^{-1}) and T_{10} (9.48 t ha^{-1}). The lowest value was registered by T_{15} (5.76). Treatment T_{11} (8.82) was found to be on par with T_6 (8.73 t ha^{-1}) and T_7 (8.99). Treatment T_4 (7.84 t ha^{-1}) was found to be on par with T_6 (7.99 t ha^{-1}) and T_3 (7.82 t ha^{-1}) were also found to be on par.

4.3.5.4. Scoring of diseases

There was significant difference among treatments with respect to disease incidence percentage (Table 17). The mean value ranged from 20.67% to 24%. Highest value (24%) was recorded by the treatment T_{15} (absolute control). Treatment T_6 (20.67%) registered the lowest mean value and was found to be on par with T_8 (21%), T_4 (21.33%), T_9 (21.33%), T_2 (22%), T_{12} (22%), T_{11} (22%), T_{10} (22%), T_3 (22.33%) and T_7 (22.33%).

4.4. Other parameters

4.4.1. Shelf life (keeping quality)

The keeping quality varied significantly with treatment (Table 18). The mean values ranged between 6 and 9 days. The highest value was recorded by T_5 (9.00 days) with the application of N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %) as inorganics which was on par with T_9 (8.67days) , T_7 (8.67 days), T_6 (8.33 days), T_8 (8.33 days), T_{10} (8 days), T_3 (8

Table 18 Keeping quality and B: C ratio as affected by treatments on bhindi

Treatments	Keeping quality	B:C ratio
T_1	6.33	3.01
T_2	7.33	2.25
T_3	8.00	2.50
T_4	7.67	2.48
T_5	9.00	2.68
T_6	8.33	2.32
T_7	8.67	2.81
T_8	8.33	3.10
T_9	8.67	3.34
T_{10}	8.00	2.76
T_{11}	7.67	2.63
T_{12}	8.00	2.68
T_{13}	7.67	2.99
T_{14}	6.33	1.34
T_{15}	6.00	1.24
CD (0.05)	1.026	0.040

T_1 Package of practice recommendation (KAU)
T_2 N (25 %) as neem cake enriched vermicompost + N (75%), P & K
T_3 N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K
T_4 P (25 %) as PSB enriched vermicompost + P (75%), N & K
T_5 NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)
T_6 N (50%) as Neem cake enriched vermicompost+ N (50%), P & K
T_7 N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K
T_8 P (50 %) as PSB enriched vermicompost + P (50%), N & K
T_9 NPK (50 %)as PGPR mix-1 enriched vermicompost + N, P & K (50%)
T_{10} N (75 %) as Neem cake enriched vermicompost + N (25%),P & K
T_{11} N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K
T_{12} P (75%) as PSB enriched vermicompost + P (25%), N & K
T_{13} N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)
T_{14} N, P, K alone as inorganics
T_{15} Absolute control

days) and T_{12} (8 days). The lowest value was recorded by T_{15} (6.33 days) which was on par with T_{14} (6.33 days) and T_1 (6.33days).

4.5. Economics

4.5.1. B: C ratio

B: C ratio was calculated by taking into consideration the cost of cultivation and returns for each treatment and the results are presented in Table 18. The mean values ranged from 1.2 to 3.34. From the analysis of data T_9 registered the highest value of 3.34. Lowest B: C ratio was reported by the treatment T_{15} (1.24) absolute control. Treatments T_{13} (2.99) was found to be on par with T_1 (3.01). Treatments T_3 (2.50) and T_4 (2.48) were also found to be on par.

Discussion

5. DISCUSSION

Soil fertility and crop production are affected by biological and chemical processes which are intimately involved in the cycling of nutrients, effect fertilizer use efficiency, reflect the microbiological activity in soil and act as indicators of soil productivity.

As soil is a part of terrestrial environment and supports all terrestrial life form, protection of soil is therefore of high priority and thorough understanding of soil physical and biological activities is a critical factor in assuring that the soil remains healthy (Srinivasalu and Rangaswamy, 2006).

Organic and inorganic fertilizers are used primarily to increase nutrient availability to plants. However they can affect the population composition and function of micro organisms and thus the soil biological regimes. The balanced fertilization of major elements viz., N, P, K could be beneficial for the growth of above ground parts and roots of plants.

A major effort is needed to develop a relative or universal index that would be interpretable under various environmental conditions. This approach involve the measurement of enzyme activities or any other biological measurements, which could be used to asses soil quality, because soil naturally varies in biological activity, (Dick,1994).

Better understanding of the role of the soil enzyme activities in maintaining the soil health will potentially provide a unique opportunity for integrated biological assessment of soil due to their crucial role in several soil biological activities, their ease of measurement and their rapid response to changes in soil management (Singher and Ewing, 2000). Although there have been extensive studies on soil enzymes, little have been reported on their roles in maintaining soil health. Thus it is authoritative to understand the roles of these enzymes and their activity to maintain soil health for future betterment of soil research and soil biology.

With overriding objective to project the beneficial effect of bio inoculants, bio fertilizers and organic amendments on the soil enzyme and microbial activities, this particular investigation was carried out. The present study was undertaken to assess the impact of IPNS on the soil biological regimes in red loam soil.

Soil samples collected from the experimental plots were subjected to enzyme activity assay to generate data for calibration and interpretation of result as independent soil quality indices. A brief interpretation of results pertaining to the study conducted, are presented in this chapter.

Much of the attention has been paid on the last few decades for the application of the nutrients in the form of inorganic fertilizers. Now there is a change in the trend with the IPNS gaining popularity. The effects of IPNS on the soil chemical and biological properties are inevitable.

5.1. Chemical properties of soil

5.1.1. pH

One of the important properties which effects the availability of soil nutrients, controls the composition and diversity of microbial community alters the equilibrium, solid phase and imparts plant response. The result of the pH of the samples from the experimental plot was furnished in Table 5.

From the data in Table 5 it was inferred that treatments imposed a significant effect on the pH of the post harvest soil. The highest pH value was registered in the absolute control plot, which was on par with the treatment involving inorganics alone and 25% N as neemcake enriched vericompst in combination with inorganics. Wide fluctuations in pH were not observed with the application of treatments due to the buffering action of manure application (Chaudhaury, 1977). The lowest value for pH was noticed by the application of 75% of N as neemcake enriched vermicompost and N (75%) PK as inorganics. This might be due to the production of acidic root exudates, which might have contributed to the lower pH in the treatment. Moreover application of neemcake might have

triggered the microbial activity, favoring the production of organic acids resulting in the low values for pH (James and Richards, 2005).

5.1.2. Electrical conductivity

Total salt accumulation in soil as measured by electrical conductivity varied significantly as inferred from Table 5. The treatment with the application of 50% P as PSB enriched vermicompost registered the highest value which was on par with the treatments receiving 50% of N as *Azospirillum* enriched vermicompost + N (50%), P & K as inorganics and 50% of N as neemcake enriched vermicompost + N (50%), P & K as inorganics. This might be due to the accumulation of soluble salts in the soil profile with the application of manures. Similar results with the application of manures were reported by Chang *et al.* 1993; Hao and Chang, 2003.

The lowest EC in the control plot might have been due to the non- accumulation of salts in soil profile. Loading of soil with high quantities of organic manures such as Beef manure was reportedly increased the EC to 3.55 dSm^{-1} and above 0.75 dsm^{-1} for the control (Maas, 1986).

5.1.3. Organic Carbon

It is inferred from the Table 5, that application of 25% of N as neemcake enriched vermicompost in combination with inorganics and 25% of *Azospirillum* enriched vermicompost in combination with inorganics had registered the highest value for organic carbon content in the post harvest soil. As compared to the absolute control, all the plots receiving enriched vermicompost along with chemical fertilizers recorded medium to high organic carbon status. An increase of 54 percent over the absolute control was observed with the application of 25% of N as neemcake enriched vermicompost in combination with inorganics and 25% of N as *Azospirillum* enriched vermicompost in combination with inorganics. This might have been due to the direct incorporation of organic matter, better root growth and more plant residues addition on realizing higher crop yields (Katyal *et al.*, 2003; Kumar and Yadav, 2003).

5.1.4. Available Nitrogen

Nitrogen is a major nutrient, which is needed for the growth of plant. Application of various treatments had significant on the availability of N in the post harvest soil (Table 6). Treatment with 75% of N as *Azospirillum* enriched vermicompost and 25% N, P, and K as inorganics had registered the highest value; which was on par with the treatments receiving 50% of NPK as PGPR mix-1 enriched vermicompost and 50% NPK as inorganics and 75% of NPK as PGPR mix-1 enriched vermicompost and 25% of NPK as inorganics. An increase of 45 percent over the absolute control was observed with the application 75% of N as *Azospirillum* enriched vermicompost and 25% N, P, and K as inorganics. Sharma and Gupta (1998) reported that supplementing organics with inorganic N fertilizers enhances the available N content of the soil due to hastened mineralization, once the requirement of N by microbes is met through inorganic nitrogen. The favorable soil condition under the treatments receiving organic manures might have helped in the mineralization of soil N leading to build up of available N content.

It is observed from the Table 6, that the treatment receiving 75% N as *Azospirillum* enriched vermicompost in combination with inorganics was also effective in improving the soil available N because of increased mineralization of N. Many reports have been documented for the successful use of *Azospirillum* for improving the soil available N, thereby increasing the growth, development and yield of important crop species (Baldlani *et al.,* 1987). Enrichment of vermicompost using *Azospirillum* enhances the decomposition and mineralization leading to the attainment of narrow C/N ratio and there by leading to increased available soil N. In this context it is believed that the application of enriched vermicompost triggers the microbial population, with the synthesis of higher microbial biomass, which releases N upon decomposition. (Banerjee *et al.,* 2006).

The significant role of PGPR mix-1 enriched vermicompost is evident from the study. PGPR can exert positive effect through the synthesis of phytohormones, nitrogen fixation and reduction of membrane potential of roots. The most notable

mechanism of PGPR in increasing the nitrogen content is by nitrogen fixation (Richardson and Hadobas, 1997). Thus, even after the plant uptake the treatments involving the application of *Azospirillum* and PGPR could sustain a higher level of available N.

The lowest value was recorded for the absolute control and the reported N-status on this plot might be attributed to the residual effect of manures and fertilizers added as well as from the native soil resources.

5.1.5. Available phosphorous

Phosphorous is one of the major essential macronutrients limiting plant growth owing to its low availability in soils (Feng *et al.*, 2004). Fertilizer P tends to be fixed soon after the application and mostly unavailable, resulting in low recovery by crops and a considerable P accumulation in soils (Alam and Ladha, 2004).

From the data presented in Table 6, it is inferred that treatment involving the application of enriched vermicompost using PSB at 75% and 50% level for P substitution were found to significantly influence the available P status and registered higher values for available P. This is consequent to the application of vermicompost enriched with P solubilizers. The enhancement of available P might be due to soluble P contributed from the mineral fertilizers added by the mineralization action of the P solubilizing bacteria, because many soil microorganisms are able to transform insoluble forms of P to usable soluble form. Increase in available P content of soil, with the addition of fertilizers along with manures was reported by Sharma *et al.*, (2005).

The effect of PSB strain (*Bacillus megaterium*) on solubilizing native P is evident from the present study. Similar results on the solubilization of P in the soil by the strains of *B. megaterium* and *B. caryophili* was also reported by Tao *et al.*, (2008).

5.1.6. Available potassium

The available K as inferred from the Table 6 was markedly influenced by the treatments. The application of vermicompost enriched with 75% of NPK as PGPR

mix-1 enriched vermicompost in combination with inorganic fertilizers which had registered the highest value and found to be on par with 50% of NPK as PGPR mix-1 enriched vermicompost in combination with inorganics. These findings corroborate with the findings of Warren and Fonteno (1993).

As opined by Tisdale *et al.,* (1995), addition of organic matter enhances the buffering capacity of soil, which represents the ability to supply nutrients to the soil. Under the high buffering capacity the total K^+ ion in the soil solution, reported a higher value for available K in the manured plots. Higher content of potassium in the soil may be attributed to its addition through potassic fertilizers, organic manures, weathering and release of labile K from organic residues (Setia and Sharma, 2007).

An increase in soil available P and K due to the application of poultry manure along with inorganic fertilizers was also reported by Babu *et al.*, 2007. However significant effect of manuring was noticed from the treatments which were significantly higher than the absolute control plot. The prominent effect of PGPR on contributing to increase available K is evident from the study and it was found to be superior to the other bio inoculants such as PSB and *Azospirillum.*

5.1.7. Available micronutrient content

Role of micronutrients in balanced plant nutrition is well established. However exploitative nature of modern agriculture involving use of high analysis NPK fertilizers coupled with limited use of organic manures and shrinking recycling of crop residues have contributed the accelerated exhaustion of micronutrients.

5.1.7.1. Fe content

From the Table 7 it is inferred that the Fe content is significantly influenced by the treatments. The increased Fe content in the plots receiving the NPK (50%) as PGPR mix-1 enriched vermicompost along with inorganics fertilizers might be due to the production of organic acids which might have favoured the increased availability of Fe^{2+} ions. This corroborated with the findings of Swarup, 1984, who reported the increased availability Fe^{2+} ions by the application of FYM.

5.1.7.2. Cu content

From the Table 7 it is obvious that treatments had significant effect on Cu content in the post harvest soil. Treatment receiving 50% NPK as PGPR mix-1 enriched vermicompost in combination with inorganics had registered the highest value. Puente *et al.*, (2004) reported that bacteria colonizing the rhizoplane of rock-weathering desert plants were found to release a significant amount of Cu along with the release of other minerals including P, K, Mg, Mn and Zn from the rocks, and were also thermo-tolerant and halo tolerant.

5.1.7. 3. Mn content

Application of treatment had significant effect on the Mn content of the post harvest soil (Table 7). Application of 25% of N as neemcake enriched vermicompost in combination with inorganics reported the highest value. Kumar *et al.* (2012) reported that DTPA- extractable Mn increased with the application of organics.

5.1.7.4. Zn content

It is inferred from the Table 7 that Zn content varied significantly with the application of treatments. Treatment receiving 75% of N as neemcake enriched vermicompost in combination with inorganics reported the highest value. This could be attributed to the direct contribution of this treatment to nutrient pool and its beneficial effects either through complexation or mobilization of native Zn. Kumar *et al.* (2010) reported that DTPA extractable Zn showed positive correlation with organic carbon content indicating that application of organics increases organic matter content that provides chelating agents for complexion of native or added zinc

5.1.7.5. B content

Treatment receiving 50% of NPK as PGPR mix-1enriched vermicompost in combination with inorganics recorded the highest value. A similar increase in the

micronutrient status in soil and increased uptake of micronutrients due to the application of bacterial PGPR consortia was reported by Rana *et al.*, (2012).

For all the five micronutrient analyzed, absolute control plot recorded the least value. Less mineralization activity due to less microbial activity and crop removal might have contributed to the least content of micronutrients for the treatment.

5.2. Soil biological properties

Soil has been a home for the living biomass conceding innumerable microbial activities leading mineralization, immobilization of nutrients which have been often the source of nutrient for autotrophs. Deterioration of soil and thereby soil health is of concern for human, animal and plant health, because air, ground water and surface water consumed by humans can be adversely affected by mismanaged and contaminated soil.

Enzymes are vital activators in life process, likewise in soil; they are known to play a substantial role in maintaining soil health and its environment. Healthy soils are essential for the integrity of terrestrial ecosystem to intact or to recover from disturbances, such as drought, climate change, pollution and human exploitation including agriculture (Ellert *et al.*, 1997). Therefore it is essential to characterize the soil for the biochemical properties and adopt management strategies to overcome the deterioration and promote rejuvenation by the addition of suitable amendments.

The most substantial index of biological activity in the soil is its enzyme activity and therefore it can give the idea of biochemical processes in the soil. Thus the enzyme activities are sensitive parameters which can be used as an early warning in changes in ecosystem, before they are detectable in other ways.

5.2.1. Urease activity

Urease is unique among the soil enzymes because it affects the fate and performance of the fertilizer urea. Urease play vital role in hydrolysis of urea

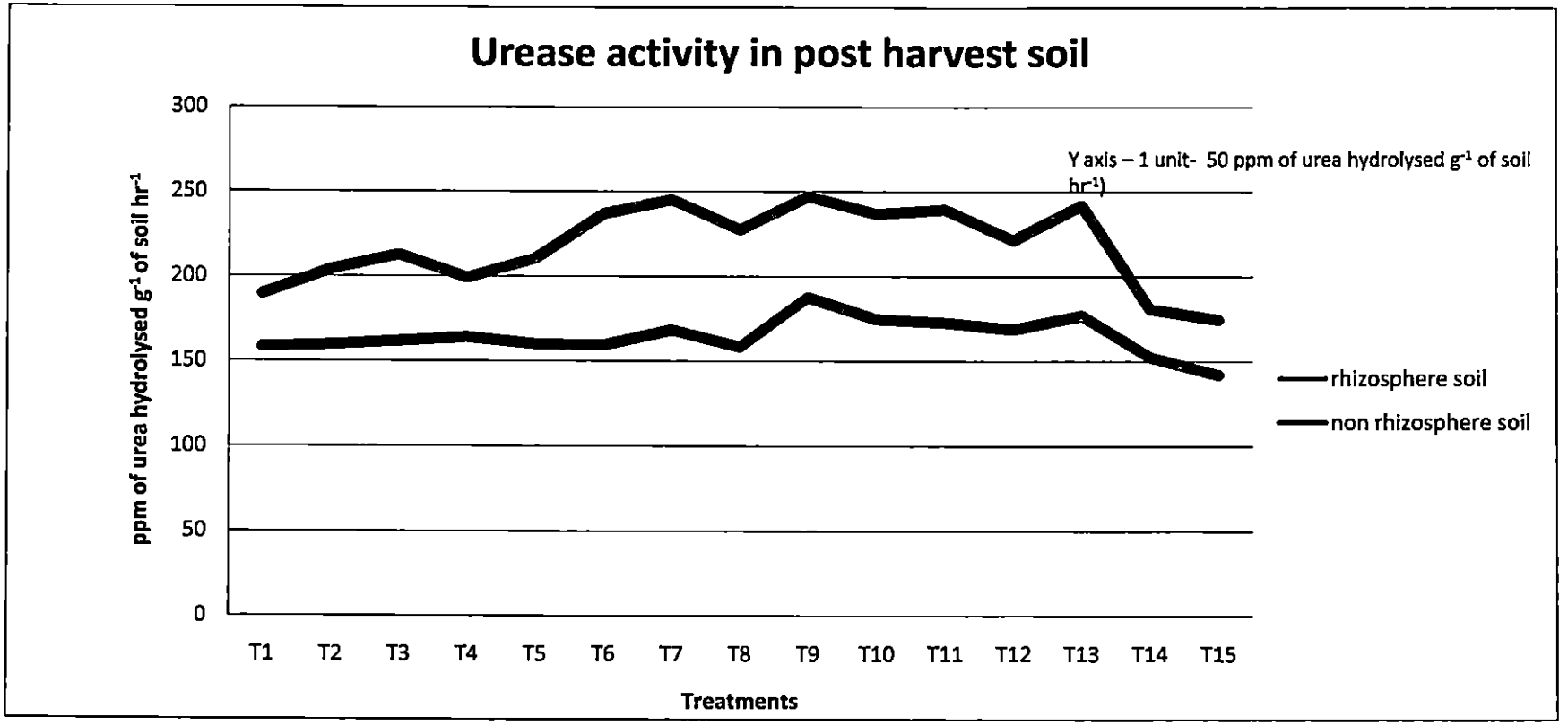

Fig. 3. Urease activity in post harvest soil

fertilizer hence it is important to uncover the other unknown factors that may affect the efficiency of this enzyme in the soil ecosystem.

5.2.1.1. Rhizosphere

From the data presented in the Table 9, Fig. 3, it is observed that the highest urease activity was reported by the treatment involving 75% N as *Azospirillum* enriched vermicompost in combination with inorganics and was superior to all other treatments.

Increased microbial population, with the addition of vermicompost enriched with *Azospirillum* along with inorganic nutrients sources might have been responsible for the sharp increase in urease activity. The results corroborated with the findings of Perotti and Pidello (1999) who reported that the application of *Azospirillum* had modified the urease activity in the soil. This trend in activation of urease activity was due to the addition of organic sources acting as sole source of C and energy. The positive effect of *Azospirillum* on increasing soil urease was also reported by Li *et al.,* (2009). Application of vermicompost also promotes release of growth promoting trigger molecules that stimulate the organisms to secrete high levels of urease enzymes (Burns, 1982). The role of inorganic nutrients is inevitable that they provide energy for the organisms to proliferate and help in the synthesis of enzyme (Skogland *et al.,* 1998). The lowest value recorded for the absolute control was obviously due to the poor nutrient content and organic carbon which fails to sustain the microflora. The red loam soil of this location is highly, impoverished depleted of bases with high Fe^{2+} and Al^{3+} content, poor in organic matter and low in microbial activity. This infertile nature might have resulted in general decline of enzyme activities and thus the urease (Aparna, 2000). It is interesting to note that each of treatment imposed varied significantly with respect to urease activity.

5.2.1.2. Non rhizosphere soil

Persual of data revealed that urease activity in non rhizosphere soil was higher with the application of 50% of NPK as PGPR mix-1enriched vermicompost in combination with inorganics (Table 10, Fig. 3). This kind of trend of increase in enzyme activities was due to addition of organic source acting as sole source of carbon and energy for the heterotrophs as also reported by Selvi *et al.,* (2004). Increased microbial population can also increase the enzymatic activity.

5.2.2. Phosphatase activity.

In soil ecosystem, soil phosphatases are believed to play critical role in P-cycle, thus influencing the availability of P in the soil. Understanding the dynamics of phosphatase is crucial for predicting their activities as their activities may in turn regulate the P availability in soil.

5.2.2.1. Rhizosphere soil

From the Table 9, Fig. 4, it is inferred that application of PSB enriched vermicompost in combination with inorganic fertilizers was found to significantly influence the phosphatase activity in rhizosphere soil. This might be attributed to the fact that higher availability of substrate phosphorus due to the solubilization from the inorganic and native P-sources resulting in higher activity. Similar results were reported by Illmer and Schinner (1995) who opined that PSB have high potential to solubilize inorganic phosphate. There are number of reports that PSB have the ability to solubilize inorganic P from soil after the inoculation in soil. The production of other metabolites by these strains beneficial to plants such as phytohormones, antibiotics or siderophores resulted in phosphate solubilization and yield stimulation (Koeppler, 1999)

Highest available P in the 50% of P as PSB enriched vermicompost in combination with inorganics treated plots might have also contributed to the availability of substrates for the enzymes to act upon. The lowest value recorded

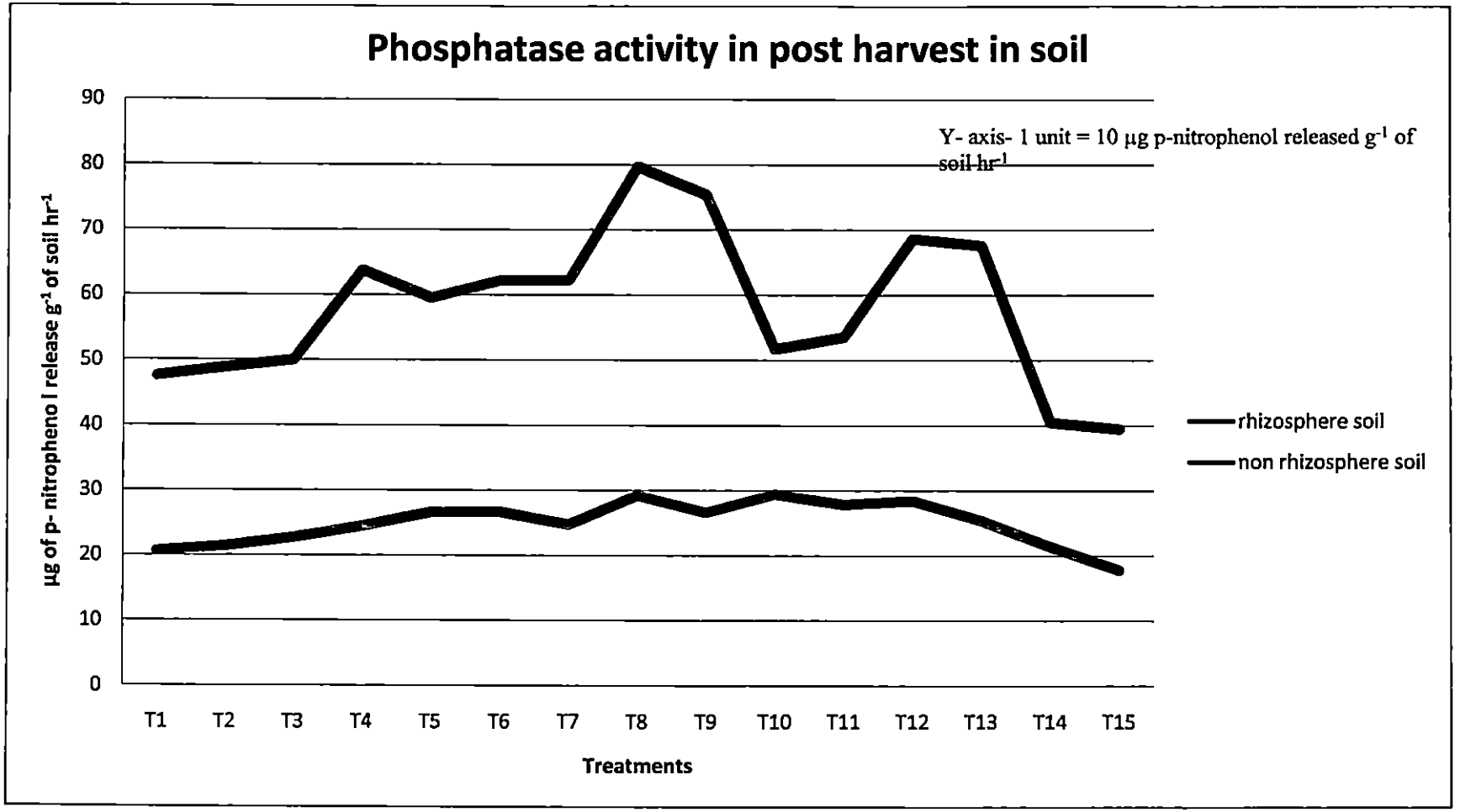

Fig. 4. Phosphatase activity in post harvest in soil

in the absolute control might be due to the non availability of P-substrates for the enzyme to act upon.

The lowest value in the absolute control plot may also be due to the bacteria colonizing P-deficient environment with low P mobilizing capacity to sustain their growth which is the basis of stress physiology paradigm as opined by Goldstein *et al.* (1999)

5.2.1.2. Non rhizosphere soil

Significant variation in phosphatase activity in rhizosphere was found due to the imposition of treatments (Table 10, Fig. 4). Highest activity was seen in treatments receiving 75% N as neem cake enriched vermicompost and 25% NPK as inorganics and 50% P as PSB enriched vermicompost and P 50% and NK as inorganics. The activity of acid phosphatase was 43% higher in plots treated with phosphate solubilizing bacteria inoculated vermicompost than in plots with uninoculated vermicompost (Busato *et al.,* 2012).

The increase in the soil phosphatase with the addition of organics could be attributed to the soil substrate enrichments by the addition of mineral fertilizers. The phosphates added through organics and fertilizers might have improved the phosphatase activity, which may ascribe to the stabilized extra cellular fraction of the enzyme (Nannipieri, 1994).

5.2.3. Protease

Protease in soil plays a significant role in N mineralization (Ladd and Jackson, 1982), an important process regulating the plant growth. Protease catalyze the hydrolysis of proteins to polypeptides and oligopeptides to amino acids, they are found in living cells, dead cells as free enzymes and adsorbed to organic and inorganic pesticides

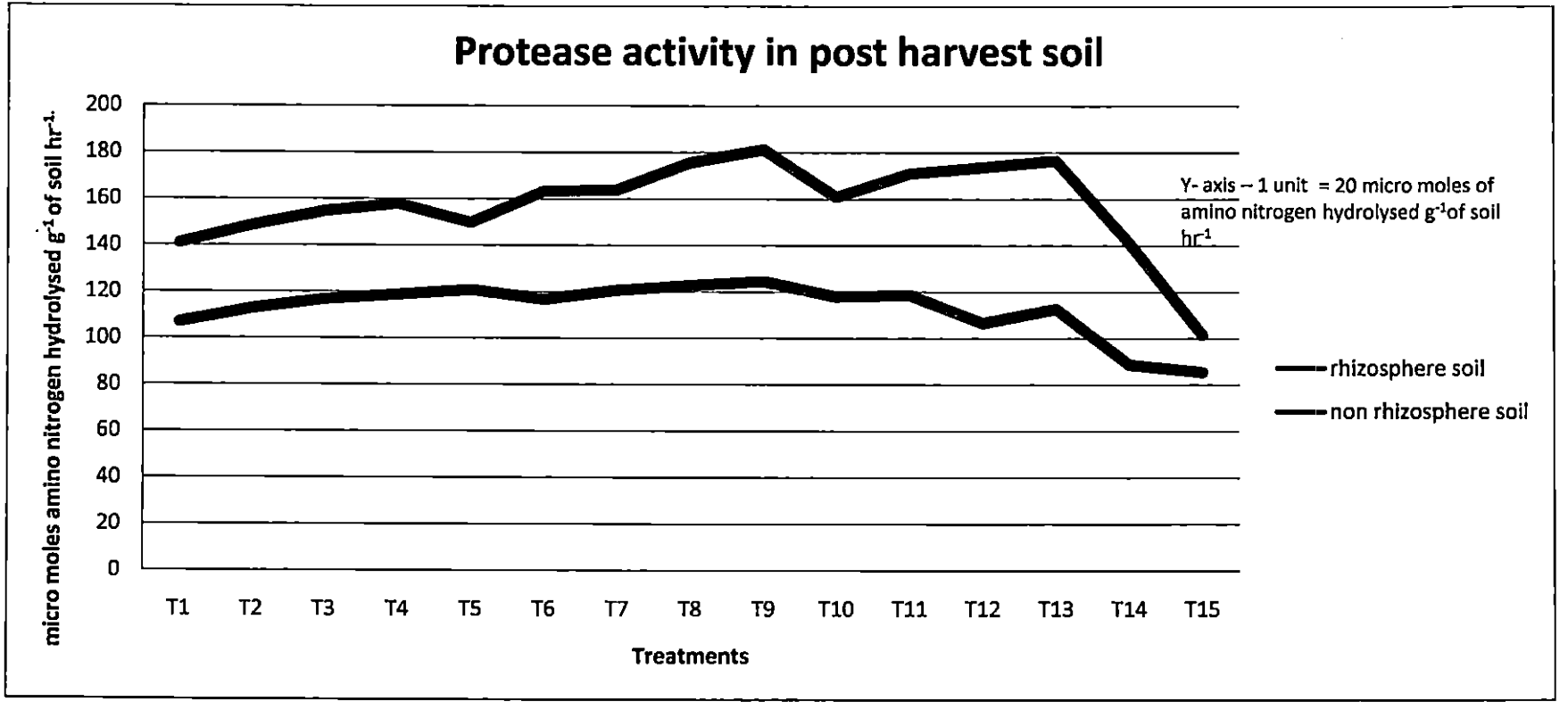

Fig. 5. Protease activity in post harvest soil

5.2.3.1. Rhizosphere soil

The results of protease activity pertaining to the application of 50% PGPR mix-1 enriched vermicompost in combination with inorganics revealed superiority over the treatments in the rhizosphere soil. It is also observed from Table 9, Fig. 5 that the response to the application of vermicompost enriched with various sources of bioinoculants is appreciable. The effect of PGPR mix-1 enriched vermicompost application was most promising in the rhizosphere resulting in an increase in the activity of protease enzyme. This may be attributed to the microbial co-operation in the rhizosphere resulting in the release of C from the organic manures added, thus accelerating the activity of proteolytic enzymes to act upon. Similar increase in activity was reported by Vivas *et al.,* (2003). The low activity in the control plot might be due to the non-availability of C sources for these proteolytic enzymes. The combined application of organic and inorganic nutrient sources might have favoured the multiplication of microflora involved in the synthesis of protease enzymes. This corroborated with the findings of Manero *et al,* (2003).

5.2.3.2. Non-rhizosphere

In the case of protease activity in the non rhizosphere soil, the application of 50% NPK as PGPR mix-1enriched vermicompost in combination with inorganics was proved to be the best treatment. Role of Phosphorous solubilising bacteria is also evident from the study. The beneficial effects of PGPR could be due to the compensating effect on the total protease pool by favouring the multiplication of a group of non specific proteolytic organisms. Similar results were observed by Sato and Omura (1987) in a study on microbial count of paddy soil in relation to enzyme activity. As observed in the rhizosphere soil, the activity was lowest in the absolute control plot where no inputs were added (Table 10, Fig. 5)

5. 2.4. Dehydrogenase

Dehydrogenase exists as an integral part of intact cells, involved in oxidative phosphorylation, and reflects in the total oxidative potential of the soil microbial community (Dick, 1997).

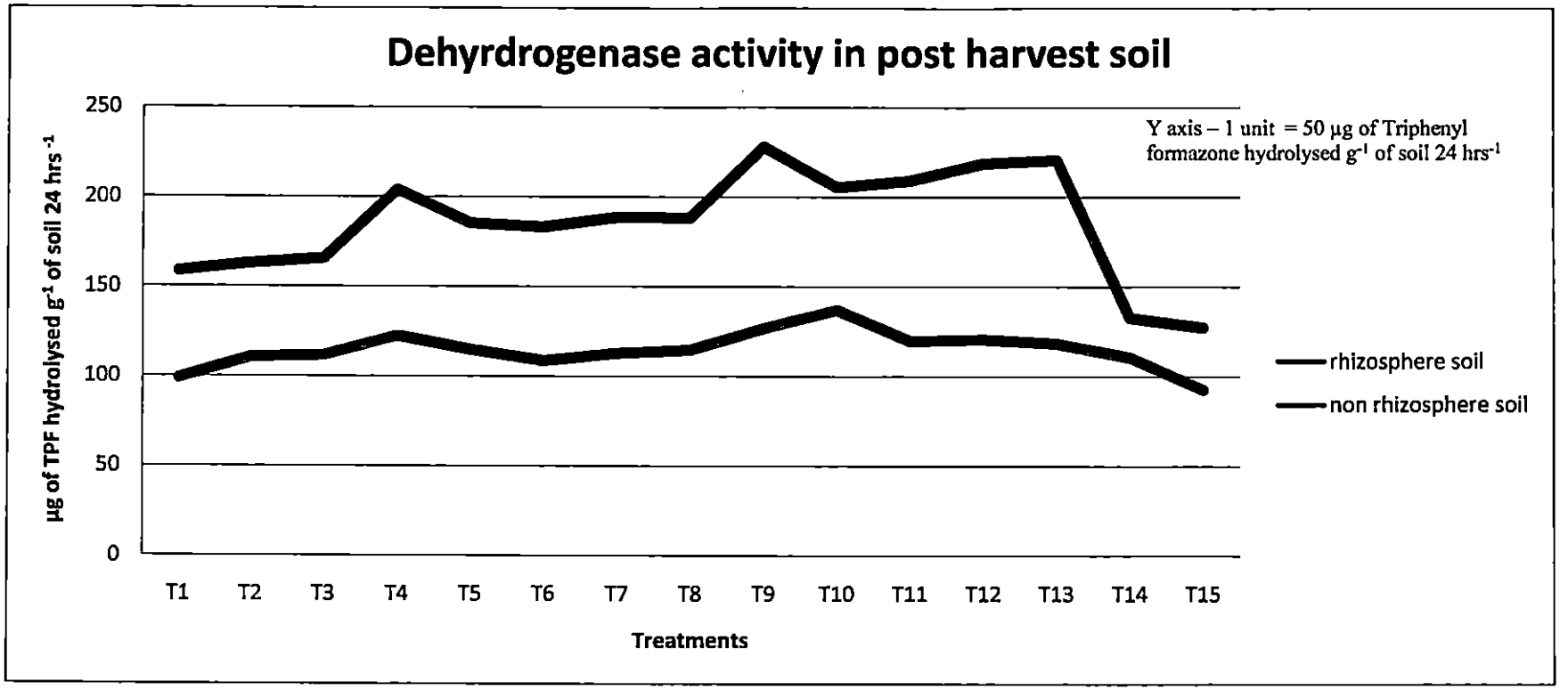

Fig. 6. Dehyrdrogenase activity in post harvest soil

5.2.4.1. Rhizosphere soil

Dehydrogenase activity was found to be the higher in the treatment receiving 50% of NPK as PGPR mix-1enriched vermicompost and 50% NPK as inorganics and on par with the treatments receiving 75% NPK as PGPR mix-1 enriched vermicompost and 25% NPK as inorganics and 75% P as PSB enriched vermicompost and P (25%) & NK as inorganics (Table 9, Fig. 6). This can be attributed that dehydrogenase activity in the soil depends on the content of soluble organic carbon (Zaman *et al.,* 2002, Kizikiya, 2008) and the increased organic matter on the surface soil.

Higher activity of dehydrogenase activity in the rhizosphere soil could be attributed to more availability of organic matter due to higher amount of organically derived carbon, in addition to rhizodeposition. These observations are in close conformity to those obtained by Patel and Varade, 1998. Sources of potential beneficial microbes in the enriched compost might possibly provide microbial diversity and activity of micro organics accompanied by better dehydrogenase activity. These findings corroborated with the findings of Nath *et al.,* (2012).

In fact, the applied organic sources were able to get mineralized rapidly; hence there was more mineralization than immobilization which consequently provided sufficient nutrition for the proliferation of microbes and their activities in terms of soil dehydrogenase (Rai and Yadav, 2011).

5.2.4.2. Non rhizosphere soil

In the non rhizosphere soil dehydrogenase activity was observed to be higher in the treatment receiving 75% N as neem cake enriched vermicompost and N (25%) & PK as inorganics. (Table 10, Fig. 6.) Krishnakumar *et al.* (2005) reported higher dehydrogenase activities were observed with the application of FYM + neem cake.

Increased dehydrogenase activity in microbial enriched vermicompost could be attributed to the increased microbial population in soil because of the greater

availability of organic substrates to act upon as reported by Panwar *et al.* (2003) and Aseri and Rao (2005).

5.2.5. Cellulase

Cellulase catalyses the conversion of insoluble cellulase in to simple water soluble mono or disaccharides, a reaction characteristic of entire cellulolytic flora and consists of 3 distinct classes of hydrolytic enzymes including β- glucosidase.

5.2.5.1. Rhizosphere soil

The application of 50% of NPK as PGPR mix-1enriched vermicompost in combination with inorganics had a significant influence on the activity of cellulase in the rhizosphere soil (Table 9, Fig. 7). Though the chemical fertilizer and organic combination were able to enhance the cellulolytic activity, substantially higher activity was expressed with application of PGPR mix-1 enriched vermicompost alone; this might be due to the phytostimulating nature of PGPR. Similar findings were reported by (Garcia-Gill *et al.*, 2000), who observed increased activity of β-glucosidase due to the application of organic amendments.

Addition of PGPR along with inorganics favored the cellulase activity in the rhizosphere due to increased availability of labile C. Higher mineralization of organic matter in the plots receiving PGPR may provide substrates for cellulolytic flora, there by reporting higher activity of cellulase (Garcia-Gill *et al.*, 2000)

The effect of inorganic fertilizers added to the experimental plots was same as that of the absolute control plot with respect to cellulase activity. This might be due to non-availability of the substrate for the cellulolytic organisms to act upon.

Chemical fertilizers serve as an immediate source of nutrients to the growth of these microorganisms where as in combination with organic manures sustained release of nutrient and substrate availability is assured (Wood *et al.*, 1980).

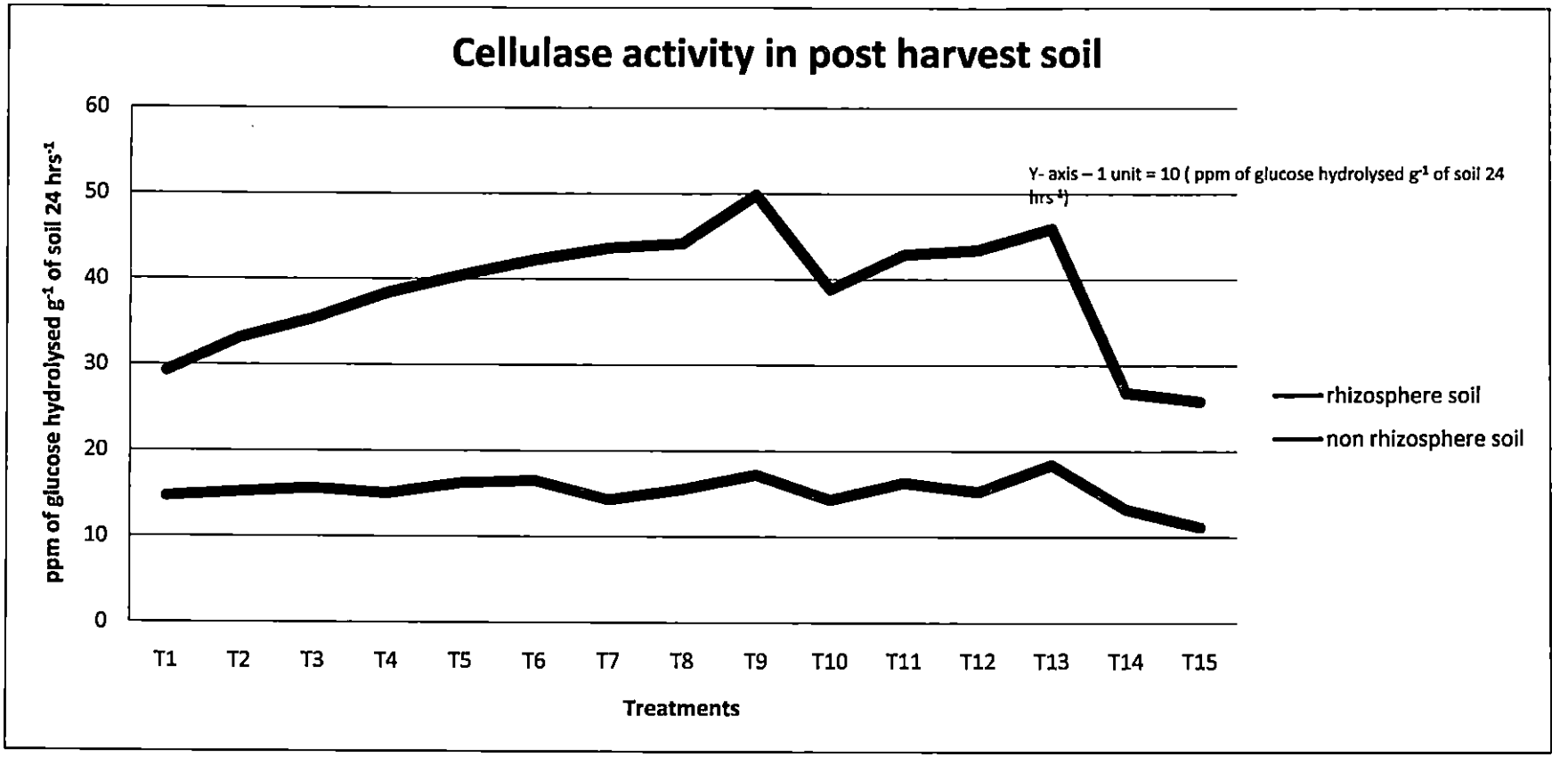

Fig. 7. Cellulase activity in post harvest soil

5.2.5.2. Non rhizosphere soil

The treatments imposed similar effect with respect to cellulase activity except for the absolute control in the non –rhizosphere soil (Table 10, Fig. 7). However higher values were noticed in plots receiving enriched vermicompost using PGPR mix-1, neemcake, PSB and *Azospirillum*. Superiority of these treatments could be attributed to the combined effect of organics and chemical fertilizers.

5.2.6. Soil respiratory activity

Respiratory activity of soil belongs among the most important characteristics of the soil biological activity. Usually measured as CO_2 emissions (in laboratory or in situ), it is a strong indicator of the soil metabolism and ecological soil functions (Santruckova, 1993). It is inferred from the Table 11, treatments receiving 75% of P as PSB enriched vermicompost in combination with inorganics and 25% of N as *Azospirillum* enriched vermicompost in combination with inorganics had registered highest values for soil respiratory activity. It might be due to the fact that organic fertilisation contributes to the soil organic matter accumulation and turnover (Kubát *et al.*, 1999). Raupp and Lockretz (1997) reported that an increased soil organic matter accumulation and turnover enhanced respiration activity in soils.

5.2.7. Calculation of enzyme kinetics (V_{max} and K_m) at fortnightly intervals

Michaelis–Menten kinetic parameters can be used to differentiate between enzyme sources. From the Table 12, it can be inferred that there exists a positive relationship between the V_{max} and K_m values for the five enzymes. It is also inferred that kinetic parameters Km and V_{max} can be readily derived from the rate of catalysis measured at varying substrate concentration. Highest V_{max} values were observed for the enzymes urease (6^{th} week), phosphatase (6^{th} week), protease (8^{th} week), dehydrogenase (2^{nd} week) and cellulase (6^{th} week) might be due to result of higher microbial activity resulting in higher enzyme activities at specific periods. These corroborated with the findings of Nannipieri and Gianfreda (1988). Since V_{max} is proportional to enzyme concentration the data suggest that the soils

may contain different amount of free and bound enzymes. V_{max} is thus always indicative of total activity of the enzyme which is evident from Table 12.

K_m values are independent of enzyme concentration and kinetically reflect apparent affinity of the enzyme for the substrate. Thus it is clear from the study that the affinity of urease for substrates was maximum at eighth week, phosphatase at sixth week, protease at eighth week, dehydrogenase at second week and cellulase at sixth week. The smaller the K_m greater the substrate affinity as proposed by Zaman *et al.*, 1999.

5.2.8. Comparison of enzyme activities between rhizosphere and non rhizosphere soils

A comparison of enzyme and microbial activities under integrated plant nutrient system between rhizosphere and non rhizosphere was attempted in this study. It is understood that rhizosphere, the soil adjacent to plant roots is significantly different from bulk soil in chemical, biological and microbiological properties. Activities of enzymes urease, phosphatase, protease, dehydrogenase and cellulase were found to be higher in rhizosphere soil. Similar results were reported by Gregory and Hinisinger, (1999)

The rhizosphere is the zone of soil influenced by roots through the release of substrates that affect microbial diversity and activity. These microbes are responsible for key environmental processes, such as biogeochemical cycling of nutrients and matter and the maintenance of plant health and soil quality (Barea *et al,* 2004). In particular, the varied genetic and functional activities of the extensive microbial populations have a critical impact on soil functions, based on the fact that microorganisms are driving forces for fundamental metabolic processes involving specific enzyme activities (Nannipieri *et al.,* 2003). These factors have contributed to the increased enzyme activities in rhizosphere.

5.2.9. Computation of Biological Fertility Index through Enzyme Activity Number (EAN)

Soil enzymes have been suggested as potential indicators of soil quality because of their essential role in soil biology. Ease of measurement and rapid response to changes in soil management (Dick *et al.,* 1994). Enzyme activity number is an index of biological fertility. Treatment T_9 with the application of NPK 50% as PGPR mix- 1 enriched vermicompost in combination with inorganics reported the highest value for enzyme activity number indicating the effect of the treatment in sustaining the soil biological health. Similar results were reported by Riffaldi *et al.* (2002) who reported higher enzyme activity number in untilled management system than the tilled management system.

5.2.10. Comparison of micro flora between rhizosphere and non rhizosphere soils.

Soil microbial populations are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural systems and natural ecosystems.

In the rhizosphere soil, the significant effect of treatments on soil bacteria was observed (Fig. 8). It is quite interesting to note that treatment involving the application of 50% of P as Phosphate solubilizing bacteria enriched vermicompost along with inorganics was similar in effect with 50% of NPK as PGPR mix-1 enriched vermicompost in combination with inorganics. This might be due to the positive co-operation between PGPR and PSB thus contributing to higher activity of bacteria. In the non rhizosphere soil, application of bio inoculants has significantly increased the bacterial count.

The rhizosphere, the zone of soil under the influence of root is characterized by high microbial diversity, activity, number of organisms and complex interactions and root (Oger *et al.*, 2004). The population and functions of microorganisms

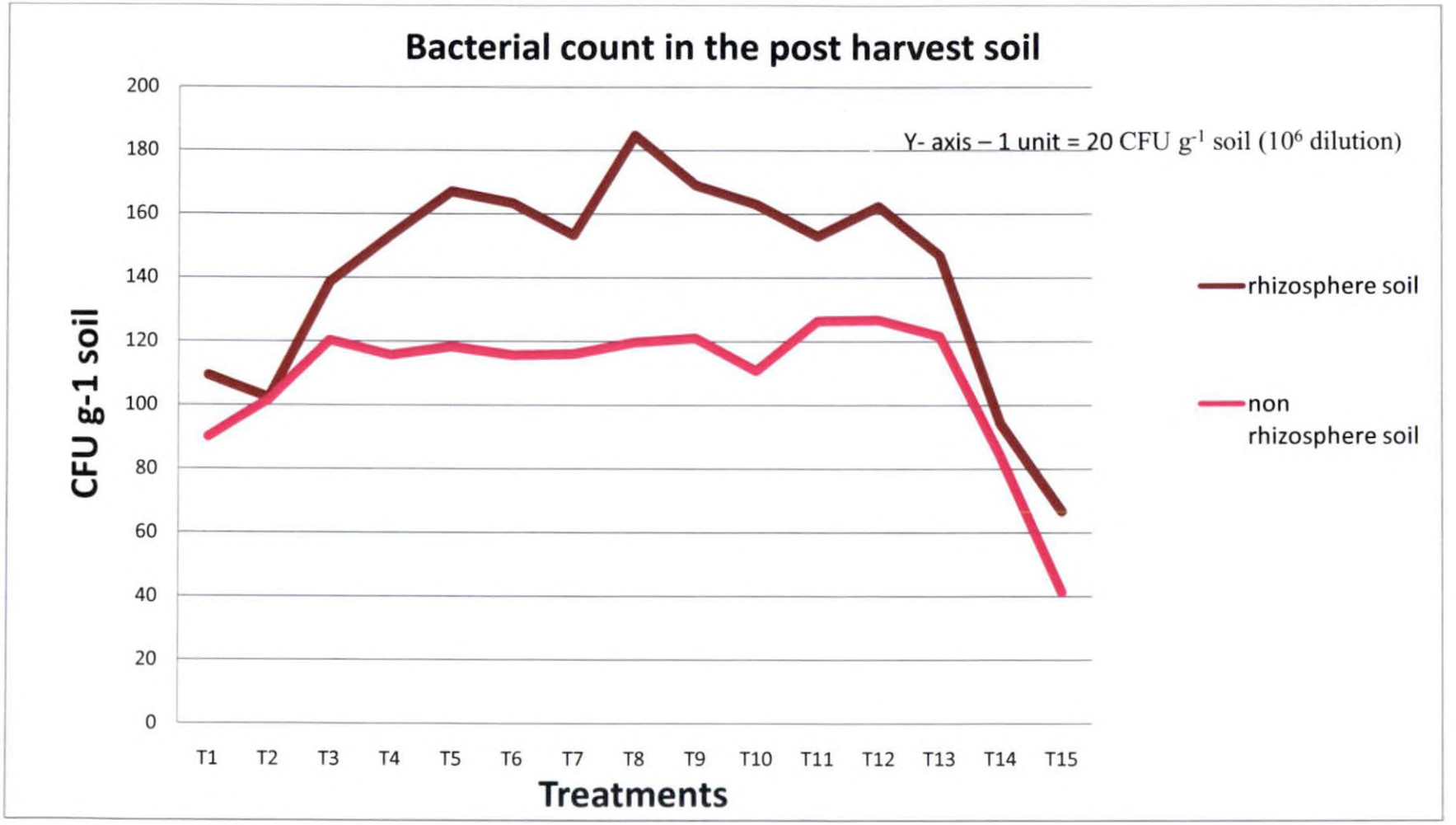

Fig. 8. Bacterial count in the post harvest soil

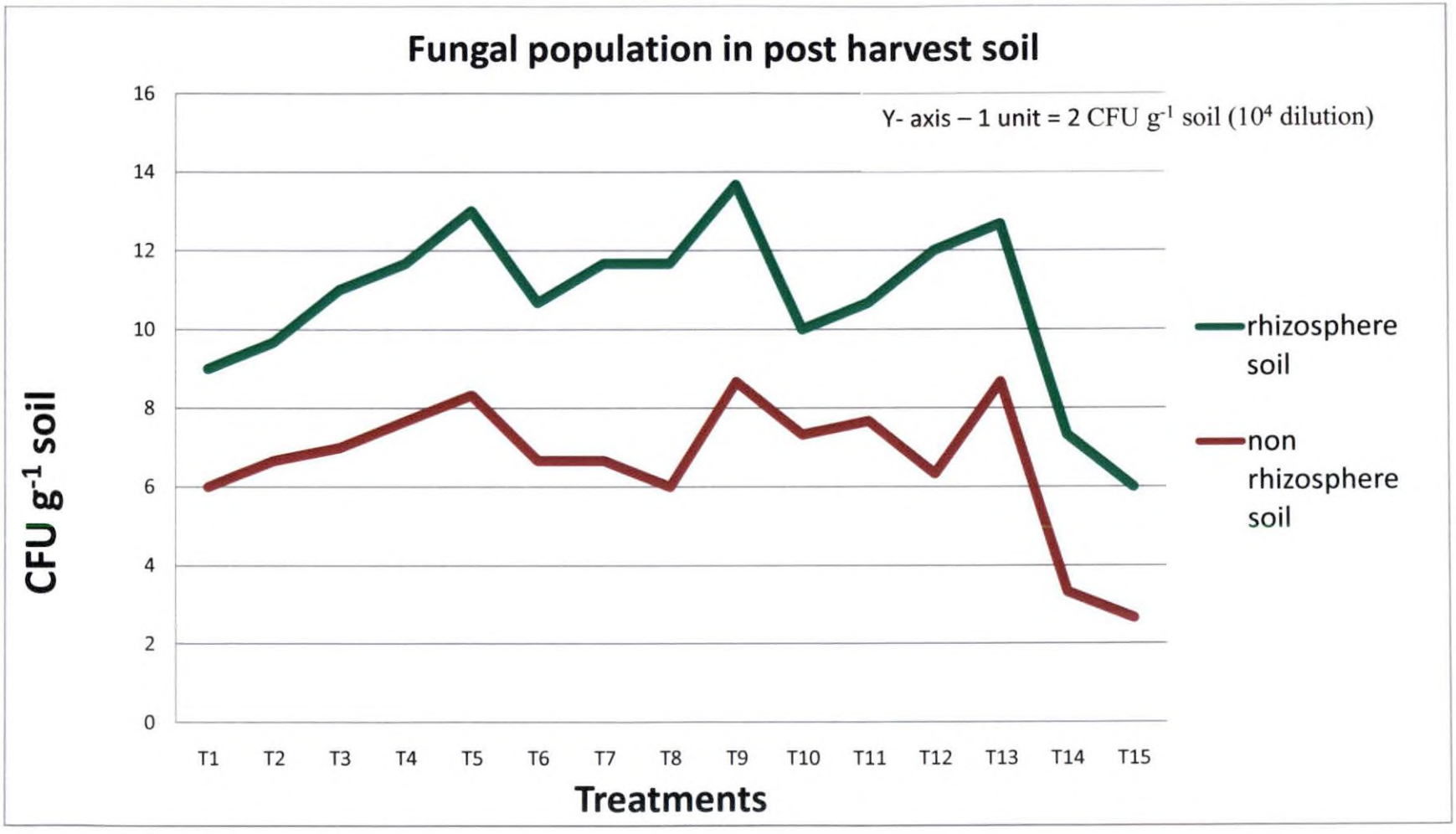

Fig. 9. Fungal population in post harvest soil

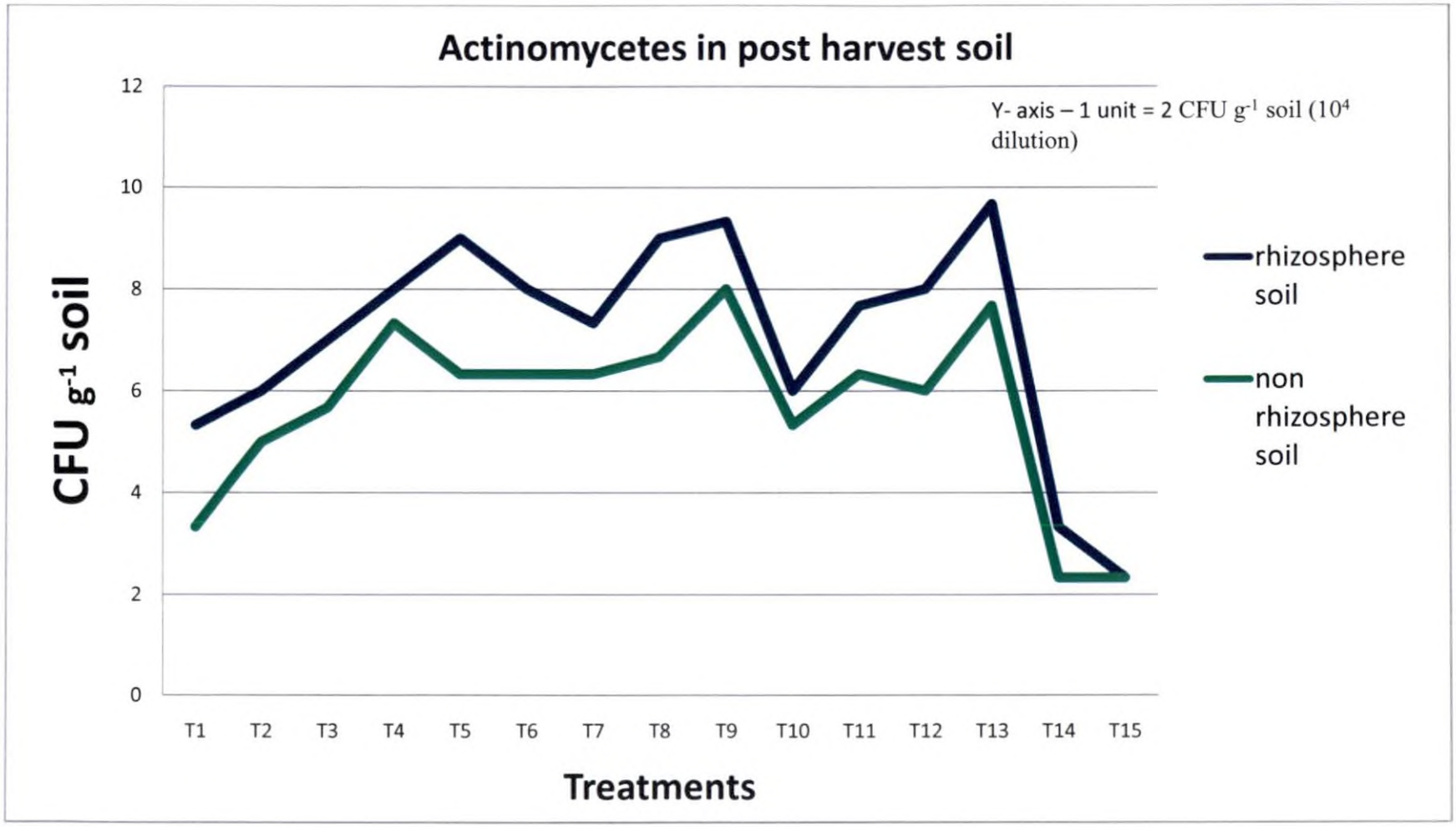

Fig. 10. Actinomycetes in post harvest soil

cannot be overlooked while considering soil health because microorganisms provide living environment to the soil and perform various functions.

In the rhizosphere soil, fungi population was higher in bio inoculants treated soils (Fig. 9). It has been recently postulated that an additional mechanism for plant growth promotion by PGPR could be their altering of microbial rhizosphere communities (Ramos *et al.* 2003).

It is inferred from the Fig. 10 that the actinomycetes population had varied significantly with treatments in both rhizosphere and non-rhizosphere soils. Application of PGPR mix-1enriched vermicompost had increased the actinomycetes population in both the soils.

From the study it is inferred that certain bacteria, fungi and actinomycetes are able to colonize the root soil environment where they carry out a variety of interactive activities known to benefit plant growth and health and soil quality. Under integrated plant nutrient system, it is clearly evident that the microflora was more in rhizosphere than in the non rhizosphere. This might be due to the differing physical, chemical and biological properties of root associated soil, bringing about a drastic change in the microbial diversity in rhizosphere environment. Similar results were reported by Toal *et al.,* (2000).

The higher activity of microflora as a whole in the rhizosphere soil (Table 14) over the non-rhizosphere soil (Table 15) might be attributed to the microbial colonization in the rhizosphere known as root colonization. Since the rhizosphere is considered as the most intense ecological habitat in soil, it is of interest to study the effects that PGPR may have on total microbial activity and bacterial population where rhizobacteria exerted a direct influence on plants.

5.3. Biometric observations

5.3.1. Plant height - at first harvest

It is inferred from the Table 16 that plant height varied significantly with the application of treatments. The application of 50% of NPK as PGPR mix-1 enriched vermicompost in combination with inorganics and application of 50% of

N as *Azospirillum* enriched vermicompost in combination with inorganics had a positive influence on plant height and their effects were found to be similar.

This might due to the fact that plant growth promoting rhizobacteria influence plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens as reported by Burd *et al.*, (2000).

5.3.2. Internodal length at final harvest

Application of treatments imposed significant effect in the internodal length (Table 16). Treatments receiving POP recommendation and 50% of NPK as PGPR mix-1enriched vermicompost in combination with inorganics registered the highest value for internodal length. Availability of nutrients, enhanced microbial population in the soil by plant growth promoting rhizobacteria might have contributed to increased internodal length.

Ibiene *et al.*, (2012) also reported that combination of PGPR including *Azotobacter sp.*, *Nitrobacter* sp., and *Nitrosomonas* sp. had significantly increased plant length, root length and internodal length in *Lycopersicum esculentus*

5.3.3. No of branches - at final flowering

Number of branches of the plant did not vary significantly with treatments (Table 16). The treatments had similar effects on the no. of branches. The plants inoculated with PGPR, showed an increase of plant height by 48.11 per cent, number of leaves by 81.22 per cent, number of branches by 55.50 per cent, biomass by 62.74 per cent and fruit yield by 97.05 per cent when compared to the control (Kirankumar, 2007).

5.3.4. Flowering stages - days to 50 % flowering

Application of 50% P as PSB enriched vermicompost in combination with inorganics resulted in the advancement of flowering in the test crop (Table 16).

The earliness of flowering may be attributed to the presence of bio-fertilizers especially inoculation with PSB which consequently lead to flower initiation and more flowering duration. This may be ascribed to easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinins to the auxiliary buds resulting in breakage of apical dominance, better sink for faster mobilization of photosynthates and early transformation of plant parts from vegetative to reproductive phase. These results are in the line with the findings of Verma (2010).

5.3.5. Fruit

5.3.5.1. No of fruits/ plant

Treatments had significantly influenced the number of fruits of bhindi plants (Table 17). Application of 75% of NPK as PGPR mix-1enriched vermicompost in combination with inorganics had registered the highest value. Availability of major, minor and secondary nutrients in enriched vermicompost might have enhanced the growth of reproductive parts. These highlight the findings of Earnapalli, (2005), who reported higher fruit yield with the application of PGPR in tomato.

5.3.5.2. Yield per plant

It is inferred from the Table 17, that yield per plant varied significantly with the application of treatments. Application of 75% of NPK as PGPR mix-1enriched vermicompost in combination with inorganics had registered higher yield per plant when compared with other treatments. These findings highlights the reports of Orhan *et al.* (2006) who reported that two plant growth promoting rhizobacteria i.e. *Bacillus* strains OSU-142 (N_2-fixing) and M3 (N_2-fixing and phosphate solubilizing) alone or in combination have the potential to increase the yield, growth and nutrition of vegetable crops under organic growing conditions.

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5.3.5.3. Yield per ha

Imposition of treatments had significantly influenced the yield per plant. (Table 17) Highest values were obtained for 75% of NPK as PGPR mix- 1enriched vermicompost in combination with inorganics. Raj *et al.*, (2003) reported that PGPR formulations showed a significant enhancement of growth and reproductive parameters such as height, fresh and dry weight, leaf area, number of tillers under greenhouse conditions and number of earheads, length and girth of earheads, and 1000 seed weight and yield of pearl millet under field conditions.

5.3.6. . Scoring of diseases

Scoring of yellow vein mosaic disease in Bhindi showed that absolute control plot had registered the highest value. The disease suppressing nature of bioinoculants had resulted in lower values in biofertilizers treated plots. Lowest value was recorded by treatment receiving 50% of N as neemcake enriched vermicompost in combination with inorganics. Ratings performed during the current studies to assess the incidence of naturally occurring foliar pathogens such as yellow vein mosaic virus led to the conclusion that the application of bio inoculants had a significant effect. This might be due to the systemic resistance by the production of antibiotics, hydrogen cyanide and siderophores by the inoculants. Similar reduction in the incidence of cucumber mosaic virus in tomato due to the application of PGPR was reported by Kokalis-burelle *et al.* (2002)

5.3.7. Shelf life (keeping quality)

Keeping quality of the okra fruits has increased with the conjunctive use of fertilizers and organics (Table 18). Padmavathiamma *et al.* (2008) reported that the quality of produce, as judged by total, reducing, non reducing sugars and shelf life of banana, was high in vermicompost treated plots. This might be due to the balanced application of nutrients which helped to retard oxidation processes responsible for increased shelf life of Bhindi fruits.

5.3.8. B: C ratio

Benefit cost ratio has recorded the highest value with the application of 50% of NPK as PGPR mix-1 enriched vermicompost in combination with inorganics (Table 18) and this could be attributed to greater availability and uptake of nutrients leading to enhanced yield of crop. These results are in accordance with those of Singh and Singh (2012) who reported a benefit: cost ratio of 2.87 with the inoculation of PGPR in pigeon pea.

Summary

6. SUMMARY

The study entitled "Effect of integrated plant nutrient system (IPNS) on the soil biological regimes in red loam soil " was carried out at College of Agriculture, Vellayani during March 2012 – June 2012 to assess the conjugal effect of manures and chemical fertilizers on dynamics of major agriculturally significant soil enzymes, available nutrient status of the soil, its relation with the activities of major soil enzymes, soil microflora, yield and yield attributes of the test crop and computation of Biological Fertility Index through Enzyme Activity Number.

Enrichment of vermicompost was carried out using *Azospirillum*, PSB and PGPR mix-1 at the rate of 2% and neemcake at a rate of 5%. Bhindi, var. Varsha uphar was used as the test crop.

The field experiment was laid out in RBD with 15 treatments and three replications and the treatment details are as follows.

T_1- Package of practice recommendation (KAU), T_2 - N (25 %) as neem cake enriched vermicompost + N (75%), P & K, T_3 - N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K, T_4 - P (25 %) as PSB enriched vermicompost + P (75%), N & K, T_5- NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%), T_6-N (50%) as Neem cake enriched vermicompost+ N (50%), P & K, T_7- N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K, T_8- P (50 %) as PSB enriched vermicompost + P (50%), N & K, T_9 - NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%), T_{10}- N (75 %) as Neem cake enriched vermicompost + N (25%),P & K, T_{11}- N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K , T_{12}- P (75%) as PSB enriched vermicompost + P (25%), N & K, T_{13}-, N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25%), T_{14} - N, P, K alone as inorganics, T_{15} - Absolute control.

The salient results emerged from the study are summarized below.

- Different treatments significantly influenced the pH and EC of the post harvest soil. The highest mean value for pH was recorded by T_{15} (Absolute

control) while the maximum value of EC was recorded by the treatment T_8 (P (50 %) as PSB enriched vermicompost + P (50%), N & K).

- Treatment T_3 recorded the maximum value for organic carbon in the post harvest soil.
- Analysis of post harvest soil for major nutrients revealed that available N, P and K increased significantly after the experiment. Availability of N was highest in the treatment T_{11} (N (75%) as *Azospirillum* enriched vermicompost + N (25%), P & K). In the case of available P, treatment T_{12} (P (75%) as PSB enriched vermicompost + P (25%), N & K) recorded the highest value. For available K, treatment T_{13} (N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %) recorded the highest value.
- In the case of micronutrient contents in the post harvest soil, Fe, Cu and B were highest in treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%)). Highest Mn content was recorded by T_2 (N (25 %) as neem cake enriched vermicompost + N (75%), P & K). Availability of Zn was highest in the treatment T_{10} (N (75 %) as Neem cake enriched vermicompost + N (25%), P & K).
- Urease activity was highest with the application of treatment T_{11} (N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K) in the rhizosphere soil. In the case of urease activity in the non-rhizosphere soil T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) registered the highest value.
- With respect to phosphatase activity, treatment T_8 (P (50 %) as PSB enriched vermicompost + P (50%), N & K) recorded the highest value in the rhizosphere soil. In the non- rhizosphere soil, the highest activity was recorded by the treatment T_{10} (N (75 %) as Neem cake enriched vermicompost + N (25%), P & K).
- For protease activity, the highest value was recorded by treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%)) both in rhizosphere and non rhizosphere soils.

- In the case of dehydrogenase activity, treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%)) recorded the highest value in the rhizosphere soil. For the non- rhizosphere soil, treatment T_{10} (N (75 %) as Neem cake enriched vermicompost + N (25%), P & K) registered the highest value for dehydrogenase activity.
- Treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) recorded the highest value for cellulase activity in the rhizosphere soil. In the non- rhizosphere soil treatment T_{13} (N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)) recorded the highest value .
- For soil respiratory activity, treatment T_{12} (P (75%) as PSB enriched vermicompost + P (25%), N & K) registered the highest value.
- V_{max} and K_m for each enzyme at fortnightly intervals were recorded after the imposition of each treatment. Highest V_{max} values for urease, phosphatase, protease, dehydrogenase and cellulase were recorded at 6^{th} week, 6^{th} week, 8^{th} week, 2^{nd} week and 6^{th} week respectively. K_m values for urease was highest at eighth week, phosphatase at sixth week, protease at eighth week, dehydrogenase at second week and cellulase at sixth week.
- Microbial count was calculated using serial dilution technique. For bacteria in the rhizosphere soil, T_8 (P (50 %) as PSB enriched vermicompost + P (50%), N & K) recorded the maximum bacterial count while in the non- rhizosphere soil, highest value of bacterial colonies was recorded by T_{12} (P (75%) as PSB enriched vermicompost + P (25%), N & K). Treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) recorded maximum number of fungal colonies in rhizosphere soil and actinomycetes in the non rhizosphere soil. In the non rhizosphere soil, maximum number of fungal colonies was observed with the application of T_9 and T_{13}. Treatment T_{13} (N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)recorded the highest values for actinomycetes in the rhizosphere soil.

- Enzyme Activity Number was highest for the treatment T_9 with the application of NPK 50% as PGPR mix- 1 enriched vermicompost in combination with inorganics indicating the effect of the treatment in sustaining the soil biological health.
- Biometric characters like plant height, internodal length, flowering stages- 50% flowering were significantly influenced by the imposition of treatments. Tallest plant with maximum number of fruits was found in treatment T_9 (NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%).With respect to internodal length T_1 (Package of practice recommendation (KAU)) recorded the highest value. Different treatments did not significantly affect number of branches at final flowering.
- Treatment T_8 (P (50%) as PSB enriched vermicompost + P (50%), N & K,) and T_{12} (P (75%) as PSB enriched vermicompost + P (25%), N & K,) reported advancement of 6 days in first flowering by half of the plant population.
- Yield characters like yield per plant and yield per hectare were significantly influenced by the treatments and the highest values were recorded by T_{13} (N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25 %)). Treatment T_{13} produced 52% increase in yield per hectare over the absolute control plot T_{15}.
- With respect to Yellow Vein Mosaic disease, treatment T_6 recorded the lowest incidence percentage. Highest disease incidence was observed with the treatment T_{15} (Absolute control).
- For keeping quality, highest value was recorded by T_5 (NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%)).
- Highest value for B: C ratio was recorded by the treatment T_9 (NPK (50%), PGPR mix-1 enriched vermicompost + N, P & K (50%))

CONCLUSION

This study identified the treatment T_9 i.e. application NPK as 50% PGPR mix-1 enriched vermicompost in combination with inorganics as the best

treatment for the cultivation of Bhindi var. Varsha uphar. Treatment T_9 increased available micro nutrient status (Fe, Cu, B), biological properties of soil viz., dehydrogenase, cellulase and protease activities; and biometric characters of bhindi i.e., plant height and number of fruits. A highest value of Enzyme Activity Number, which is an index of biological activity, was also registered by the treatment T_9, revealing its superiority over other treatments. The treatment has recorded highest value for B: C ratio. T_9 found to be the best treatment both in sustaining soil biological activity and economic returns.

FUTURE LINE OF WORK

From the investigation it can be confirmed that the treatment T_9 (50% of NPK as PGPR mix-1 enriched vermicompost + N, P & K (50%) as inorganics) which gave the highest B: C ratio could be taken as the best treatment as far as the sustainable production is concerned. On Farm Trial (OFT) and Multi Location Trials (MLT) are to be carried out at farmers' fields for confirming the results. Future lines of work can be focused on the development of technology of enriched composts fortified with major, secondary and micronutrients in a balanced rate to maximize the crop yield and to sustain the productivity of the soil. From the present study, isolation of compost DNA can also be attempted.

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EFFECT OF INTEGRATED PLANT NUTRIENT SYSYTEM (IPNS) ON THE SOIL BIOLOGICAL REGIMES IN RED LOAM SOIL

by

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Abstract of the thesis

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ABSTRACT

The research entitled "Effect of integrated plant nutrient system (IPNS) on the soil biological regimes in red loam soil" was a study undertaken in the Dept. of Soil Science and Agrl. Chemistry during the period from March 2012 to June 2012. The objective of the study was to assess the conjugal effect of manures and chemical fertilizers on dynamics of major agriculturally significant soil enzymes, available nutrient status of the soil, its relation with the activities of major soil enzymes, soil microflora, yield and yield attributes of the test crop and computation of Biological Fertility Index through Enzyme Activity Number.

Field experiment using bhindi as test crop consisted of 15 treatments. The treatments were laid out in RBD with three replications. The treatments were T_1- Package of practice recommendation (KAU), T_2 - N (25 %) as neem cake enriched vermicompost + N (75%), P & K, T_3 - N (25 %) as *Azospirillum* enriched vermicompost + N (75%), P & K, T_4 - P (25 %) as PSB enriched vermicompost + P (75%), N & K, T_5- NPK (25 %) as PGPR mix-1 enriched vermicompost + N, P & K (75%), T_6- N (50%) as Neem cake enriched vermicompost+ N (50%), P & K, T_7- N (50%) as *Azospirillum* enriched vermicompost + N (50%), P & K, T_8- P (50 %) as PSB enriched vermicompost + P (50%), N & K, T_9 - NPK (50 %) as PGPR mix-1 enriched vermicompost + N, P & K (50%), T_{10}- N (75 %) as Neem cake enriched vermicompost + N (25%),P & K, T_{11}- N (75 %) as *Azospirillum* enriched vermicompost + N (25%), P & K , T_{12}- P (75%) as PSB enriched vermicompost + P (25%), N & K, T_{13}-,N, P, K, (75 %) as PGPR mix-1 enriched vermicompost + N, P & K (25%), T_{14}- N, P, K alone as inorganics, T_{15}- Absolute control

Results revealed that maximum available N content and urease activity in rhizosphere soil were recorded with the treatment T_{11}. Treatment T_9 increased available micro nutrient status (Fe, Cu, B), biological properties of soil viz., dehydrogenase, cellulase and protease activities; and biometric characters of bhindi i.e., plant height and no. of fruits. Results showed significant variations in electrical conductivity, phosphatase activity and bacterial count and advancement of flowering days with the application of treatment T_8. The highest available K, actinomycetes and yield attributes including yield per ha and yield per plant was noticed with the application of treatment T_{13}.

V_{max} and K_m for each enzyme at fortnightly intervals were recorded after the imposition of each treatment. Highest V_{max} values for urease, phosphatase, protease, dehydrogenase and cellulase were recorded at 6^{th} week, 6^{th} week, 8^{th} week, 2^{nd} week and 6^{th} week respectively. K_m values for urease was highest at eighth week, phosphatase at sixth week, protease at eighth week, dehydrogenase at second week and cellulase at sixth week. Comparison of biological properties such as enzyme activities and microflora between rhizosphere and non-rhizosphere soils had shown variation highlighting the rhizosphere effect in the test crop. A highest value of Enzyme Activity Number, which is an index of biological fertility, was registered by the treatment T_9, revealing its superiority over other treatments.

Conclusion

Treatment T_9 with the application of NPK (50 %), PGPR mix-1 enriched vermicompost + N, P & K (50%) was found to be the best treatment both in sustaining soil biological fertility and economic returns. The treatment has recorded highest values for Enzyme activity number as well as for B: C ratio.

Appendices

APPENDIX I

Weather Parameters during field experiment (March 2012- June 2012)

Standard weeks	Maximum temperature (°C)	Minimum temperature (°C)	Maximum Relative Humidity (%)	Minimum Relative Humidity (%)	Rainfall(mm)
9	31.5	23.2	94.3	62.1	0
10	31.2	24.7	88.6	66.3	4.5
11	31.4	21.0	98.3	69.1	4.0
12	32.2	24.0	93.7	63.6	0
13	32.2	23.6	93.4	61.6	4.5
14	32.6	24.7	89.9	64.9	1.5
15	32.7	24.7	92.6	65.9	5.5
16	33.0	25.9	85.3	68.0	4.5
17	30.1	25.0	92.3	79.9	15.6
`18	30.6	25.4	92.9	73.6	6.3
19	31.0	25.5	88.1	72.7	8.0
20	31.5	26.1	91.4	74.3	22.0
21	31.5	25.8	91.7	72.1	0
22	31.5	26.1	90.0	70.6	1.0
23	31.3	24.7	91.4	71.1	3.6
24	30.4	23.9	93.6	72.4	7.0
25	29.4	24.3	94.4	77.0	3.5.0
26	29.8	23.8	87.0	74.0	6.0

APPENDIX II

Composition of media for microbial enumeration

1. Enumeration of Bacteria

Media: Nutrient Agar

Composition

1. Peptone - 5gm
2. NaCl - 5gm
3. Beef extract - 3 gm
4. Agar - 20 gm
5. pH - 7.0
6. Distilled water - 1000 ml

2. Enumeration of Fungi

Media: Rose Bengal agar

Composition

1. Glucose - 3gm
2. $MgSO_4$ - 0.2 gm
3. K_2HPO_4 - 0.9 gm
4. Rose Bengal - 0.5 gm
5. Streptomycin - 0.25 gm
6. Agar - 20 gm
7. Distilled water - 1000 ml

3. Enumeration of Actinomycetes

Media: Kenknight's Agar

Composition

1. Dextrose - 1gm
2. KH_2PO_4 - 0.1 gm
3. $NaNO_3$ - 0.1 gm
4. KCl - 0.1 gm
5. $MgSO_4$ - 0.1 gm
6. Agar - 15gm
7. Distilled water - 1000 ml