

**THE EFFECT OF ORGANIC FERTILIZERS ON SOIL
QUALITY, NUTRIENT AVAILABILITY AND QUALITY OF
BLACK PEPPER**

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IN CHEMISTRY

By

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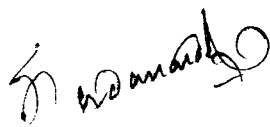
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CERTIFICATE

I hereby certify that the thesis entitled **“The Effect of Organic Fertilizers on Soil Quality, Nutrient Availability and Quality of Black Pepper”** submitted to the University of Calicut by Mrs. Rubina M R in partial fulfillment for the award of the degree of Doctor of Philosophy in Chemistry is a bonafide record of the research work carried out by her at Indian Institute of Spices Research, Calicut, Kerala, under my guidance. No part of the work has formed the basis for the award of any other degree or diploma previously. All sources of help received by her during the course of this investigation have been duly acknowledged.

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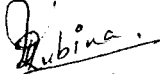

A K SADANANDAN

DECLARATION

I, Rubina M R hereby declare that the thesis entitled “**The Effect of Organic Fertilizers on Soil Quality, Nutrient Availability and Quality of Black Pepper**”, submitted by me for the award of the degree of **Doctor of Philosophy** in Chemistry to the University of Calicut is an authentic record of the research work carried out by me at the Indian Institute of Spices Research, Calicut, Kerala, under the guidance of Dr. A K Sadanandan, ICAR Emeritus Scientist and Rtd. Principal Scientist & Head, Division of Crop Production and Post Harvest Technology, Indian Institute of Spices Research, Calicut. This thesis or part of it has not been submitted to any university for the award of any degree or diploma.

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RUBINA M R

LIST OF ABBREVIATIONS

Abbreviation	Expansion
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
Fe	Iron
Mn	Manganese
Zn	Zinc
Cu	Copper
CaCl ₂	Calcium Chloride
DTPA	Diethylene Triamine Penta Acetic Acid
OM	Organic Matter
M	Mean of with and without organic spray
N	Normal
Ck	Check
FYM	Farm Yard Manure
VC	Vermicompost
LC	Leaf compost
NC	Neem Cake
APA	Acid Phosphatase Activity
DHA	Dehydrogenase Activity
MUB	Modified Universal Buffer
CC	Coir Compost
W	With organic spray
WO	Without organic spray
HA	Humic Acid
FA	Fulvic Acid
OC	Organic Carbon
B/C	Benefit cost ratio
EDTA	Ethylene Diamine Tetra Acetic Acid
NaHCO ₃	Sodium bicarbonate

TPF	TriphenylFormazan
TTC	TriphenylTetrazoliumchloride
MOP	Muriate of Potash
CEC	Cation Exchange Capacity
Fe ₂ O ₃	Iron oxide
Al ₂ O ₃	Aluminium Oxide
AAS	Atomic Absorption Spectrophotometer
E ₄	Extinction Coefficient at 465nm
E ₆	Extinction Coefficient at 665nm
MBC	Microbial Biomass Carbon
BD	Bulk Density
WHC	Water Holding Capacity
HC	Hydraulic Conductivity
h	Hour
min	Minute
g	gram
mg	milligram
kg	kilo gram
L	Litre
SE	Standard Error of Mean
CD	Critical Difference at 0.05 level
r	Coefficient of simple correlation
R ²	Regression Coefficient
*	Significant at 0.05 level
**	Significant at 0.01 level
NS	Non Significant

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INTRODUCTION

Rubina M R “The effect of organic fertilizers on soil quality, nutrient availability and quality of black pepper” Thesis. Department of Chemistry, University of Calicut, 2005

INTRODUCTION

Chapter 1

INTRODUCTION

The role of organic fertilizers on the availability of plant nutrients in soil is well known from the dawn of modern agriculture. However, the mechanism of nutrient availability, brought about by the organic matter and the uptake of nutrients resulting in bumper yield of many crops besides alleviating the deficiencies and nutrient disorders are not well understood. With the advance of civilization, the need to fertilize the land became evident and resulted in the application of fertilizers providing N, P and K needed for the plant. Later on it was realized that mere N, P and K were not enough and that the addition of organic fertilizers are vital for the plant growth, yield and quality of the produce.

The present academic study aims at investigating the supremacy of organic fertilizers over inorganic and the superiority of particular organic manure in rendering nutrients available, from a variety of sources of organic fertilizers taken up for investigations in different typical black pepper growing soils.

World wide there has been an increasing awareness about using the natural resources in a renewable fashion so that the productivity of the land can be sustained for the generation to come. In recent years, there has been a growing demand in western countries for organically produced agricultural produces. The increasing concerns about the use of environmentally hostile agrochemicals and the spurt in prices of fertilizers in India have provided the much needed push for organic agriculture.

Organic fertilizers are materials derived from animal, human and plant residues which contain nutrients in complex organic forms. They are the by-products from the processing of animal or vegetable substances that contain

sufficient plant nutrients to be of value as fertilizers. Addition of high organic matter content to the soil through organic manures supports the growth and multiplication of beneficial soil microorganisms like Nitrogen fixers, phosphate solubilizers, phosphate mobilizers and plant growth promoting rhizobacteria.

These soil microorganisms survive with organic matter that they break down first into humus, then humic acid and ultimately into basic elements required by plants. Many of them including Rhizobia, Azotobacter, Azospirillum fix atmospheric nitrogen and many of them convert soil minerals into forms available to plants e.g. phosphate solubilizing bacteria convert fixed phosphates into available form. Bio-organic manures and composts can add life to soil, which has become lifeless with the indiscriminate use of inorganic fertilizers (Shroff and Pawar 1998). So healthy soil includes bacteria, fungi, actinomycetes, algae, protozoa, nematodes etc.

The soil organic matter is one of the most complex materials existing in nature and contains naturally occurring organic compounds of plant and animal origin. Humus represents the heterogeneous complex of numerous compounds of plant, animal and microbial origin and their decomposition products. This has been divided into two groups, non-humic substances and humic substances (Kononova 1966). Non-humic substances include various nitrogenous and non-nitrogenous compounds like proteins and their degradation products, carbohydrates, fats, waxes, resins, pigments and numerous low molecular weight compounds. On the other hand, humic substances are not related to any of the existing known groups of organic chemistry and form a large part of total reserves of humus. These are yellow or brown to black coloured, acidic polydisperse substances of relatively high molecular weight. Based on solubility, the humic substances are broadly divided into three classes: (a) fulvic acid (acid and alkali soluble), (b) humic acid (alkali soluble and acid insoluble) and (c) humin (insoluble in both acid and alkali).

The soil organic matter is the storehouse of all the energy material required by most of the soil microorganisms. The soil physical properties are so much dependent on organic matter that the content of the latter in soil can very well serve as an index of the soil condition. The biological activity of an agricultural soil will be in a state of dynamic equilibrium which can be altered by changes in temperature, water content, or by the addition of more usable organic material. Regular additions of organic materials help in maintaining the tilth and productivity of the soil and reduce soil erosion, run off and leaching.

Assessment of soil organic matter is therefore a valuable step towards identifying the overall quality of soil. Soil quality assessment is based on the relation between composition and functioning of soil. It is defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health (Doran and Parkin 1994).

Indiscriminate use of inorganic fertilizers causes decline in organic carbon status. However, when organic manures are added or organic fertilizers are added in conjunction with chemical fertilizers, it results in build up of organic carbon in the soil. The black pepper growing soils are predominantly lateritic soils which have high content of Fe and Al which causes P fixation (Mathew 1984). The present study was therefore taken up to study the effect of organic fertilizers on transformation and availability of nutrients, maintaining soil fertility, improving black pepper productivity and quality of the produce.

India is one of the largest grower and exporter of black pepper. During 2003-04, in India the production of black pepper was 71,000 tonnes. India exported 16,700 tonnes of black pepper valued at Rs. 143 crores, emphasizing the importance of pepper in Indian economy. The balance of

production is used for internal consumption. To meet the national and international demand, an annual growth rate of 6-8% is envisaged.

In black pepper, oleoresin and piperine content are important parameters in quality. It would be worthwhile pursuing studies to know the effect of organic manuring with regard to production, soil quality and the quality of the king of spices. "Organic pepper" will add to the economy of the nation and will also safeguard the health of the people.

Hence the present study was taken up with the following specific objectives:

- To study the influence of different organic fertilizers on soil physical parameters.
- To study the soil availability and uptake pattern of nutrients due to the application of organic fertilizer.
- To investigate and characterize the organic matter build up of black pepper growing soil.
- To study biological index of soil fertility based on soil dehydrogenase and acid phosphatase activities.
- To study the effect of organic fertilizers on P- adsorption.
- To study soil quality as influenced by organic fertilization and
- To assess the effect of treatments on crop growth, yield and quality of black pepper.

Laboratory incubation experiments, green house and field studies were taken up. Relevant review of work done and results of research carried out from April 1998 to September 2004 on the above aspects are discussed in the thesis.

REVIEW OF LITERATURE

Rubina M R “The effect of organic fertilizers on soil quality, nutrient availability and quality of black pepper ” Thesis. Department of Chemistry, University of Calicut, 2005

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REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

The literature pertaining to the influence of organic fertilizers on the physico-chemical properties of the soil, transformation and availability of nutrients in different soil types, uptake of nutrients by black pepper, dehydrogenase activity, humic and fulvic acid fractions in soil, soil quality and crop qualities are reviewed in this chapter, in detail.

2.1. Influence of organic fertilizers on soil physical properties

Organic manures increase the humus content of the soil, which improves the soil physical properties by causing an increase in aggregation (Elson 1943; Sommerfeldt and Chang 1985; Sharma *et al.* 2000) and water holding capacity (Acharya *et al.* 1988) and a decrease in soil bulk density (Hafez 1974). The decrease in bulk density can be ascribed to an increased volume of micropores as well as a decreased particle density in soil amended with organic manure (Schjonning *et al.* 1994). According to Pagliai and Vignozzi (1998), application of organic manures improves the soil physical properties by improving porosity and aggregate stability and reducing the formation of surface crusts.

Studying the influence of continuous long-term application of manures and fertilizers on the physical properties of sandy loam soil, Muthuvel *et al.* (1982) reported a decrease in bulk density with increase in organic matter content due to better aggregation. A decrease in bulk density has also been reported by Khaleel *et al.* (1981) and Anderson *et al.* (1990) after addition of manures.

According to Mathan and Thilagavathi (1997), the decreased bulk density might be due to higher application and advanced decomposed organic matter and formation of better stable aggregates. The greatest influence of

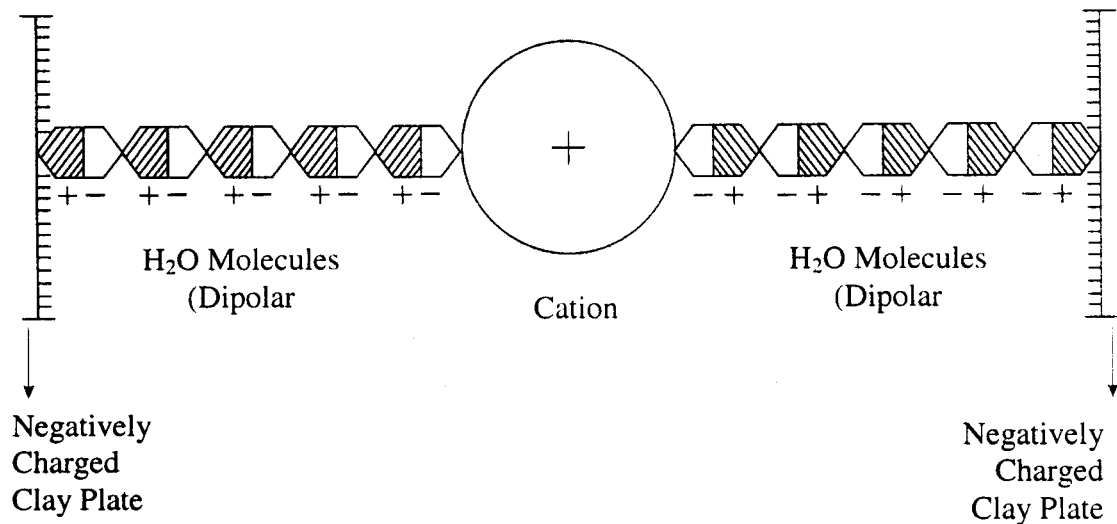
organic matter on water holding capacity is attributed to the structural changes in pore size both within and between the soil aggregates (Larson and Clapp 1984). Studies conducted by Lourduraj (1997) revealed that coir pith application favorably influences soil physical properties like hydraulic conductivity and moisture holding capacity in groundnut growing soil.

High carbonaceous material in organic manures contributes towards enhancing the water holding capacity of the soil (Selvi Ranganathan and Augustine Selvaseelan 1997). Tiwari *et al.* (1998) and Patidar and Ali (2004) observed that incorporation of FYM results in improvement of soil physical properties, water-holding capacity, porosity and bulk density of soil. Similar findings were reported by Suja *et al.* (2004) in white yam cultivation. The experiment carried out by Tennakoon (1990) using goat manure showed improvement in water holding capacity of soil.

The results of physical analysis of soils amended with organics in horticultural crops indicated that apart from ameliorating the poor physical properties of the compacted soil, additions of the composted organic amendments significantly increased soil pH, organic carbon content and the available supplies of phosphate and Mg in the soil (Mishra and Sharma 1997; Wein and Allen 1997; Tiwari *et al.* 1998).

Studies have revealed that soil physical properties improve significantly in soils amended with organic manure. The hydraulic conductivity and soil porosity increase whereas bulk density decreases. Soil aggregation contributes to the fertility, because it is crucial to soil porosity, aeration and infiltration of water. Organic agriculture results in continuous build up of soil organic carbon, available P and microbial biomass in the organic system, resulting in the improvement of the hydro physical environment (Singh *et al.* 1997; Kairon *et al.* 1998). The decrease in bulk density could be attributed to the fixing of the low density materials with dense mineral fraction of the soil

(Srikanth 2000). The mechanism of structure formation has been given as follows by Edwards and Bremner (1967) and Prabhakaran Nair (1987).



The mechanism of structure formation: As water molecules evaporate, the negatively charged clay plates are pulled closer to each other, and with cementing agents, soil structure develops (Prabhakaran Nair 1987).

2.2. Influence of organic fertilizers on transformation, availability and uptake of nutrients

The changes as a result of organic agriculture affect nutrient availability to crops either directly by contributing to nutrient pool or indirectly by influencing the soil chemical and physical environment (Sean Clark *et al.* 1998). Soil organic matter, as well as added organic fertilizers, act not only as a source of nutrients but also influence the availability of nutrients in different ways.

Transformation of essential nutrients is due to mineralization as a result of decomposition of organic plant and animal residues, immobilization due to the reduction of the oxidized forms of the elements or assimilation and immobilization in microbial protoplasm, increased availability resulting from the oxidation of reduced forms or vice versa and chemical transformations

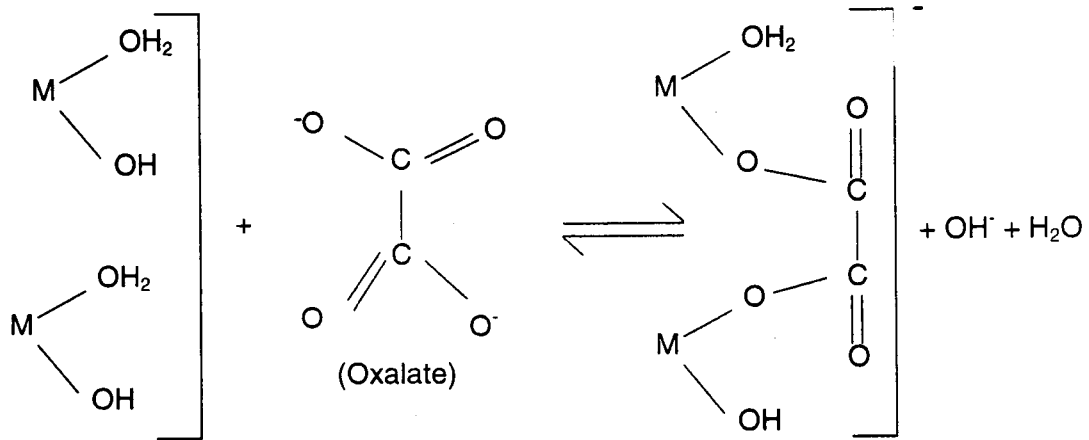
occurring indirectly through the production of organic acids or chelating agents (Alexander 1961).

During the process of decomposition of organic materials in soil, organic acids formed in the transformation cycle of soil organic matter and also those excreted by soil organisms can contribute to the solubilization of nutrients from the mineral components of soil or parent material. In reality, organic agriculture is a consistent system approach based on the perception that tomorrow's ecology is more important than today's economy. Without ecology, there is no economy (Arun 2004).

The results of chemical and physical analysis of soils amended with organics in horticultural crops indicated that apart from ameliorating the poor physical properties of the compacted soil, additions of the composted organic amendments significantly increased soil pH, organic carbon content and the available supplies of phosphate and Mg in the soil (Wein and Allen 1997). Singh and Chauhan (2002) recorded relatively high accumulation of organic carbon, on FYM application in soil.

Sharma *et al.* (1997) have reported moderating effect of FYM on soil pH. Often there is an initial increase in pH over the first one or two months of the residue decomposition followed by a decline to above or below the initial pH level (Asghar and Kanehiro 1980). Similarly there was improvement in the soil pH towards neutrality after the application of vermicompost in the soil growing Chinaaster (Nethra *et al.* 1999). An increase in pH on application of organic manures has also been reported by (Hoyt and Turner 1975; Hue *et al.* 1986; Iyamuremye and Dick 1996; Noble *et al.* 1996; Wong *et al.* 1999).

The mechanism for the organic fertilizer induced increase in pH is specific adsorption of humic material or organic acids on to Al and Fe (M) hydroxides with the consequent release of OH⁻ ions (Hue 1992)-



According to Kairon *et al.* (1998) and Patidar and Ali (2004) incorporation of FYM increased organic carbon, available P and K status of soil. Incorporation of FYM significantly increased the available P in a study conducted by Mathur *et al.* (1998) in a sandy loam soil. Similarly Patil *et al.* (1993) and Mercik *et al.* (1995) observed significant increase in organic carbon content of soil on organic fertilization. Studies on the transformations and availability of soil P fractions and phosphate sorption behaviour in a Typic Ustochrept showed that P availability increased due to FYM application (Anonymous 2000). Increased availability of soil Phosphorus has been reported by Venkatesh *et al.* (2003) in turmeric cultivation on applying P sources in conjunction with FYM.

Muthuvel *et al.* (1982) and Yaduwanshi *et al.* (1985), have reported that application of FYM alone or in combination with fertilizers check the depletion of exchangeable K to some extent as compared to control, but increased the K status over control. This may be attributed to the fixation of applied K as well as dissolution of K bearing minerals. The micro-organisms and organic decomposition products affect the availability of K through liberation of organically bound K by decomposition processes as well as by the solubilization of insoluble forms present in soil minerals.

Bear (1976) reported that the micro-organisms and organic decomposition products affect the availability of K through liberation of organically bound K by decomposition processes as well as by the solubilization of insoluble forms present in soil minerals. FYM is reported to be not only a direct source of K but also aid in minimizing the leaching loss by retaining K^+ ions on exchange sites (Bansal 1992; Sadanandan *et al.* 2001).

The organic manure vermicompost produced by earthworms is a mixture of worm castings, organic material including humus, live earthworms, their cocoons and other organisms. A worm cast consists of organic matter that has undergone physical and chemical breakdown through the activity of the muscular gizzard that grinds the material to a particle size of 1-2 microns. The nutrients present in the worm cast are readily soluble in water for the uptake of plants (Bhawalkar and Bhawalkar 1993).

Leita *et al.* (1996) stated that, soil organic matter is the most effective cation exchanger and thus stores the nutrients against leaching. Further, active biodegradation and resultant production of CO_2 can lead to dissolution of nutrients in soils. The triggering of oxidation-reductions and increased chelation capacity brought about by addition of organic matter dictate transformation and availability of micronutrients in soils. Bioavailability of metals in soil is strongly influenced by the amount and the quality of organic matter which can interact with the metals forming complexes and chelates of varying stability.

Use of organic materials such as farmyard manure, cakes of plant origin, vermicompost, and microbial biofertilizers on one hand, and exploiting the synergism between citrus-vesicular arbuscular mycorrhizal fungus on the other hand, are important components of the bio-organic concept of citrus cultivation. Organic fertilizer treated trees had better plant growth and uptake of nutrients like P, Ca, Zn, Cu, and Fe (Srivastava *et al.* 2002). Reddy *et al.* (2002) have reported higher uptake of Nitrogen with application of FYM. Data

published by Rajkhowa *et al.* (2000) attributes the steady and increased availability of nutrients from vermicompost for the increased uptake of nutrients by green gram. In a study carried out by Patel (1996) the P and S uptake in mustard was significantly high when FYM was incorporated in the soil.

Organic fertilization increased several soil fertility parameters (N, P, K, Ca, and Mg) and the improved fertility status provided conducive microclimatic conditions for dominance of earthworms which could increase enzymatic activities as well as microbial biomass in a study conducted by Lopez Hernandez (2004). Organic amendments seem to be an alternative for sustaining agriculture in Venezuelan Amazonia which is dominated by soils with poor structure because fertilizer and other agricultural items are expensive and need to be imported from distant regions (Lopez Hernandez *et al.* 1986).

Organic manures improved the availability of Zn and Mn in soils, but their effects were more pronounced on the soil Zn supply (Yang *et al.* 1990). Tomar and Gupta (1992) have reported that organic matter affects Mn transformation in soils due to production of complexing agents, decrease in redox potential of soil, stimulation in microbial activity and availability of P. Koppen and Eich (1991) reported decrease in Mn content in soil with increasing pH. Similarly application of FYM increased availability of B and the plant height of sunflower (Sharma *et al.* 1999). Compost additions caused significant increases in pH, organic matter content, EDTA extractable Cu, Zn, and Fe and had a positive effect on the water retention capacity (Martinez *et al.* 1999).

Singh and Swarup (2000) have reported the synergistic effect of FYM on improving efficiency of doses of NPK and correcting deficiency of Zn and S. FYM besides supplying additional quantities of N, P, K has beneficial effect on the physical properties and biological condition of the soil. In

addition organic fertilizers have proved useful in correcting the deficiencies of several other nutrients also. It has been observed that application of FYM over a major period led to a build up of plant available Zn (Katyai and Randhawa 1983). FYM application not only reversed the decline in P status of soil with cropping (Brar and Bhajan Singh 1983), but a distinct rise in P fertility was also seen. Chattopadhyay *et al.* (1993) observed that organically fertilized soils maintained significantly higher available nutrients throughout the crop growth period than the inorganically fertilized soils.

2.3. Influence of organic fertilizers on crop growth, yield and quality

In vegetables and fruits, role of organic manures for improving productivity and quality is well documented. The influence of vermicompost on growth and yield of turmeric varieties was studied at Indian Cardamom Research Institute, Regional Station, Sakleshpur, Karnataka. The results indicated that influence of vermicompost was pronounced in all the varieties tried. The superiority of organic manures in increasing the yield and improving the quality (curcumin content) of turmeric rhizomes has been studied by Sadanandan and Hamza (1996).

Rani and Sathiamoorthy (1997) reported greatest growth enhancement when 50% of the N fertilizer was substituted by FYM and neem cake and 50% of the P fertilizer requirement by sterameal and biofertilizer mixture was applied in papaya. Sood and Lal (2004) reported that application of vermicompost saved 25% of NPK fertilizers in potato cultivation. Increased oil and protein content was obtained on application of FYM by Balasubramanian (1997) in groundnut. Arumugam *et al.* (1994) have reported beneficial effect of organic manuring in palmyrah as application of FYM increased the yield and quality of the crop. Similarly influence of organics in green gram cultivation was studied by Reddy *et al.* (1992).

Hsieh *et al.* (1996) reported better soil fertility factors such as lower salinity, higher organic matter, higher available phosphorus and microelements and the application of effective microorganisms (EM) in organic treatments caused higher yield of broccoli. Similar observations were recorded by Gao *et al.* (2001) in sweet pepper. Compared with inorganic fertilizer, mixed organic fertilizer and organic-mineral complex fertilizer greatly promoted the growth and development of sweet pepper. The yield increased by 43.3-109.8%, the content of vitamin C increased by 39.1-59.5%.

According to Sadanandan and Hamza (1998), application of organic cakes increased nutrient availability, yield and oleoresin production of ginger. Similarly goat manure has been found to increase the nut yield, copra weight, nuts/bunch and female flowers/tree in coconut (Tennakoon 1990). Barley yields obtained with organic amendments were, in general, similar to, or even higher, than those obtained with mineral fertilizer application (Marcote *et al.* 2001).

Vermicompost incorporation alone or in combination with *G. intraradices* or *A. brasilense* increased the photosynthetic rate, dry matter accumulation and tomatillo yield. A synergic effect was observed between the vermicompost and *G. intraradices*; increasing dry weight and yield by 120 and 26%, respectively, compared with the control. It is concluded that the arbuscular mycorrhiza and vermicompost could be very helpful in the organic farming of tomatillo (Velasco Velasco 2001).

Zn accelerates the protein and amino acid synthesis thereby increasing the content of oil, oleoresin and piperine in pepper. Kumaresan *et al.* (1985) have reported beneficial effect of Zn application and the reason attributed is that Zn is involved in many metallo-enzymes in plants. And the enzymes like carbonic anhydrase plays important role in photosynthesis. Zn is also said to involve in the protein synthesis through RNA.

Singh *et al.* (1997) reported that FYM produced very high gross yield of rabi onion. Vermicompost increased the yield of roots and sugar, and enhanced the content of sugar in the roots and decreased weed infestation (Kopczynski *et al.* 1999). Organic manures promoted tuber weight and length in white yam (Suja *et al.* 2004). Yadav *et al.* (1992) obtained better yield of green gram on addition of compost and biofertilizers in soil.

The importance of vermicompost in improving the soil fertility, increasing growth and yield of crops are very well established by many workers (Radha Kale and Kubra Bano 1986; Mulongoy and Bedoret 1989; Hullegalle and Ezumah 1991; Bawalker 1992). Vermicompost increased the yield of roots and enhanced the content of sugar in the roots of sugar beet (Kopczynski *et al.* 1999). Nethra *et al.* (1999) reported beneficial effect of vermicompost in China aster cultivation. High quality cucumbers and carrots were obtained on application of vermicompost in soil by Yang *et al.* (1990). Vadiraj *et al.* (1996) reported that vermicompost significantly increased the yield and growth of turmeric.

Vermiwash is prepared by agitating heavy population of earthworms in water. The extract contains major nutrients, Vitamin B₁₂ and hormones secreted, by earthworms. Earthworms produce bacteriostatic substances which protect the crop from bacterial infection. It is sprayed on crops for better growth, yield and quality (Kalloo 2004). Lozek and Fecencko (1998) and Lozek and Gracova (1999) have reported increase in yield of tomato and winter wheat on foliar application of vermisol special produced by extraction of vermicompost. Vermiwash application on tomato seedlings has been reported to increase yields by 7.3%. Similarly crop quality was greater in carrot and cucumber with vermicompost than with mineral fertilizers on the basis of studies conducted by Kolodzziej and Kostecka (1994).

A substantial increase in yield of groundnut has been reported on the application of FYM in soil by Pawar and Kadam (2000). High sugarcane and

sugar yield on application of organic manures was reported by Khandagave (2002). Similarly Sinha *et al.* (1980) have observed higher uptake of nutrients and yield of rice on incorporation of neemcake in soil. With chicken manure, the requirement for mineral fertilizer was lower, but higher and better quality tuber production was obtained (Romero *et al.* 2000). Lourduraj (1997) reported that coir pith application favourably increased soil nutrient status and pod yield in groundnut. Higher levels of secondary defence compounds were found in groundnut leaves from plots that received organic manures. The studies provide insight into the manipulation of the host plant ecosystem through host plant nutrition to induce resistance in plants. The studies reduce the cost of inputs towards fertilizers and insecticides through organic agriculture and promote the ability of organic agriculture to attain sustainable plant protection (Krishnamoorthy and Ravi Kumar 1973; Gaur and Sadasivan 1981; Rao *et al.* 2001).

2.4. Effect of organic fertilizers on soil biological properties

Organic fertilizers optimize soil microorganisms, increase the activity of enzymes in the soil and improve soil fertility. The soil is a living medium in which micro flora and micro fauna play an active role. Inputs of organic material not only effect available nutrient content of the soil, but also the development of beneficial microorganisms. Soil organisms contribute to the maintenance of soil quality in that they control the decomposition of plant and animal materials, biogeochemical cycling including nitrogen fixation, the formation of soil structure and the fate of organics applied to soils. Since they are one of the most sensitive biological markers available, they should be useful for classification of disturbed or contaminated systems (Abdel Aziz *et al.* 1998). A high quality soil is biologically active containing a stable cross-section of microorganisms.

The activity of soil enzymes may be inhibited by addition of certain organic amendments. Perucei *et al.* (1984) found that the addition of organic residues (tomato, maize and wheat straw) inhibited several soil enzymes

assayed. Soil enzyme activities may also be increased by the addition of organic materials (Balasubramanian *et al.* 1972; Zantua and Bremner 1976; Nannipieri *et al.* 1983). This increased activity has been attributed to the increased microbial biomass.

The effects of fertilizing with oilseed cake on the biological properties of root region soil under *Phyllostachys pubescens* forest were investigated in a trial in bamboo forest in Lin'an City, Zhejiang Province, China. Application of oilseed cake caused a significant increase in the numbers of bacteria and fungi. Application of pure mineral (NPK) fertilizer had no significant effect on either soil bacterial or fungal numbers or enzyme activity (Xu Qiu Fang *et al.* 2000).

Organic amendments enhanced beneficial soil microorganisms, reduced pathogen populations, increased soil organic matter, total carbon, and cation exchange capacity (CEC), and lowered bulk density thus improving soil quality (Bulluck 2002). The incorporation of compost, FYM, cereal residues/straw and green manure is known to influence favourably the physical, chemical, physico-chemical and biological properties of soil (Subba Rao 1988; Haynes and Naidu 1998; Kishorbhai *et al.* 2000). The large increase in microbial population due to the incorporation of organic residues in soil may be attributed to the mineralization of carbon and other elements contained in the residues (Russell 1988; Mukherjee *et al.* 1991). The microorganisms through their activities in the soil provided valuable nutrients to the plants in the soil. Thus organic agriculture helps in keeping agricultural production at a higher level and makes it sustainable.

Nair and Rao (1977) recorded increased activity of N fixers like *Beijerinckia*, Phosphate solubilisers like *Pseudomonas* species and *Aspergillus niger* and other organisms like *Escherichia* sps., *A. Flavus* and *A. fumigatus*, which produce growth promoting substance in coconut based organic agriculture systems. Nambiar and Ghosh (1984) found that the height and production of coconut was improved by the addition of organic manures

and showed increased vigour and earlier flowering. According to Sarapatka (2004) soil phosphatase activity was directly dependent on the content of organic substances in the soils (C, N forms) which may be influenced by farming activities.

The most generalized and accepted way of evaluating the biological activity of soil is its enzyme activity. According to Hayes and Swift (1978), soil organic matter refers to the non-living heterogeneous mixture of organic components arising from the microbial and chemical transformations of organic debris. It is one of our most important natural resources and from antiquity, man has recognized that soil fertility may be maintained or improved by adding organic manures (Allison 1973). Viable microbial counts of phosphate dissolving bacteria and dehydrogenase activity in soil were greatly enhanced by organic manuring. There was a highly positive linear correlation between viable microbial counts and dehydrogenase activity in the control soil.

Significant increases in the availability of mineral N and P occurred after organic manuring (Estefanous *et al.* 1997). Soils freshly amended and soils previously amended with organic manures registered significantly greater microbial biomass and enzyme activity than unamended control. Enzymes were activated to different degrees by organic manures. A significant and positive relationship of enzyme activity with organic C and total N suggest that addition of organic manure to soils increased C turnover, N availability and microbial activity which in turn led to greater enzyme synthesis and accumulation in the soil (Dinesh *et al.* 1998).

It has been established that chiefly enzymes, which are extra cellular and are of plant roots origin, catalyse several biochemical changes involving plant nutrient transformations and organic matter decomposition. Dehydrogenase plays a significant role in the oxidation of soil organic matter. According to Tabatabai (1984) dehydrogenases activity can be considered as

a major indicator of the total microbial activity in soil. Phosphatase may also be used for biochemical soil characterization.

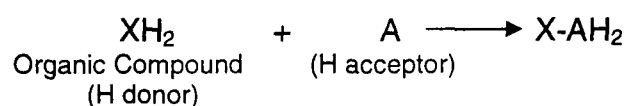
The addition of organic matter caused an increase in dehydrogenase in the active microbial biomass in field experiments in Tuscany, Italy (Masciandaro 2000). Soil microorganisms, which are typically associated with the organic fractions of the soil, are expected to influence the mobilization - immobilization equilibria of metals by changing the chemical composition of their immediate microenvironment (Beveridge 1988).

The higher soluble sugar (including sucrose, glucose and fructose) and ascorbic acid contents, and lower nitrate concentration in plants grown with organic fertilizer indicated the positive effect of this fertilizer on leaf quality. The organic fertilizer had a positive effect on the soil microbial population, resulting in enhanced soil biomass C and N contents, and dehydrogenase [oxidoreductase] activity (Xu HL *et al.* 2000).

Soil phosphatase activity is directly dependent on the content of organic substances in the soils (C, N forms) which may be influenced by farming activities. The determination of soil enzyme content is important to soil fertility ratings but the information on soil enzyme activity cannot serve as sole criteria for soil fertility evaluation (Chander *et al.* 1997).

Biological activity/index of a soil is the function of number of organisms present in soil and their physiological efficiency. The rate of respiration can be used as an index of biological activity of soil as it reflects the physiological efficiency of the organisms. Monitoring of dehydrogenases that are respiratory enzymes and integral part of all soil organisms, will give a measure of biological activity of soil, at a given time.

Biological oxidation of organic compounds is a dehydrogenation process and there are many dehydrogenases, which are highly specific.



The dehydrogenase enzyme systems play a significant role in the oxidation of soil organic matter, as they transfer H from substrates to acceptors. Dehydrogenase systems are an integral part of the microorganisms. The results of the assay of dehydrogenase activity would show the average activity of the active population (Skujins 1976). The dehydrogenase activity depends on the total metabolic activity of soil microbes, so its value in different soils containing different populations do not always reflect the total numbers of viable microorganisms isolated on a particular medium (Skujins 1976).

A group of enzymes that catalyze the hydrolysis of both esters and anhydrides of H_3PO_4 are known as Phosphatases. These enzymes are important in soil organic P mineralization and plant nutrition. Acid phosphatase is predominant in acid soil.

Addition of organic manure to soils enhances soil organic C status and Microbial activity, which subsequently enhance soil enzyme synthesis and accumulation. This in turn would bolster the soil's capability to cycle and provide nutrients for crop growth. Soil microbial biomass and enzyme activity are sensitive to even short term organic manuring (Dinesh *et al.* 2000). Increased activities of several enzymes have been shown with organic amendments, green manure and municipal refuse.

The microbial biomass carbon in the soil increased with application of organic N sources (Smith and Paul 1991). But the microbial biomass concentration is effected by concentration of Zn exceeding nutritional requirements because it exerts biocidal effect on microorganisms.

2.5. Influence of organic fertilizers on soil organic matter

Organic matter is the key constituent of cultivated soils having a profound influence on the physico-chemical and biological characteristics of the soil medium. Assessment of soil organic matter is a valuable step

towards identifying the overall quality of a soil. Maintaining or enhancing soil quality is a key factor in sustaining the soil resources of the world (Gregorich *et al.* 1994).

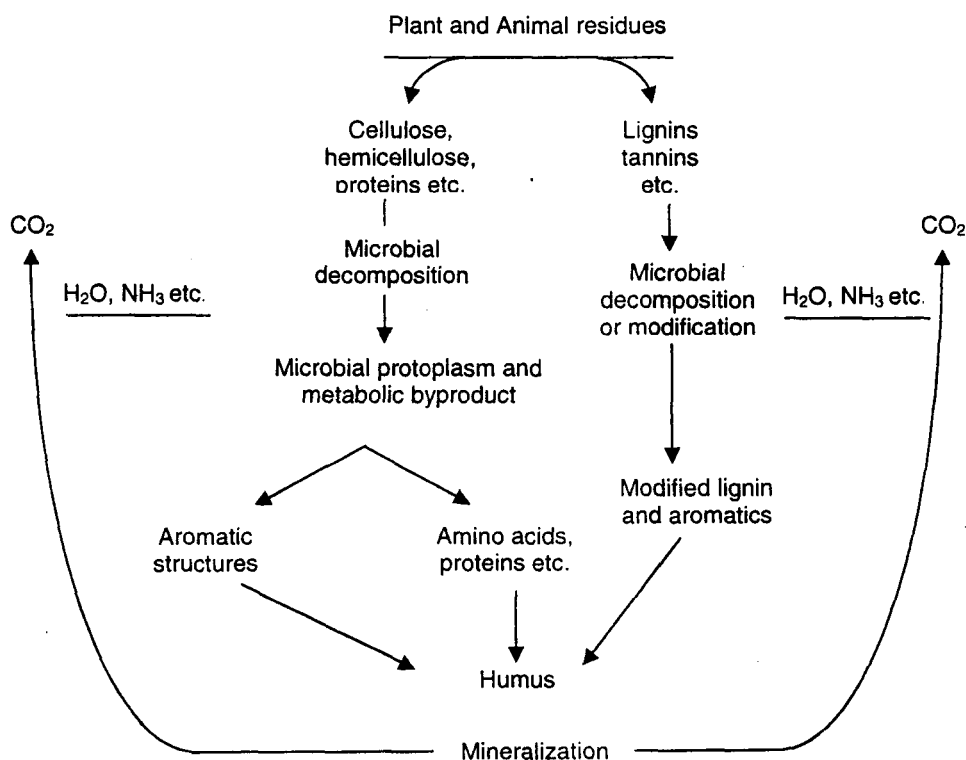
In acid soils the use of organic matter plays a very important role in nutrient availability because the exchange capacity of the soil is increased due to organic matter added resulting in the predominance of organic colloids (Sadanandan 2000). Humic substances which represent differently extractable fractions of the soil organic matter exert various effects on soil as a site for plant growth. Humic acid and fulvic acid are subjected to degradation or transformation by soil micro organisms (Filip *et al.* 1998).

According to Yang *et al.* (2004) farmyard manure application increased humic acid and humin fractions at a higher rate than chemical fertilizers. The humification process of soil organic matter can follow two main directions. Direct humification occurs without soluble phases. The fresh organic matter principally lignin, changes slowly by moderate oxidation and binds progressively with the passage of time. This part of the organic matter still does not change greatly from fresh organic matter and is called "inherited humin".

Indirect humification is an important mechanism of humification that occurs through solubilization of more or less soluble low molecular weight polyphenols of several origins microbiological formation during the decomposition and breaking down of high molecular weight compounds and biosynthesis at the expense of amino acids, proteins or sugar (Flaig *et al.* 1964) directly from litter compounds. Most of the extractable humic compounds namely, fulvic and humic acids result from indirect humification. An important part of the non-extractable humin also originates by this process.

In soil two major decomposition processes occur simultaneously: (a) the breakdown of fresh organic substrates and (b) the decomposition of the native soil organic matter or humus (Hayes and Swift 1978). Plant or animal residues added to soil are initially broken down to their basic structural units

by extracellular enzymes. These are in turn absorbed and oxidized by microorganisms. During these decomposition processes the constituent elements (carbon, nitrogen, phosphorus, and sulphur) in the residue are liberated in forms available to the plant. At the same time, however, appreciable amounts of these elements are being immobilized during the formation of new microbial tissue or metabolic byproducts. These are further decomposed and mineralized. During the decomposition process microbial activity gradually decreases as the more resistant structures and byproducts of microbial activity accumulate. So, the essential nutrients remain temporarily immobilized as these resistant materials are complexed into the soil humus fraction. In spite of these changes soil organic fraction remains stable with regard to amount and nature. This equilibrium is due to the balance between mineralization and immobilization processes (Bear 1965). In addition to the numerous transformations of N, S, and P achieved by microorganisms, many of the other minor nutrients necessary for plant nutrition are also affected by the metabolic activities of soil organisms.



Cycle of organic matter decomposition and formulation of humic substances in soil (Stevenson 1965).

Humic acids are high molecular weight, negatively charged polymers built from amino acids and aromatic compounds. The phenolic compounds are derived from microbial synthesis; the amino acids are chiefly direct synthetic products of microorganisms or degradation products of proteins synthesized by microorganisms. Humic acids are strongly adsorbed on negatively charged clay particles even though the organic acid is also negatively charged. Humic acids are apparently held by positive charges on the edges of the clay or more likely through Al or Fe or their oxides present on clay. Fulvic acids are heterogeneous group of substances consisting of phenolic compounds probably similar to humic acids but of lower molecular weight, together with a group of polysaccharides and other materials, the nature of which is largely unknown (Allison 1972).

Additions of organic materials significantly alter the soil microbial populations. In acid soils fungi are active. By the degradation of plant and animal remains, the fungi participate in humus formation from raw organic residues. The high value of E_4/E_6 ratio of humic acid shows low degree of aromatic condensation and large proportion of aliphatic groupings in the humus (Biplab and Chakraborty 1980). The increase in accumulation of fulvic acid in the lateritic soils is attributed to humid high rainfall and continuous addition of litter and subsequent humus synthesis (Malewar *et al.* 1998). An increase in the ratio of microbial biomass C to soil organic C during the initial stages of decomposition of FYM when added to the soil indicates that the biomass increased in response to the addition of organic amendments (Anderson and Domsch 1986).

The bioavailability of metals in soil is strongly influenced by the amount and quality of organic matter, which can interact with the metals forming complexes and chelates of varying stability. Among the secondary nutrients, Ca and Mg are complexed mainly in the humic and fulvic fractions of organic matter and thereby influence the soil reactions and other chemical properties

of the soil (Schnitzer and Skinner 1969). When these two elements released from the minerals are not taken up by crops, they are likely to be adsorbed by humus, thereby preventing the loss of these two nutrients by leaching (Allison 1973; Parfit 1978).

The effect of organic materials seems to be dependent not only upon the relative amount of the reactive components but also upon the stability of the organic compounds involved. Humic acids have a great influence on soil fertility (Lee and Bartlett 1976). Soil aggregate stability was improved and maintained with time more by hydrophobic than by hydrophilic components of organic matter. Long lasting stability of soils can be thus achieved by addition of hydrophobic humic material with hydrophobic organic wastes (Alessandro and Joe 1999). Low molecular weight organic acids are as active in mineral weathering as humic acids and fulvic acids but because of their low concentrations in soils, their overall effectiveness appears to be limited. The ability of humic acids and fulvic acids to form water soluble metal-organocomplexes is directly related to the high contents of COOH and phenolic OH groups of humic acids and fulvic acids. In the surface layers of soils organic matter bonded to clay particles through association with Al or Fe plays a major role in the formation of stable soil aggregates. The other possible organic binding agents are polysaccharides, waxes and other hydrophobic organics which help in binding soil particles. These hydrophobic materials resist biodegradation and chemical decomposition and have long residence time in soils. They exert beneficial effects on the structure and stability of soil aggregates (Schnitzer 1992).

Organic manures like farmyard manure, neem cake and vermicompost recorded lower incidence of *A. modicella* than the conventionally fertilized control and showed positive correlation with leaf nitrogen. In coconut gardens, according to Venkitaswamy (1996), organic agriculture brings down the insect pest and diseases.

2.6. Effect of organic fertilizers on P-adsorption

The P deficiency is one of the critical soil constraints to crop production in the red soil regions. Strong adsorption and occlusion of phosphate by Fe and Al reduces the utilization efficiency of applied and indigenous soil P to crops (Lin 1995). Yang *et al.* (1994) reported that application of organic manures increased the availability of indigenous soil P and fertilizer P to plants in the red soils. This could be partially attributed to the enhanced microbial activities resulting from increased supply of energy materials for soil microorganisms.

Significant increase in available P content was also observed by application of phosphatic fertilizers in combination with FYM (Sharma *et al.* 1984). Das *et al.* (1991) reported that application of FYM resulted in tremendous increase in available P status of soil after 12 days of incubation and drastically decreased afterwards. The extremely high value of P after 12 days of incubation might be attributed to the build up of available P owing to the application, which forms soluble complexes with native P in soils. Another possibility is that organic matter might have decreased the activity of Fe^{3+} and Al^{3+} by forming stable complexes and thus reducing phosphate fixation (Dhargave *et al.* 1991; Sheeba and Chellamuthu 1999). It is reported that phosphate fixing capacity of soil is reduced by coating of sesquioxide particles by humus which forms a protective cover (Tisdale and Nelson 1978). According to Stevenson (1982) available P increased in the post harvest soil due to P and FYM application, which might be due to increased availability of phosphate by chelating the phosphate fixing cations and exchange of adsorbed PO_4^{3-} by organic anions.

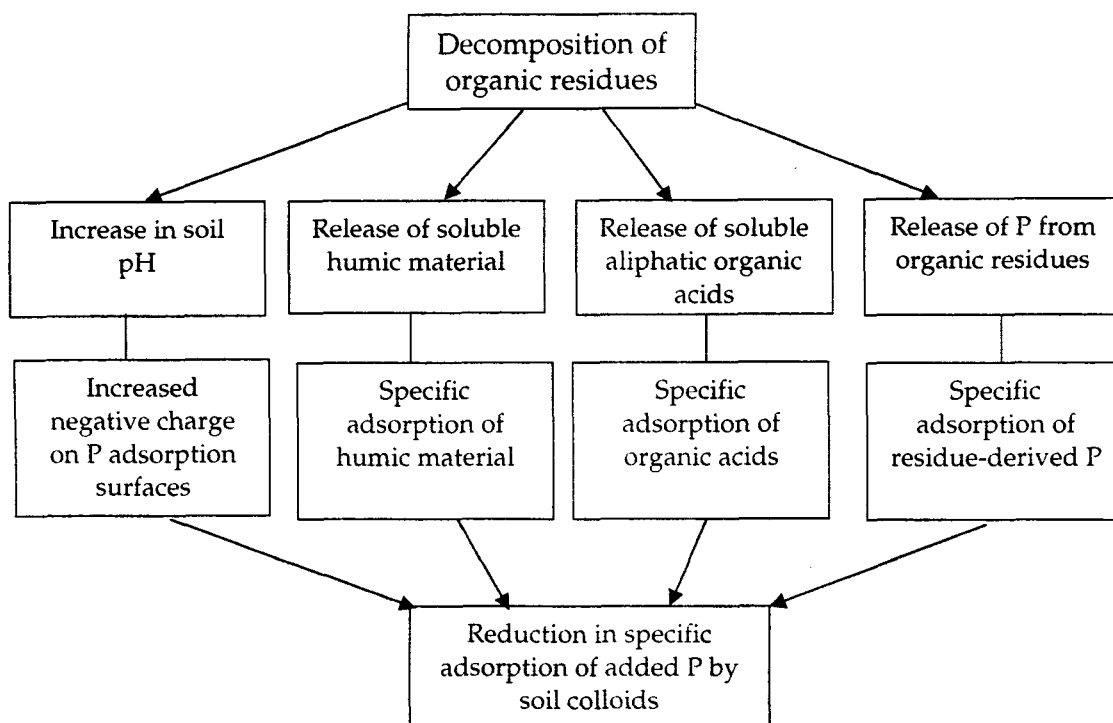
General trend is that fertilizer P could be reduced without affecting the yield of crops if it is supplemented with FYM (Rokima and Prasad 1991; Sharma and Singh 1991; Yadav *et al.* 1991). Better performance of crop in

the form of increased dry matter has been reported by Dhargave *et al.* (1991) on applying FYM with inorganic fertilizer, which might be due to increased availability of soil P. Adsorption of added P decreased significantly with incorporation of both FYM and biogas slurry. Desorption of P was relatively higher in soils amended with organic manures as compared to control (Lopez *et al.* 1986; Brar *et al.* 1999). High and low energy phosphate adsorbing surfaces have been accounted for by Holford and Mattingly (1975) in calcareous soil.

Singh and Sarkar (1986) studied the effect of FYM on P availability and reported that FYM improved the P availability in the soil. The beneficial effect of FYM in doing so in acid soils may be due to the formation of phospho-humic complexes which can be more easily assimilated by plants and coating of sesquioxide surface humus to form protective cover that reduces P fixing capacity of an acid soil (Patiram 1994; Damodar *et al.* 1999).

The effect of organic manures and soil organic matter on availability of phosphate is mainly due to the complex formation of bi- or trivalent cations which inhibit the formation of non-available forms of inorganic phosphates. The organic acids produced in soils by microorganisms prevent the precipitation of phosphate by Fe and Al. The effectiveness increases with the number of carboxylic groups and decreases with chain length.

The same action has been reported with humic substances, phenolic compounds and several sugars. The effect is observed chiefly after the addition of easily decomposable organic materials and under calcareous soil conditions (Bear 1965). A model of the processes that can induce a reduction in P adsorption and increased P availability when organic residues are added to soils is shown below (Haynes and Mokolobate 2001).



A conceptual model of the major processes that lead to a reduction in P adsorption and increased P availability when organic residues are applied to soils (Haynes and Mokolobate 2001).

2.6. Soil quality

Soil quality is the capacity of the soil to function within eco-system and land use boundaries, to sustain biological productivity, maintaining environmental quality and promote plant and animal health (Doran and Parkin 1994). It is the degree of fitness of a soil for a specific use. It is the sustained capability of a soil to accept, store and recycle water, nutrients and energy (Anderson and Gregorich 1983).

The quality of any soil depends in part on the soil's natural or inherent composition (inherent soil quality) which is a function of soil formation factors (eg. parent material and topography) and also to changes related to human use and management (dynamic soil quality) (Pierce and Larson 1993).

Quantifying soil quality requires that a minimum data set be defined, comprising measures of various soil attributes as critical properties (Larson and Pierce 1991). Properties of top soil, thickness, bulk density, penetration resistance, CEC, pH, E_{Ce}, organic carbon, total N and P, available N, P, K, Ca, Mg and S are used to define the physical and chemical aspects of soil quality. According to Hamza (2000) soil pH correlates significantly and positively with soil zinc in moderate and high black pepper yielding locations. Significant correlation of available zinc with organic carbon was reported by Singh *et al.* (1999). Similarly soil copper was positively correlated with soil zinc at moderate and low yielding locations of pepper growing soil, but at high yielding locations antagonistic effect was seen. This finding by Hamza (2000) was supported by the findings of Saha *et al.* (1996). Khan *et al.* (1997) reported high correlations of DTPA extractable Zn, Cu, Fe, and Mn with their total amounts. Leaf N has been reported to show negative correlation with leaf Zn.

The two biological parameters mostly used are soil respiration and nitrogen mineralization. Microbial biomass, nitrification rates, activity of key soil enzymes, vesicular arbuscular mycorrhizal population and soil faunal activity are important indicators of soil health. A set of basic soil quality indicators proposed by Doran and Parkin (1994) are-

Soil organic matter attribute	Methodology	Soil organic matter quality indicator	Attributes that can be estimated
Organic carbon and total nitrogen	Wet/dry oxidation	Carbon and nitrogen mass and balance	Soil structure and nutrient supply
Mineralizable carbon and nitrogen	Soil incubation	Total metabolic activity of soil organisms. Net flux of inorganic nitrogen from mineralization and immobilization	Microbial activity Nutrient supply
Microbial biomass	Fumigation extraction or substrate induced respiration	Carbon and nitrogen in microbes Labile carbon and nitrogen	Soil structure Nutrient supply
Carbohydrates	Hot-water extraction	Soil structure Labile carbon	Soil structural stability

Soil organic matter which is a key attribute of soil quality is the primary source of and a temporary sink for plant nutrients in agro ecosystems and is important in maintaining soil tilth, aiding the infiltration of air and water retention, reducing erosion. Assessment of soil organic matter is therefore a valuable step towards identifying the overall quality of soil. Organic matter itself can be characterized by measuring several different components which are involved with various soil processes. Thus the multi-faceted role of soil organic matter must be taken into consideration in the assessment of soil quality. This requires the identification of separate minimum data sets to characterize organic matter for the following functions: soil structure, nutrient storage and biological activity.

Soil organic carbon and nitrogen contents provide a measurement of a soil's total inventory of organic matter, while microbial biomass, carbohydrates and soil enzymes reflect forms of labile organic matter. These properties also have a functional role in soil and thus may provide information relating to the magnitude of that function in nutrient storage, biological activity and soil structure.

Soil quality cannot be measured directly, but must be inferred from soil quality indicators (Larson and Pierce 1991 and Seybold *et al.* 1997). Soil quality indicators are measurable soil attributes that influence the capacity of the soil to perform crop production. Many soil attributes, however, are highly correlated (Zueng-Sang Chen 1999). Soil quality assessment is therefore based on the relation between composition and functioning of soil. According to Rice *et al.* (1996) microbial biomass, a dynamic component of soil organic matter makes a good indicator of changes in soil quality.

Positive relationship between total N content and enzyme activity indicates higher C turnover in soils amended with organic manures compared to control (Saviozzi *et al.* 1997). The increase in dehydrogenase activity

indicates that larger biomass of microbes was accompanied by an increase in their activity (Dhillon *et al.* 1996; Sneh Goyal *et al.* 1999). The high significant correlation between soil pH and the enzyme activities were reported by Aoyama and Nagumo (1996). The organic fertilizers increased the soil pH and enrich the soil with soil organic matter thus stimulating soil biological activity (Marinari *et al.* 2000).

The enzyme activities were highly correlated with the soil organic carbon, total N and microbial biomass carbon as per research conducted by Dinesh *et al.* (2000). Hence soil enzyme activity can be used as an index to characterize the status of soil fertility (Abdel Maksoud *et al.* 1997).

Soil enzymes are molecular sub-systems of soil organisms that can be used as indicators of soil quality if their activities are affected by environmental variables and farming practices. Enzymes are biological catalysts that lower the energy required to activate biochemical reactions. Enzyme activities are critical indicators of organic matter quality because enzymes control nutrient release for plant and microbial growth (Burns 1978; Skujins 1978).

Soil quality is thus the capacity of a soil to retain, disperse and transform chemical and biological materials and thus function as an environmental filter. Maintaining or enhancing soil quality is a key factor in sustaining the soil resources of the world. High quality soils will not only be better producers of food and fibre for the world's growing population but also play a major role in stabilizing natural ecosystems and in enhancing air and water quality.

A window of hope has finally opened in the form of a growing demand for organic food, world wide and this opportunity should not be missed and could be exploited for benefit of Indian farmers. In black pepper oleoresin and piperine content are important parameters in quality. It would be worthwhile pursuing studies to know the effect of organic manuring with regard to spices production, soil quality and the quality of the king of spices.

MATERIALS AND METHODS

Rubina M R “The effect of organic fertilizers on soil quality, nutrient availability and quality of black pepper ” Thesis. Department of Chemistry, University of Calicut, 2005

10

MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

The particulars regarding the experimental details, Green house experiment, Laboratory incubation study, Field experiments and details relating to the analytical methods followed are presented in this chapter.

3.1. Soils

The soils collected for the Greenhouse experiment, incubation and enzyme study and of field experiment represented the typical red lateritic, Ustic humitropept collected from the IISR Experimental Farm Peruvannamuzhi, Calicut district, Kerala state, Ustic Humitropept of Pulpally, Tropic Argiustoll of Wayanad district, Kerala and Ultisol Haplustult of Coorg district, Karnataka state (Table 1).

The details of the Greenhouse experiment, incubation and enzyme study conducted and the methods employed for soil and plant nutrient analysis, determination of microbial biomass carbon, dehydrogenase and acid phosphatase activity, oleoresin and piperine determination in black pepper are given below.

3.1.1. Soil Analysis

Physico-chemical and biological characterizations were taken up from the samples collected from the different experimental plots.

3.1.2. Soil physical properties

a. Water Holding Capacity

The maximum water holding capacity of the composite soil samples was determined according to the procedure of Keen and Reczkowaski (1921).

To previously weighed Keen's cup with filter paper discs, 2 mm sieved soils was added and packed by gentle tapping till three fourth full. The cups were then placed on a flat dish containing water to a depth of 0.6 cm and the soil was thus allowed to equilibrate for 20 h. The cups were then removed and the sides were wiped with filter paper to remove excess water and weighed. From the weight difference recorded, the maximum water holding capacity was computed.

b. Bulk density

Soil bulk density was determined by core sampler method (Blake 1965). Bulk density is the ratio of the mass of dried soil particles to the total volume of soil (including soil particles and pores) in its natural undisturbed state. Undisturbed soil core was excavated from the field by a core sampler. From the known volume of the core and the weight of oven dry soil, the bulk density was calculated. Bulk density is then worked out as below,

$$\text{Bulk density} = \frac{\text{Mass (Dry wt of soil)}}{\text{Volume (volume of soil)}}$$

The result is expressed as g cm^{-3}

c. Hydraulic conductivity

The hydraulic conductivity of the soil was measured by constant head (undisturbed soil) method (Klute 1965).

Soil colour was studied in moist soil using Munsell's Colour Chart (Munsell 1990) (Table 2).

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Table 1. Background information of the experimental plots

Sl. No.	Agro-ecological region	State	District	Village	Year	Pepper Variety
1.	West coast plains and Ghat region	Kerala	Calicut	Peruvannamuzhi	1998-2001	Panniyur-1 Karimunda
2.	West coast plains and Ghat region	Karnataka	Coorg	Boikeri Ashoka Plantations	1998-2001	Panniyur-1

Table 2. Soil physical characteristics of the experimental plots

Location	Colour	Bulk density g cm ⁻³	Texture	Sand	Silt	Clay
				(%)		
Calicut (Peruvannamuzhi)	7.5 YR ⁴ / ₄	1.3	Clay	27	15	58
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Pulpally	10 YR ² / ₂	1.0	Sandy clay loam	50	17	33
Wayanad	7.5 YR ⁴ / ₃	1.2	Sandy clay	44	11	45

Using Munsell's soil colour chart

3.1.2. Chemical analysis of soil

The soil samples were analyzed for pH, using dry soil- water paste in the ratio 1:2 (Jackson 1967), Organic carbon (Walkely and Black method of Nelson and Sommers 1982), Bray-1 P (Jackson 1967), Exchangeable K, Ca and Mg by neutral 1 N ammonium acetate extraction (Jackson 1967). The nutrient concentration was determined by AAS-20 (CSTPA 1974). The micronutrients Fe, Mn, Zn and Cu were extracted using DTPA (diethylene triamine penta acetic acid) (Lindsay and Norvell 1978).

3.1.4. Soil Enzyme Study

Peruvannamuzhi and Wayanad soils were used for enzyme study. These soils were incubated with FYM, neem cake, coir compost, vermicompost and urea. The soils were analysed at regular intervals to determine microbial biomass carbon, dehydrogenase, and acid- phosphatase activities. The microbial biomass carbon was determined by chloroform-fumigation method.

a. Dehydrogenase activity

The method used for determination of dehydrogenase activity is that developed by Klein *et al.* (1971). This method involves the colorimetric determination of 2, 3, 5- triphenyl formazan (TPF), produced by the reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC) by soil microorganism.

Tetrazolium salts are quaternary NH_4^+ salts and have high degree of water solubility. The TPF produced from the reduction of TTC in soils is extracted with methanol and colorimetrically determined.

In this method 20 g of air-dried soil is mixed with 0.2 g of CaCO_3 and 6 g of the mixture is placed in 3 test tubes. To each tube, 1ml of 3% aqueous solution of TTC and 2.5 ml of distilled water is added and incubated at 37°C . After 24 hours, 10 ml of methanol is added and tube shaken for one hour.

The suspension is filtered and the filtrate is diluted to 100 ml volume with methanol. The intensity of reddish colour is measured by using a spectrophotometer at a wavelength of 485 nm using 1 cm cuvette with methanol as blank. The amount of TPF Produce is calculated by reference to a calibration graph prepared from TPF standard.

b. Acid-phosphatase

For assay of acid phosphatase activity in soil Tabatabai and Bremner's method (1969) was used. It involves colorimetric estimation of the p- nitro phenol released when soil is incubated with buffered sodium p- nitrophenyl phosphate solution and toluene.

In this method, 1 g of soil is placed in a 50ml Erlenmeyer flask and 0.2 ml of Toluene, 4 ml of MUB (modified universal buffer pH 6.5), 1 ml of p-nitro phenyl phosphate solution added and the flask is swirled for a few seconds to mix the contents. The flask is stoppered and placed in an incubator at 37°C. After one hour, 1 ml of 0.5 molar CaCl₂ and 4 ml of 0.5 molar NaOH added and the flask is swirled for a few seconds and the soil suspension is filtered through Whatman no. 2 folded filter paper. The yellow colour intensity of filtrate is measured using a photoelectric colorimeter.

c. Microbial biomass carbon

As soil micro organisms play an important role in the retention and release of nutrients and energy any attempt to assess nutrient and energy flow in soil systems must take into account the role of soil microbial biomass. The chloroform fumigation method followed by Jenkinson and Powlson (1976) was adopted. In the fumigation–extraction method, a direct measurement of C in microbial biomass is done. Overnight fumigation of chloroform is done to kill all the microbes in soil samples. The microbial biomass constituents released by CHCl₃ fumigation treatment is extracted directly through chemical extractants and the readily oxidisable C contained in the extractant is measured through standard chemical procedures.

3.1.5. P-adsorption study

Phosphorus adsorption by selected pepper growing soils was studied according to the procedure described by Fox and Kamprath (1970). The Phosphorus (Bray-1 P) in equilibrium solution was determined colorimetrically by Jackson's method (1967).

3.1.6. Fractionation of organic matter

The method followed by Stevenson (1965) was adopted for fractionation of soil organic matter.

Major acid fractions (Humic substance)

Humic and fulvic acids were estimated by separation after extracting with 0.5 N NaOH. Their differential solubility in alkali, acid and alcohol was adopted as the criterion for separating them into empirical groups (Stevenson 1965).

Humic Acid (HA)

Acid washed (0.01 N HCl) 2.5 g soil sample was taken in a polypropylene centrifuge bottle and 200 ml of 0.5 N NaOH was added. The mixture was shaken for 12 hrs on a mechanical shaker and centrifuged at 3000 rpm for 10 min. Dark coloured supernatant liquid was filtered and the pH of the solution was adjusted to 1.0 with concentrated HCl. Additional 200 ml of 0.05 N NaOH was added to the residual soil, shaken, centrifuged and filtered. The residue was dispersed in 200 ml distilled water, centrifuged and the supernatant liquid was added to the previous extracts and the pH was adjusted to 1.0 with concentrated HCl and the humic acid was allowed to settle.

The supernatant liquid in the acidified extract was fulvic acid. This was siphoned off. The suspension was transferred to a polyethylene bottle and the HA was centrifuged off at 3000 rpm for 10 min. HA was redissolved in 0.5 N NaOH and reprecipitated with concentrated HCl. This purification was repeated several times. The supernatant liquid in each case was transferred to the original acid filtrate. HA was washed with distilled water until free of chloride. The HA extracted was dried in a rotary evaporator and ground to a fine powder. This was weighed and reported as percentage of HA in soil and organic matter.

Fulvic Acid (FA)

The acid extract collected in the HA preparation was FA. A known aliquot was taken, evaporated and dried. The residue was weighed and reported as percentage of FA on moisture and ash free basis and also as percentage of organic matter.

E_4/E_6 ratio

To find out the E_4/E_6 ratio of HA and FA, 25 mg of the acid was dissolved in 0.05 N sodium bicarbonate solution and the optical density was measured at 465 and 665 nm in Shimadzu UV – Visible Spectrophotometer and the ratio was calculated.

3.2. Leaf and Berry Analysis

The crop was harvested at maturity, in both the years, and pepper yield per pot was recorded. The leaf and berry samples were collected and used for chemical characterization for different nutrients. The pepper berry samples were used for the quality analysis *viz.* oleoresin, piperine and nutrient uptake computation.

3.2.1. Leaf Analysis

Leaf samples were collected from the individual vine at middle one-third portion of the vine as per standard procedure (Sadanandan and Rajagopal 1989). The youngest matured leaf from the fruiting laterals from all the directions of the vine was collected, pooled and used for chemical analysis of the plant nutrients.

The samples were washed with tap water, 0.1% detergent, 0.1 N HCl, distilled water, and double distilled water and dried at 70°C in hot air oven. The dried samples were powdered in cyclotec mill, weighed and digested using 9:4 nitric acid - perchloric acid mixture and made upto 100 ml. The aliquots were analyzed for P as per vanadomolybdate method (Jackson 1967). The K, Ca, Mg, Fe, Mn, Zn and Cu were analyzed after dilution using atomic absorption spectrophotometer (Black 1965).

3.2.2. Berry Analysis

The pepper spikes, representing each vine were collected and cleaned as per the procedure followed for leaf sample processing. The pepper samples were dried at 70°C and powdered using Cyclotec grinding mill.

The nutrient content in the berry was estimated for major, secondary and micronutrients by following the same procedure adopted for leaf analysis. From the data the nutrient uptake by berry was computed.

Pepper Oleoresin

The procedure developed by ASTA (1968) was followed for analysis of oleoresin and piperine content in black pepper. The powdered berry samples were used for the estimation of oleoresin.

Ten grams of powdered pepper (0.5 mm mesh) was taken in a chromatographic column plugged with cotton. Forty ml acetone was added and kept overnight. The extractant was drained in to a pre weighed beaker. Again, 20 ml acetone was added in the column and kept for 30 min., drained into the same beaker and evaporated on water bath. The mass was then kept in oven at 105°C for constant weight. From the weight obtained oleoresin, percentage was calculated (ASTA 1968).

Piperine

One hundred milligrams of powdered pepper was taken in 100 ml volumetric flask, made up to volume with acetone. From this 1 ml was taken and diluted to 10 ml in acetone and absorbance was taken at 337 nm using UV/Visible spectrophotometer (Shimadzu, UV-160A). From piperine standard curve the percentage piperine was computed (Genest *et al.* 1963).

3.3. Soil Quality

Soil quality is defined as the degree of fitness of a soil for a specific use. It is the sustained capability of a soil to accept, store and recycle water, nutrients and energy (Anderson and Gregorich 1983).

Soil quality cannot be measured directly, but must be inferred from soil quality indicators (Larson and Pierce 1991 and Seybold *et al.* 1997). To identify soil quality factors, the method adopted by Zueng Sang Chen (1992) was followed. For studying the soil quality parameters correlation matrix with different soil attributes was worked out.

Soil quality was assessed by determining correlation between soil physical parameters water holding capacity, bulk density and hydraulic conductivity; soil chemical properties pH, organic carbon, P, K, Ca, Mg, Fe,

Mn, Zn and Cu; and soil biological properties, microbial biomass carbon, dehydrogenase activity and acid phosphatase activity.

3.4. Green house Experiment

The experiment was laid out in earthen pots of size 30 cm. The soil was air dried, processed, passed through 2 mm sieve and weighed accurately to 10 kg and filled in each pot lined with polythene sheet. The bush black pepper saplings of 3-4 months old were planted at the rate of one each per pot. The varieties Subhakara (cv. Karimunda) and Panniyur-1 were used as test crop. The treatments (Table 3) were superimposed. All the treatments received a uniform dosage of NPK @ 1, 0.5, 2 g/pot in all the pots at bimonthly intervals per year (Plate 1). The quantity of organic fertilizers applied was based on their N content (Table 4). To adjust the balance of P and K, to be received for each treatment, Rock Phosphate and Muriate of Potash were added. The pots were arranged in Randomized Block Design. Each treatment was replicated four times. The pots were irrigated @ 1-2 litres water day⁻¹. The FYM and neem cake used for the experiment was provided by IISR, Calicut. The vermicompost was produced in the IISR Experimental Farm, Peruvannamuzhi using the African earthworm species *Eudrilus eugeniae* (Plates 2 and 3). The leaf compost was also produced in the farm using leaf litter and Glyricidia leaf. No plant protection measure was resorted to, as there was no incidence of pest and disease. Organic sprays of vermi wash and neem oil (0.2%) were sprayed alternatively at bimonthly intervals. Vermiwash was collected by agitating earthworms in water (Plate 4).

3.5. P-adsorption study

Phosphorus adsorption by selected pepper growing soils was studied according to the procedure described by Fox and Kamprath (1970). An incubation experiment was carried out using black pepper growing soil of Pulpally and Peruvannamuzhi, Calicut to determine the availability of Phosphorus as a result of applying organic fertilizers. The treatments

imposed were FYM, vermicompost and coir compost alone and in combination with 25 mg and 50 mg of P supplied as SSP. P-adsorption was studied in the test soil samples by equilibrating them with graded P concentration of 0, 2.5, 5, 10, 15, 20, 30, 40, 60 and 80 ppm P in 0.01 molar CaCl_2 as KH_2PO_4 in 50 ml polypropylene centrifuge tubes for 24 h equilibration.

The Phosphorus concentration was determined in the supernatant after equilibration by Bray's method (Jackson 1965). The amount of adsorbed P was calculated from the difference between P initially added and P remaining in solution. The extent of adsorption was studied by plotting Langmuir's adsorption isotherm and Freundlich adsorption isotherm for soil of Peruvannamuzhi. The adsorption data were fitted to the Langmuir equation and Freundlich equation:

$$\text{Langmuir equation: } (c/x/m) = (1/kb) + (c/b)$$

where, c = equilibrium P concentration (mg P L^{-1}), x/m = amount of P adsorbed (mg P kg^{-1}) and k = constant related to bonding energy of sorption (Lmg^{-1}) and

$$\text{Freundlich equation: } x/m = k C^{1/n}$$

Where, k and n are empirical constants.

For pepper growing soil of Pulpally the adsorption data were fitted to the Binary Langmuir equation and Freundlich equation.

Binary Langmuir equation or two surface Langmuir equation:

$$Q = [b_1 k_1 C / (1 + k_1 C)] + [b_2 k_2 C / (1 + k_2 C)]$$

Where, Q is the amount of P adsorbed, C is the equilibrium P concentration, b_1 and b_2 are the high and low affinity maxima of P adsorption and k_1 and k_2 are related to high and low affinity binding energies of P adsorption.

3.6. Soil Enzyme Study

For the incubation study, the soils of Peruvannamuzhi and Wayanad were used. One kg soil was incubated with FYM, neem cake, coir compost, vermicompost and urea at rates equivalent to the dosage for black pepper and mixed thoroughly. Three replicate samples of each treatment were assayed for enzyme activity at 30th, 60th, 90th and 120th day of incubation. The soil were analysed at regular intervals to determine MBC, dehydrogenase, and acid- phosphatase activity.

3.7. Field experiments

The field experiments were laid out in Randomized Block Design (RBD) with six treatments (Table 5). The quantity of organic fertilizers applied was based on their N content. All the treatments received a uniform dosage of NPK @ 100:40:140 kg/ha per year. The sources of N, P₂O₅ and K₂O were Urea, Rock Phosphate and Muriate of Potash respectively. All recommended package of practices (POP) for pepper and coffee prescribed were followed. The coffee was manured with N:P₂O₅:K₂O @ 120:90:120 kg ha⁻¹ year⁻¹ (Plate 5).

Soil samples (0-15 cm), from the basin of individual vines, were collected just before superimposing the treatments and immediately after the harvest of the crop, to evaluate the soil nutrient status. Soil samples were collected from individual basin of the vine to a depth of 15 cm from the surface using a soil auger.

3.8. Statistical analysis

The data obtained from the different experiments were subjected to statistical scrutiny using statistical software - SPSS (Statistical Package for Social Science) - Standard Version. The possible interrelationships among the essential parameters were determined, to find out the influence of various treatments. Correlations were also worked out between the nutrients in plant and soil to study the strength of their relationship.

Table 3. Treatment details of the experiment

	Treatment details
Layout	Split plot design
Main plot	With and without organic spray
Sub plot	6 treatments
T ₁	Check
T ₂	Farm yard manure
T ₃	Neem cake
T ₄	Leaf compost
T ₅	Vermicompost
T ₆	Recommended fertilizers (pop-package of practices)
Replications	4

Table 4. Nutrient composition of organic fertilizers used in the study

Organic fertilizers	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
	%						ppm			
FYM	0.6	0.20	0.4	1.3	0.39	0.12	5730	518	40	20
Neem Cake	3.0	0.08	1.7	0.5	0.22	0.10	3046	227	17	18
Leaf Compost	0.6	0.60	0.5	2.9	0.41	0.12	3820	286	59	15
Vermi Compost	0.7	0.30	0.3	3.3	1.10	0.08	3860	268	43	19
Coir Compost	2.0	0.01	0.1	0.2	0.06	0.07	2269	56	15	5.2
Vermi wash (ppm)	20	18.0	766	13	7.3	Trace	0.13	0.39	0.03	0.05

Table 5. Soil chemical characteristics of the experimental plots

Experiment	Soil	pH	OC %	P	K	Ca	Mg	Fe	Mn	Zn	Cu
				ppm							
1. Green house experiment	Peruvannamuzhi	5.5	0.8	2.6	100	398	78	12	3	3.0	1.5
2. Field experiment	Coorg	6.2	2.5	14.5	555	1601	449	40	51	1.2	2.4
3. Enzyme Study	Wayanad	5.8	1.4	31.0	149	1041	186	43	33	1.4	3.0
4. P-adsorption study	i) Pulpally	5.4	1.7	31.0	210	794	188	50	28	2.1	3.8
	ii) Peruvannamuzhi	5.5	1.1	32.0	70	324	42	26	19	1.1	1.7



Plate 1. Bush black pepper (cv. Panniyur-1 and Karimunda) cultivation in green house with mist irrigation

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Plate 2. Earthworm species *Eudrilus eugeniae* used for composting



Plate 3. Vermicomposting in progress



Plate 4. Vermiwash collection used for spraying treatment



Plate 5. General view of field experiment

MATERIALS AND METHODS

Rubina M R “The effect of organic fertilizers on soil quality, nutrient availability and quality of black pepper ” Thesis. Department of Chemistry, University of Calicut, 2005

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MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

The particulars regarding the experimental details, Green house experiment, Laboratory incubation study, Field experiments and details relating to the analytical methods followed are presented in this chapter.

3.1. Soils

The soils collected for the Greenhouse experiment, incubation and enzyme study and of field experiment represented the typical red lateritic, Ustic humitropept collected from the IISR Experimental Farm Peruvannamuzhi, Calicut district, Kerala state, Ustic Humitropept of Pulpally, Tropic Argiustoll of Wayanad district, Kerala and Ultisol Haplustult of Coorg district, Karnataka state (Table 1).

The details of the Greenhouse experiment, incubation and enzyme study conducted and the methods employed for soil and plant nutrient analysis, determination of microbial biomass carbon, dehydrogenase and acid phosphatase activity, oleoresin and piperine determination in black pepper are given below.

3.1.1. Soil Analysis

Physico-chemical and biological characterizations were taken up from the samples collected from the different experimental plots.

3.1.2. Soil physical properties

a. Water Holding Capacity

The maximum water holding capacity of the composite soil samples was determined according to the procedure of Keen and Reczkowaski (1921).

To previously weighed Keen's cup with filter paper discs, 2 mm sieved soils was added and packed by gentle tapping till three fourth full. The cups were then placed on a flat dish containing water to a depth of 0.6 cm and the soil was thus allowed to equilibrate for 20 h. The cups were then removed and the sides were wiped with filter paper to remove excess water and weighed. From the weight difference recorded, the maximum water holding capacity was computed.

b. Bulk density

Soil bulk density was determined by core sampler method (Blake 1965). Bulk density is the ratio of the mass of dried soil particles to the total volume of soil (including soil particles and pores) in its natural undisturbed state. Undisturbed soil core was excavated from the field by a core sampler. From the known volume of the core and the weight of oven dry soil, the bulk density was calculated. Bulk density is then worked out as below,

$$\text{Bulk density} = \frac{\text{Mass (Dry wt of soil)}}{\text{Volume (volume of soil)}}$$

The result is expressed as g cm^{-3}

c. Hydraulic conductivity

The hydraulic conductivity of the soil was measured by constant head (undisturbed soil) method (Klute 1965).

Soil colour was studied in moist soil using Munsell's Colour Chart (Munsell 1990) (Table 2).

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Table 1. Background information of the experimental plots

Sl. No.	Agro-ecological region	State	District	Village	Year	Pepper Variety
1.	West coast plains and Ghat region	Kerala	Calicut	Peruvannamuzhi	1998-2001	Panniyur-1 Karimunda
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Mn, Zn and Cu; and soil biological properties, microbial biomass carbon, dehydrogenase activity and acid phosphatase activity.

3.4. Green house Experiment

The experiment was laid out in earthen pots of size 30 cm. The soil was air dried, processed, passed through 2 mm sieve and weighed accurately to 10 kg and filled in each pot lined with polythene sheet. The bush black pepper saplings of 3-4 months old were planted at the rate of one each per pot. The varieties Subhakara (cv. Karimunda) and Panniyur-1 were used as test crop. The treatments (Table 3) were superimposed. All the treatments received a uniform dosage of NPK @ 1, 0.5, 2 g/pot in all the pots at bimonthly intervals per year (Plate 1). The quantity of organic fertilizers applied was based on their N content (Table 4). To adjust the balance of P and K, to be received for each treatment, Rock Phosphate and Muriate of Potash were added. The pots were arranged in Randomized Block Design. Each treatment was replicated four times. The pots were irrigated @ 1-2 litres water day⁻¹. The FYM and neem cake used for the experiment was provided by IISR, Calicut. The vermicompost was produced in the IISR Experimental Farm, Peruvannamuzhi using the African earthworm species *Eudrilus eugeniae* (Plates 2 and 3). The leaf compost was also produced in the farm using leaf litter and Glyricidia leaf. No plant protection measure was resorted to, as there was no incidence of pest and disease. Organic sprays of vermi wash and neem oil (0.2%) were sprayed alternatively at bimonthly intervals. Vermiwash was collected by agitating earthworms in water (Plate 4).

3.5. P-adsorption study

Phosphorus adsorption by selected pepper growing soils was studied according to the procedure described by Fox and Kamprath (1970). An incubation experiment was carried out using black pepper growing soil of Pulpally and Peruvannamuzhi, Calicut to determine the availability of Phosphorus as a result of applying organic fertilizers. The treatments

imposed were FYM, vermicompost and coir compost alone and in combination with 25 mg and 50 mg of P supplied as SSP. P-adsorption was studied in the test soil samples by equilibrating them with graded P concentration of 0, 2.5, 5, 10, 15, 20, 30, 40, 60 and 80 ppm P in 0.01 molar CaCl_2 as KH_2PO_4 in 50 ml polypropylene centrifuge tubes for 24 h equilibration.

The Phosphorus concentration was determined in the supernatant after equilibration by Bray's method (Jackson 1965). The amount of adsorbed P was calculated from the difference between P initially added and P remaining in solution. The extent of adsorption was studied by plotting Langmuir's adsorption isotherm and Freundlich adsorption isotherm for soil of Peruvannamuzhi. The adsorption data were fitted to the Langmuir equation and Freundlich equation:

$$\text{Langmuir equation: } (c/x/m) = (1/kb) + (c/b)$$

where, c = equilibrium P concentration (mg P L^{-1}), x/m = amount of P adsorbed (mg P kg^{-1}) and k = constant related to bonding energy of sorption (Lmg^{-1}) and

$$\text{Freundlich equation: } x/m = k C^{1/n}$$

Where, k and n are empirical constants.

For pepper growing soil of Pulpally the adsorption data were fitted to the Binary Langmuir equation and Freundlich equation.

Binary Langmuir equation or two surface Langmuir equation:

$$Q = [b_1 k_1 C / (1 + k_1 C)] + [b_2 k_2 C / (1 + k_2 C)]$$

Where, Q is the amount of P adsorbed, C is the equilibrium P concentration, b_1 and b_2 are the high and low affinity maxima of P adsorption and k_1 and k_2 are related to high and low affinity binding energies of P adsorption.

3.6. Soil Enzyme Study

For the incubation study, the soils of Peruvannamuzhi and Wayanad were used. One kg soil was incubated with FYM, neem cake, coir compost, vermicompost and urea at rates equivalent to the dosage for black pepper and mixed thoroughly. Three replicate samples of each treatment were assayed for enzyme activity at 30th, 60th, 90th and 120th day of incubation. The soil were analysed at regular intervals to determine MBC, dehydrogenase, and acid- phosphatase activity.

3.7. Field experiments

The field experiments were laid out in Randomized Block Design (RBD) with six treatments (Table 5). The quantity of organic fertilizers applied was based on their N content. All the treatments received a uniform dosage of NPK @ 100:40:140 kg/ha per year. The sources of N, P₂O₅ and K₂O were Urea, Rock Phosphate and Muriate of Potash respectively. All recommended package of practices (POP) for pepper and coffee prescribed were followed. The coffee was manured with N:P₂O₅:K₂O @ 120:90:120 kg ha⁻¹ year⁻¹ (Plate 5).

Soil samples (0-15 cm), from the basin of individual vines, were collected just before superimposing the treatments and immediately after the harvest of the crop, to evaluate the soil nutrient status. Soil samples were collected from individual basin of the vine to a depth of 15 cm from the surface using a soil auger.

3.8. Statistical analysis

The data obtained from the different experiments were subjected to statistical scrutiny using statistical software - SPSS (Statistical Package for Social Science) - Standard Version. The possible interrelationships among the essential parameters were determined, to find out the influence of various treatments. Correlations were also worked out between the nutrients in plant and soil to study the strength of their relationship.

Table 3. Treatment details of the experiment

	Treatment details
Layout	Split plot design
Main plot	With and without organic spray
Sub plot	6 treatments
T ₁	Check
T ₂	Farm yard manure
T ₃	Neem cake
T ₄	Leaf compost
T ₅	Vermicompost
T ₆	Recommended fertilizers (pop-package of practices)
Replications	4

Table 4. Nutrient composition of organic fertilizers used in the study

Organic fertilizers	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
	(%)						(ppm)			
FYM	0.6	0.20	0.4	1.3	0.39	0.12	5730	518	40	20
Neem Cake	3.0	0.08	1.7	0.5	0.22	0.10	3046	227	17	18
Leaf Compost	0.6	0.60	0.5	2.9	0.41	0.12	3820	286	59	15
Vermi Compost	0.7	0.30	0.3	3.3	1.10	0.08	3860	268	43	19
Coir Compost	2.0	0.01	0.1	0.2	0.06	0.07	2269	56	15	5.2
Vermi wash (ppm)	20	18.0	766	13	7.3	Trace	0.13	0.39	0.03	0.05

Table 5. Soil chemical characteristics of the experimental plots

Experiment	Soil	pH	OC %	P	K	Ca	Mg	Fe	Mn	Zn	Cu
				(ppm)							
1. Green house experiment	Peruvannamuzhi	5.5	0.8	2.6	100	398	78	12	3	3.0	1.5
2. Field experiment	Coorg	6.2	2.5	14.5	555	1601	449	40	51	1.2	2.4
3. Enzyme Study	Wayanad	5.8	1.4	31.0	149	1041	186	43	33	1.4	3.0
4. P-adsorption study	i) Pulpally	5.4	1.7	31.0	210	794	188	50	28	2.1	3.8
	ii) Peruvannamuzhi	5.5	1.1	32.0	70	324	42	26	19	1.1	1.7



Plate 1. Bush black pepper (cv. Panniyur-1 and Karimunda) cultivation in green house with mist irrigation

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Plate 2. Earthworm species *Eudrilus eugeniae* used for composting



Plate 3. Vermicomposting in progress



Plate 4. Vermiwash collection used for spraying treatment



Plate 5. General view of field experiment

DISCUSSION

Rubina M R “The effect of organic fertilizers on soil quality, nutrient availability and quality of black pepper ” Thesis. Department of Chemistry, University of Calicut, 2005

DISCUSSION

Chapter 5

DISCUSSION

Results of laboratory, green house and field experiments conducted in the present investigations have been briefly enumerated in the preceding chapter. From those results it might be possible to draw a few broad conclusions. It was, however, not possible to explain and interpret some of the results for which more work were called for. The significant findings obtained from these studies are discussed below in broad categories by taking into account the various view points expressed by previous workers.

- Effect of organic sources on soil physical properties
- Effect of organic sources on soil chemical properties
- Effect of organic sources on biological properties
- Effect of organic sources on soil organic fractions
- Effect of organic sources on P-adsorption
- Effect of organic sources on soil quality
- Effect of organic sources on yield and quality of black pepper
- Economics

5.1. Effect of organic sources on soil physical properties

a. *Bulk density*

Organic matter content strongly affects the soil fertility by increasing the availability of plant nutrients, by improving the soil physical structure and the WHC. Humic acids have a great influence on soil fertility (Lee and Bartlett 1976), because it possesses peculiar chemical and physical characteristics by means of which it interacts with various soil components.

Soil structure would have developed due to the organic matter added through the organic fertilizers. The increased microbial activity strongly influences aggregation. Microbial gums, fats, waxes and fungal mycelia act

as cementing agents. It has been reported by Prabhakaran Nair (1988) that when there is good aggregation, a high proportion of pore space results allowing occupation of these pore spaces by water and air. Such structures are suitable for improved crop growth. Adsorption of organic matter on clay surfaces by van derWaal's forces occurs when humus polymer comes close to a clay surface. According to Theng (1979) the forces of attraction become important, particularly if the polymer is flexible and can bend to touch a large area of the clay. These forces become stronger and the adsorption firmer as the system is dried, for this removes water from between adjacent surfaces, allowing closer contact to be made.

At 90th day, the bulk density was seen to decrease in general with the application of organic fertilizers from 1.01 g/cc in absolute control to 0.71 g/cc in FYM treatment (Table 21). At harvest there was further decrease in bulk density in FYM treatment to 0.65 g/cc (Table 22). Similar results were reported by Muthuvel *et al.* (1982) on application of organic manures in a sandy loam soil due to better aggregation. The stable soil aggregates may be formed due to the action of organic matter bonded to clay particles through association with Al or Fe. The other possible organic binding agents are polysaccharides, waxes and other hydrophobic organics which help in binding soil particles. These hydrophobic materials are resistant to biodegradation and chemical decomposition and are present in soil for a long time. Hence there has been further decrease in bulk density at crop maturity in the experimental plot. The organic fertilizers exert beneficial effects on the structure and stability of soil aggregates (Schnitzer 1992).

A decrease in bulk density has also been reported by Khaleel *et al.* (1981) and Anderson *et al.* (1990) owing to the addition of organic manures. Several other workers have attributed the decreased bulk density to higher application of organic fertilizers and advanced decomposition of organic matter and formation of better stable aggregates (Mathan and Thilagavathi 1997; Hafez 1974; Patidar and Ali 2004).

b. Water holding capacity

The water retention capacity of the soil is more pronounced with high organic matter content, basically due to its qualitatively higher net negative charges and the dipolar nature of water molecules. Besides, the greatest influence of organic matter on water holding capacity was attributed to the structural changes in pore size both within and between the soil aggregates (Larson and Clapp 1984; Sharma *et al.* 2000). Organic manures increase the humus content of the soil, which improves the soil physical properties by causing an increase in water holding capacity. (Hafez 1974; Patidar and Ali 2004).

The water holding capacity was highest in vermicompost treatment which was on par with FYM. At crop maturity stage, the build up of organic matter in the soil further improved the soil physical properties. Water holding capacity increased in vermicompost treatment (Table 22). Water holding capacity of soil increases as the contents of inorganic and organic salts increase. Water can be retained by small and medium sized pores and this shows that soil structure directly affects water holding capacity of a soil. The ability of a soil to hold water is related to surface area and pore space volume.

Another plausible explanation for increased water holding capacity in organic fertilizer applied plots is that the organic matter of the applied organics might act as a barrier for the diffusion of vapour from the soil, resulting in minimum evaporation loss (Rajalingam *et al.* 2003). Besides, high carbonaceous material in these organic manures might have contributed towards enhancing the water holding capacity of the soil (Selvi Ranganathan and Augustine Selvaseelan 1997).

5.2. Effect of organic sources on soil chemical properties

5.2.1. Soil pH

The soil chemical analysis data revealed that at 90th day of treatment application, the soil pH increased for the organic treatments (Table 7). The pH increased from 5.6 in absolute control to neutral in the organic treatments. Continuous application of organic fertilizers increased the soil pH significantly as compared to NPK fertilizers treated soil (Table 9). This may be attributed to buffering from bicarbonate and organic acids released from organic sources by microbial action (Joann *et al.*, 2000). The moderating effect of FYM on soil acidity could be attributed to decrease in the activity of exchangeable Al³⁺ ions in the soil solution due to chelation by organic molecules and formation of alumino phosphate complexes (Sharma *et al.* 1997).

The higher pH in vermicompost treatment could be due to mineralization of organic N to NH₄⁺ and consequent consumption of protons. Addition of composted organic amendments has also been reported to significantly increase the soil pH (Wein and Allen 1997; Martinez *et al.* 1999).

The rise in soil pH on addition of organic fertilizers might be due to microbial decomposition and decarboxylation of organic acid anions (Barekzai and Mengel 1993; Mengel 1994; Yan *et al.* 1996; Tang *et al.* 1999). Decarboxylation of organic acid anions results in both consumption of protons and release of CO₂:



Yan *et al.* 1996 have reported increases in soil pH following the addition of malate and citrate which showed high correlation with CO₂ evolved as a result of decomposition of these two anions.

Another possible mechanism for the organic fertilizer induced increase in pH, is specific adsorption of humic material or organic acids on to Al and Fe hydrous oxides with the consequent release of OH⁻ ions (Hue *et al.* 1986; Iyamuremye and Dick 1996).

5.2.2. Soil organic carbon

Organic agriculture affect nutrient availability to crops either directly by contributing to nutrient pool or indirectly by influencing the soil chemical and physical environment (Sean Clark *et al.* 1998). In acid soils the use of organic manures plays a very important role in nutrient availability because the exchange capacity of the soil is increased due to organic matter added resulting in the predominance of organic colloids (Sadanandan 2000). Among the organic sources studied the vermicompost treatment showed greater soil availability of organic carbon as compared to the other organic treatments. The organic matter in vermicompost would have transformed to organic carbon.

Earthworms ingest organic materials together with inorganic soil particles. The ingested inorganic matter is macerated, mixed with inorganic soil particles, passed through the gut and excreted as a cast, which is finely ground (Lee 1985). The higher organic carbon status in vermicompost treatment may be due to the greater surface area exposed to the microbial population by maceration, which facilitates further decomposition.

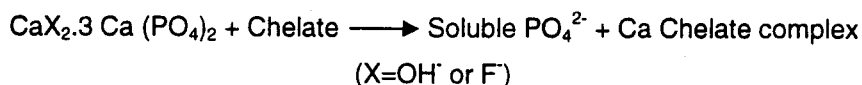
The organic carbon build up was significantly high in vermicompost treatment whereas it was very low in absolute control (Table 9). The increase in organic carbon content could be attributed to the easily available biodegradable organic matter in the organic sources, which would have also stimulated the microbial activity (Subbiah and Kumaraswamy 2000). In a field study conducted in Orissa to investigate the use of vermicompost in cabbage and tomato, soil bulk density decreased, whereas hydraulic conductivity

increased and soil organic carbon was highest in the treatment as in the present study (Chaudary *et al.* 2003).

5.2.3 Soil available Phosphorus

The leaf compost and vermicompost treatments showed greater soil availability of P. At harvest soil P availability was lower as compared to that of 90th day (Tables 7 and 9). This may be attributed to continuous crop uptake of the nutrient.

Organic acids may be involved in the formation of structures of metallo organic complexes. The action of organic chelates in solubilizing insoluble phosphates and phosphate materials has been attributed to the formation of complexes with Ca, Fe or Al thereby releasing the phosphate in water soluble form (Somani and Dadhich 2005).



Application of organic manure alone or in combination with phosphatic fertilizer helps both in proper nutrition of crop and maintaining fertility of soils. The result of a field experiment revealed that the rate of application of phosphatic fertilizer could be reduced by application of FYM (Chahal *et al.* 1984). Chaudhary *et al.* (1981) reported that application of FYM helped in releasing native soil P. Transformation of added phosphatic fertilizer is also reported to be influenced by level of organic matter in soils. Various researchers observed the increase in available P in soils due to addition of organic residue with and without fertilizers (Bairathi *et al.* 1974; Dhillon and Dhillon 1991, Misra and Das 2000; Srinivasan *et al.* 2000)

The application of FYM would have resulted in increase in available P status of soil due to the formation of fulvic acid and other chelating agents

which form soluble complexes with native P in soils. Similarly, significant increase in available P content was also observed on application of phosphatic fertilizers in combination with FYM (Sharma *et al.* 1984; Das *et al.* 1991). Another possibility for increased availability of P is that increased pH might have decreased the possibilities of fixation or precipitation as insoluble Al and Fe oxides and hydroxides. General trend is that the dose of fertilizer P could be reduced without affecting the yield of crops, if it is supplemented with FYM (Sharma and Singh 1991; Yadav *et al.* 1991).

The beneficial effect of FYM in doing so in acid soils might be due to the formation of phospho-humic complexes which can be more easily assimilated by plants and coating of sesquioxide surface by humus to form protective cover that reduces P fixing capacity of acid soils (Mack Drake 1965).

5.2.4. Soil available Potassium

Among the organic sources the leaf compost and vermicompost treatment showed greater soil availability of potassium at 90th day of treatment application. At harvest FYM, leaf compost and vermicompost treatments were on par (Tables 7 and 9).

The depletion of exchangeable K can be checked to some extent as compared to control by the application of FYM alone or in combination with fertilizers. This also increases the K status over control (Muthuvel *et al.* 1982). This may be attributed to the fixation of applied K as well as dissolution of K bearing minerals. Release of CO₂ and organic acids from the organic manures possibly act on insoluble soil minerals, thereby releasing nutrients into soil solution. This could be the reason for increase in available K in FYM and leaf compost treated pots.

In the vermicompost treatment ingestion of organic matter by earthworms would have increased the exchangeable K content. The ingested

organic matter is macerated, mixed with inorganic soil particles, passed through the gut and excreted as a cast. The higher available K in the vermicompost treated soil may be due to the greater surface area exposed to the microbial population by maceration which facilitates further decomposition (Lee 1985).

have increased the availability of micronutrients as reported by Devarajan *et al.* (1980).

According to Prasad *et al.* (1984) the application of FYM raises the Zn and Fe content of maize plants from deficiency to sufficiency levels and results in higher yield in soil with moderate to acute Zn and Fe deficiency. Organic manures in general improved the availability of Zn and Mn in soils. Zn accelerates the protein and amino acid synthesis thereby increasing the content of oil, oleoresin and piperine in pepper. Many workers (Kumaresan *et al.* 1985; Balasubramanyan *et al.* 1997) have reported beneficial effect of Zn application and the reasons attributed are that Zn is involved in many metallo-enzymes in plants. And the enzymes like carbonic anhydrase plays important role in photosynthesis. Zn is also said to involve in the protein synthesis through RNA. These might have been some of the reasons for getting quality enhancement in black pepper.

Tomar and Gupta (1992) have reported that organic matter affects Mn transformation in soils due to production of complexing agents, decrease in redox potential of soil, stimulation in microbial activity and availability of P.

5.3. Effect of organic sources on biological properties

The effect of addition of organic manure on microbial biomass and activities of some enzymes in pepper growing soils of Wayanad and Peruvannamuzhi were studied (Table 32). The incorporation of organic manures provided a conducive environment for microbial proliferation due to increased organic carbon. The microbial biomass carbon which is a measure of microbial population was also greater in amended soils. Similar result was reported by Dinesh *et al.* (2000).

Selected soil enzymes i.e., dehydrogenase and acid-phosphate activity were assayed. Enzyme activities were also consistently greater in the

amended soils. Greater enzyme activity in the amended soils was the result not only of a large microbial biomass, but also of higher amounts of endo enzymes and greater enzyme production by this microbial biomass. Besides, increased levels of accumulated enzymes in the soil and more importantly, direct contribution of enzymes by the organic manures themselves, might be responsible for greater soil enzyme activity (Dinesh *et al.* 1998).

Enzyme activity increased on application of vermicompost (Tables 32 and 33). Similar results were reported by Masciandaro *et al.* (1997). Viable microbial counts of phosphate dissolving bacteria and dehydrogenase activity in soil were greatly enhanced by organic manuring (Estefanons *et al.* 1997). Variation in enzyme activities among the amended soils might possibly be due to variation in the organic matter content, as well as the type of organic manure added to the soils.

The FYM and vermicompost treatments showed significant increase in enzyme activity in both Wayanad and Peruvannamuzhi soils. Higher organic C levels stimulate microbial activity and provide a favourable environment for enzyme synthesis and accumulation in the soil, since organic constituents are thought to be important in forming stable complexes with free enzymes (Marumoto *et al.* 1982).

Addition of organic manure to soils enhances soil organic C status and microbial activity which subsequently enhance soil enzyme synthesis. This in turn bolsters the soil's capability to cycle and provide nutrients for crop growth (Marinari *et al.* 2000). Soil fertility is markedly affected by microbial activity. According to Boyle and Paul (1989) the microbial biomass controls soil organic matter mineralization and is a source and sink of nutrients (Franzluebbers *et al.* 1994).

The increased level of enzyme activity in the organic amended soil may be a reflection of the increased protective sites within the soil as a result of

enhanced humus content. Decomposition of plant or animal residues in soil releases, essential nutrients such as nitrogen, phosphorus and sulphur required for both plant and microbial growth. Skujins (1976) reported that the total enzyme activity of a soil depends on the level of extracellular enzymes present, the amount of active enzymes within dead cells and the level activity associated with living cells.

Soil enzymes mediate biochemical transformations involving organic residue decomposition and nutrient cycling in soil. Incorporation of organic materials in soil promotes microbial and soil enzyme activity (Balasubramanian *et al.* 1972; Zantua and Bremner 1976; Nannipieri *et al.* 1983).

Soil is a dynamic living system where all biochemical activities proceed through enzymatic processor (Tabatabai 1982). Many of the enzymes added to soils by decaying microbial tissues and by plant and animal residues are partially degraded by proteases with the remainder being incorporated into soil humus (Mc Laren 1975). This increased activity has been attributed to the increased microbial biomass.

The increase in dehydrogenase and acid phosphatase activity initially may be due to the release of a promoter by the decay of organic amendments that stimulated soil organisms to secrete high levels of enzymes. This promotion of enzyme activity has been suggested by Burns (1982).

If the increased enzyme production presumably by microbes stimulated by a promoter molecule, then perhaps a feed back mechanism is also present to terminate the production of enzymes in a situation where adequate energy sources are available. This may explain why organic applications incorporated during the latter time period failed to increase the enzyme activity. In a soil receiving constant or regular organic additions, the process

of promotion and suppression may be balanced resulting in a relative constant level of enzyme activity (Martens *et al.* 1992).

Organic amendments applied to the soil enhance favourable soil conditions (Khaleel *et al.* 1981). The mechanism by which organic amendments improve the physical structure of soil is not well understood, although the effects of organic additions are universally recognized. It has been shown that the addition of organic materials to sterile soil results in little or no structural improvement indicating about the contribution and suggests that the organic residue decomposing organisms were major contributors to the soil enzyme activity.

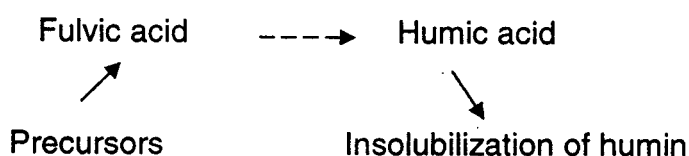
Soil enzyme activity increased as the soil structure improved upon the addition of organic fertilizers. Though the enzymes were assayed at the 30th, 60th, 90th and 120th day of incubation the data pertaining to 30th and 60th day is given as peak activities were observed at this stage and enzyme activities showed identical trends at all stages of incubation. The readily available C fraction of the organics supported the development of microbial biomass later on, a slow decline in the microbial biomass was observed due to exhaustion of available C substrate. These values are comparable to those reported by Sparling (1985) and Anderson and Domsch (1986).

Soil enzyme activities have been used as indices of microbial activity in soil by several workers. In general there is an increase in microbial growth and enzyme activities with addition of organic fertilizers but these activities subsequently decline as the available C is exhausted (Sparling *et al.* 1981; Nannipieri *et al.* 1983). Greater dehydrogenase and acid phosphatase activity in long-term manured soils is an indicator of higher organic C levels which sustain higher microbial biomass and enzyme activities (Dormaar and Sommerfeldt 1986).

5.4. Effect of organic fertilizers on soil organic fractions

In the greenhouse experiment, at 90th day of treatment application, the fulvic acid fraction of the soil organic matter was greater than that of humic acid (Table 30). The lower molecular weight humic acid has been reported to form predominantly hydrophilic complexes with metal ions. Humic acid fraction is composed of medium molecular weight aromatic compounds. The increase in the accumulation of fulvic acid in the lateritic soils is attributed to humid high rainfall and continuous addition of litter and subsequent humus synthesis (Malewar *et al.* 1998).

According to Yang *et al.* (2004) farmyard manure application increased humic acid fractions at a higher rate than chemical fertilizers. The humification may be due to slow moderate oxidation of the fresh organic matter principally lignin. This part of the organic matter which does not change greatly from fresh organic matter is called "inherited humin". Humification might also occur through solubilization of more or less soluble low molecular weight polyphenols of several origins microbiological formation during the decomposition and breaking down of high molecular weight compounds and biosynthesis at the expense of amino acids, proteins or sugar directly from litter compounds (Flaig *et al.* 1964). Most of the extractable humic compounds namely, fulvic and humic acids result from indirect humification. An important part of the non-extractable humin also originates by this process.



The fulvic acid, although primarily considered to be humic acid precursors, may be humic acid degradation products as well. It is probable that fulvic acids can be adsorbed by clay, but the size of their molecule suggests that the forces of attraction would be less than for the larger humic acid constituents (Anderson 1979). The relatively lesser stability of humic

acid which is prone to changes under environmental as well as soil conditions most probably might have given rise to the formation of fulvic acid in the green house experiment. Humic acids are thought to result from condensation and polymerization reactions involving increase in primary productivity of the soils and also because of a better environment of humus formation. The high E_4/E_6 ratio shows that humic acid forms water soluble complexes with metal ions (Table 30).

At crop harvest in the pot experiment, there was a decrease in the humic and fulvic acid fractions of the soil organic matter, which could be due to the assimilation of the chelated nutrient by plants (Table 30). There was a build up of humic acid after three years of treatment application but the fulvic acid fraction decreased with the passage of time. This may be because fulvic acid is the most labile fraction of the soil organic matter, but humic acid is intermediate in microbial attack as compared to other fractions of soil organic matter.

In the field experiment, the experimental plot was rich in organic matter as shown by the humic and fulvic acid fractions in the absolute control, 3% and 3.2%, respectively (Table 31). The percentage of organic matter increases with increase in altitude of the site due to the gradual change in the temperature and moisture which favours the formation of humus like substances. Unlike in the green house experiment, here the humic acid content was higher than fulvic acid. The proportion of the humic acid increase would have been due to the addition of litter from the intercrop coffee in the experimental plot. Similar findings have been reported by Santhy and Muthuvel (1995). Anandan (1988) also reported increased presence of HA than FA in soils of hilly region. In the present study the experimental plot is situated at high altitude.

The E_4/E_6 ratio can be used to characterize humic substances (Kumada 1965; Kononova 1966; Chen *et al.* 1977; Ghosh and Schnitzer

1979). The E_4/E_6 ratio supports the formation of hydrophilic complexes by humic acids (Table 30). The high value of E_4/E_6 ratio of humic acid shows low degree of aromatic condensation and large proportion of aliphatic groupings in the humus (Biplab and Chakravarti 1980).

In the field experiment, at 90th day, among the organic treatments the E_4/E_6 ratio was low for vermicompost treatment, followed by FYM and leaf compost treatments. The lower E_4/E_6 ratio indicates that humic substances had undergone greater degree of humification due to initial proliferation of microbial activity, but at crop maturity E_4/E_6 ratio increases, which might be due to the constant addition to the organic matter through the litter from intercrop. The absolute control and NPK fertilizer treatment had lowest E_4/E_6 ratio, indicating higher humification of the inherent organic matter in the soil in which additional organic matter was not added (Malewar 1998).

5.5. Effect of organic sources on P-adsorption

The availability of phosphorus as a result of applying organic fertilizers in selected black pepper growing soils was studied. P deficiency is one of the critical soil constraints to black pepper production in the lateritic soil regions. Phosphorus adsorption was studied in the test soil samples by equilibrating them with graded P concentration.

5.5.1. Effect of organic sources on P-adsorption in Pepper growing soil of Pulpally

The adsorption isotherm Fig. 10 shows that, among the organic fertilizers, the coir compost treatment has high adsorption rates, possibly because coir compost has less P (<0.01%) and its microbial population immobilizes more P compared to other organic sources aggravating the problem. When 25 mg P was added, adsorption was still high in coir compost treatment. For 190 mg kg⁻¹ adsorbed P, solution P for coir compost further decreased, even less than 15 mg L⁻¹.

The adsorption data fitted best in the Freundlich adsorption equation with R^2 value ranging from 0.896** in VC treatment to 0.968** in VC + 50mg P. The simple Langmuir model does not adequately describe P adsorption by pepper growing soil of Pulpally. Hence, the two-surface Langmuir equation was used (Holford *et al.* 1974). Fig. 11 depicts the two straight lines obtained from a plot of Q/C vs. Q which shows that the isotherm data fit the equation well. The two straight lines account for the two type of adsorption sites – High energy binding sites with adsorption maxima b_1 and bonding energy constant k_1 and low energy binding sites with values b_2 and k_2 (Yaobing and Michael 2000).

According to Frossard *et al.* (1995) adsorption of P by soil minerals and organic compounds initially proceeds by a rapid, exothermic ligand–exchange reaction with functional groups at mineral surfaces. Subsequently slower reactions such as liquid state diffusion into micropores, solid state diffusion or discrete precipitation may occur.

The adsorption maxima ' b_1 ' and b_2 shows a wide difference ranging from $b_1 = 40.35$ mg/kg to 122.98 in the initial state of P-adsorption and $b_2 = 279.2$ to 998.3 mg/kg in the final stage of P-adsorption. The k_2 value shows tremendous decrease as a result of addition of organic fertilizers in general in all the treatments. The k_2 value for low energy binding sites showed that adsorption considerably decreased on FYM application on adding higher level of 50mg P whereas, adsorption increased in the other two organic treatments at higher level of P.

Data Fig. 12 showed significant correlation of P-adsorption with ex-Fe and ex-Al. Similar results have been reported by Singh and Singpuri (1986). The correlation with Organic Carbon was significant and negative. From this it can be inferred that organic carbon added through organic fertilizers

decreases P adsorption whereas the Fe and Al available in the soil fix P as phosphate aggravating the problem of P fixation.

The decrease in adsorption maxima 'b' on application of organics denotes lowering of P-adsorption by the organic matter added to the soil through the organic fertilizers (Sayin *et al.* 1990). The humic substances would have probably occluded the P adsorption sites. Due to strong adsorption and occlusion of phosphate by Fe and Al oxides, the utilization efficiency of applied and indigenous soil P to crops is very low (Lin 1995). The decrease in adsorption on applying organic fertilizers may be attributed to the chelation of phosphate fixing cations Al^{3+} and Fe^{3+} by the organic matter. The clay minerals found in the surface layers of soil are frequently coated with a skin of poorly crystalline hydrous oxides of Fe and Al, so that there are numerous opportunities for the formation of this sort of linkage.

The Freundlich constants obtained for pepper growing soil of Pulpally describes adsorption of P better (Murthy *et al.* 1996). Freundlich constant $1/n$ and k are reported in Table 36 along with corresponding regression values. Freundlich constant k is maximum in coir compost treatment at higher level of P and minimum in the control soil. But the k values denote considerable decrease in adsorption of P with the addition of organics like FYM and vermi compost when compared to coir compost treatment. This could be partially attributed to the enhanced microbial activities resulting from increased supply of organic matter for soil microorganisms in general for all the organic treatments but the immobilization of P would have been more in the coir compost treatment.

The high value of P after incubation with 25 mg P in the organic treatments may be attributed to the build up of available P owing to the application, which forms soluble complexes with native P in soils. Another possibility is the decrease in fixation or precipitation of insoluble Al and Fe oxides and hydroxides. General trend is that the fertilizer P could be reduced

if it is supplemented with organic fertilizers (Sharma and Singh 1991; Yadav *et al.* 1991).

5.5.2. Effect of organic sources on P-adsorption in Pepper growing soil of Peruvannamuzhi

Langmuir isotherm Fig. 14 showed that over a range of equilibrium P concentration, the ability of the soil to adsorb added P decreased due to organic fertilizers application. Among the soil properties exchangeable Al and Fe had a highly positive and significant relationship with phosphate sorption as shown in Fig. 15. The R^2 value for the simple Langmuir equation showed that the classical Langmuir isotherm reflected better the P adsorbed in the pepper growing soil of Peruvannamuzhi. The adsorption maxima b (mg/kg) and binding energy constant k (L/mg) values for Langmuir adsorption isotherm and the Freundlich constants $1/n$ and k are reported in Table 37 along with the corresponding regression values. The high b and k value for the control shows maximum adsorption in the soil of Peruvannamuzhi.

The decrease in adsorption on adding organic sources was more in the soil of Peruvannamuzhi as justified by the k values for simple Langmuir equation. The k value was lowest for FYM+50 mg P. The coir compost treatment in this soil showed very low adsorption at high level of P. This is contrary to the result obtained for the soil of Pulpally where the adsorption is maximum on coir compost addition. This could be explained on the basis of clay content in both the soils. The clay content was high in Peruvannamuzhi soil (58%) whereas, in the Pulpally soil it was only 33.4 % Lekha (1997). According to Singh and Singpuri (1986) large amount of clay, Al or Fe oxides and hydroxides are the factors responsible for increased P adsorption. Hence in the Peruvannamuzhi soil the high clay content would have resulted in higher adsorption. Similar results have been reported by several workers (Milap Chand *et al.* 1995; Tomar *et al.* 1995; Dey and Bhattacharya 1996).

The R^2 value for Langmuir isotherm for the soil of Peruvannamuzhi showed that the P adsorption fits best in Langmuir equation. The Freundlich equation can also be used for describing the P adsorption in the soil reasonably accurately (Table 37). The Freundlich constant k and $1/n$ show considerable decrease with addition of organics. The incorporation of FYM had a spectacular influence in decreasing the adsorption at high levels of P. This must be due to the replacement of phosphate by humate and coating of sesquioxide surfaces by humus to form a protective cover that reduces the P fixing capacity of an acid soil. (Pritam *et al.* 1994). Sayin *et al.* (1990) have attributed the decrease in P adsorption to soil organic matter which occludes important adsorption sites and hinders P adsorption.

In the presence of FYM, the adsorption of P reduced with the increased levels of P application. But the adsorption of P increased in coir compost applied system (Fig. 9). When initially no P was incubated, in Peruvannamuzhi soil the adsorption was maximum in the absolute control which had a solution P less than 5 mg L^{-1} for 170 mg kg^{-1} P adsorbed. But the incorporation of organics in the soil increased the P concentration in the soil. Similarly, significant increase in available P content was also observed by application of phosphatic fertilizers in combination with FYM by Sharma *et al.* (1984). Das *et al.* (1991) reported that application of FYM resulted in tremendous increase in available P status of soil after 12 days of incubation and drastically decreased there after.

In the Peruvannamuzhi soil, P-adsorption is high. For '0' level of P incubation, P is less adsorbed, than in Pulpally soil. Solution P is higher for FYM addition and unlike in Pulpally soil, control shows high adsorption. The clay mineral in Peruvannamuzhi soil probably provides more surface area for adsorption of organic matter and enzymes and exchangeable cations for precipitation (Tomar 2000). But when 25 mg P is added, during incubation, adsorption is highest in coir added system and adsorption rate is highly decreased in the case of FYM addition (Table 37). According to Stevenson (1965), if the P content of the added organic residue is low, the available form

of P would be immobilized by the microbes. Hence in the coir compost treatment the decrease in P would have been due to immobilization of available P by microbial proliferation.

In a similar study conducted by Oehl *et al.* (2002) in alfisols, FYM improved the P use efficiency upto 36% from fertilizer source and 93 to 99% from native source. The beneficial effect of FYM in doing so in acid soils may be due to the formation of phospho-humic complexes which can be more easily assimilated by plants and coating of sesquioxide surface humus to form protective cover that reduces P fixing capacity of an acid soil.

The organic matter in soil can influence P- adsorption and availability. According to Stevenson (1994) high molecular weight humic polymers that are negatively charged can form strong electrostatic bond with metal hydrous oxide surfaces. This is possible because positive sites exist on the amphoteric Fe and Al oxide surfaces. The electrostatic competition would have resulted in a decrease in adsorption of P (Moshi *et al.* 1974; Perrott 1978).

Appelt *et al.* (1975) reported that HA and FA did not decrease P sorption by volcanic ash derived soils. The addition of humic and fulvic acid to these soils would have resulted in the formation of organic matter – hydroxyl Al complexes which constituted new P adsorption surfaces. Similarly in the present study on addition of coir compost, the newly added humic material would have been adsorbed to oxide surfaces thus reducing P adsorption. Some of the humic material would have reacted with the soluble and exchangeable Al thus forming new P adsorption sites. Similar results were reported by Traina *et al.* (1986) and Han and Thompson (1999) who attributed the maximum P adsorption potential of soil to organic acids produced by increased microbial activity which extract Al from an acidic soil and create new P sorption sites in the form of Al - organic acid complexes.

5.6. Effect of organic fertilizers on yield and quality of black pepper

Piperine content was significantly high in vermicompost treatment followed by FYM and NPK treatments, which were on par. Oleoresin content was more or less same in vermicompost and NPK treatments followed by FYM treatment which was significantly high as compared to neem cake applied plots (Table 19). According to Madhu Dwivedi *et al.* (1991) the nutrient elements are the constituents of metabolites and act as catalyst in several metabolic activities in plants, which enhance the quality of the crop. Hence, the increased availability of nutrients through organic fertilizers in the soil and subsequent uptake would have improved the quality of black pepper as compared to the control.

The increased availability of micronutrients especially Zn on addition of organic fertilizers would have also improved the quality of pepper. The micronutrient Zn is essential for synthesis of auxins and proteins, seed production and maturity (Tandon 1987). Geetha and Sivaraman Nair (1990) observed that spraying of ZnSO₄ solution increased the oleoresin content and yield of black pepper.

Nambiar and Ghosh (1984) and Liyangage (1989) reported that better water retentivity and favourable environment for root development was provided by the addition of organic manure and that increased the nutrient availability. The increase in piperine and oleoresin content of black pepper may be attributed to higher nutrient uptake and better growth of pepper vines on addition of organic fertilizers similar to the findings of Maheswarappa *et al.* (2000). Better yield and quality of pepper (*Capsicum annum*) has been reported by Norman *et al.* (2004) on addition of vermicompost due to increased microbial biomass and dehydrogenase activity.

The mean yield recorded over three consecutive years was significantly high in vermicompost and FYM treatments, which was higher

than the inorganic NPK applied treatment (Table 20). The increase in yield can be attributed to the beneficial effects of the humus contributed by organic manures. The humus might have improved the physical condition of the soil making a favourable environment for increased uptake of nutrients by black pepper. Vadiraj *et al.* (1996) have reported the increase in yield of turmeric on application of vermicompost. In ginger Sadanandan and Hamza. (1998) have reported increased rhizome and oleoresin yield and better curcumin content in turmeric on addition of organic fertilizers.

The vermiwash spray on bush black pepper further enhanced the yield of black pepper and crop quality (Tables 19 and 20). The spraying of vermiwash which was collected by agitating heavy population of earthworms contains major nutrients and possibly hormones also, would have improved the black pepper quality. Similar observations were reported by Kalloo (2004). Lozek and Gracova (1999) and Lozek and Fecenko (1998) have reported increase in yield of tomato and winter wheat on foliar application of vermisol special produced by extraction from earthworms.

5.7. Effect of organic sources on soil quality

Maintaining or improving the soil quality can provide economic benefits in the form of increasing productivity, more efficient use of nutrients and pesticides, improvements in water and air quality and amelioration of green house gas emission (USDA-Economic Research Service 1997).

Soil quality cannot be measured directly, but must be inferred from soil quality indicators (Larson and Pierce 1991 and Seybold *et al.* 1997). Soil quality indicators are measurable soil attributes that influence the capacity of the soil to perform crop production. Many soil attributes, however, are highly correlated (Zueng-Sang Chen 1999). An accurate assessment of soil quality may be achieved by evaluating several soil attributes simultaneously using

statistical procedures that account for correlation among soil attributes (James and McCulloch 1990; Johnson and Wichern 1992; Brejda *et al.* 2000).

Johnson and Wichern (1992) proved that by adopting factor analysis and covariance matrix large data could be analyzed for drawing meaningful conclusions regarding soil quality factors. The soil attribute selected may perhaps be few in identifying soil quality parameters. Further study is needed to determine how and why some of the soil attributes are not related to soil quality.

5.7.1. Interrelationship of soil and plant nutrients with yield of black pepper

In the green house experiment significant correlation was obtained (Table 38) for organic carbon with all the soil attributes-soil pH, P, K, Ca, Mg, Fe, Mn, Zn, Cu. The build up of organic matter in soil due to addition of organics increased the soil pH which released nutrients continuously either by the action of microbes or from that inherent in the organic fertilizers. Soil pH is negatively correlated with soil Mn. Similar result was reported by Uday and Ashwani (1999). The positive correlation of soil P with Zn indicates synergistic interaction between P and Zn at lower levels (Raghubir *et al.* 1995). The berry nutrients Mg and Fe showed positive correlation with K concentration. Berry Mn showed strongly negative correlation with N, Ca, and Fe (Table 39). The increased uptake of N improved the morphological characteristics of bush pepper which might have caused reduction in other nutrients due to dilution effect. N-uptake and P-uptake correlated positively with N, P concentration respectively in pepper leaf indicating better uptake of nutrients due to increased availability of phosphorus in soil. Olsen's P was significantly correlated with P uptake ($r = 0.54^{**}$) in a similar study carried out by Vig *et al.* (2000).

The piperine and oleoresin content of berry showed positive correlation with N and K concentration of leaf. Strong positive correlation was obtained between N, P concentration and yield of black pepper. The incorporation of organics in soil resulted in build up of organic carbon resulting in increased uptake of N and K. Similar results have been reported by Sadanandan (2002). The incorporation of organic fertilizers in soil ameliorated soil physical and biological properties and increased soil nutrient availability, this in turn increased the uptake of major nutrients which had a synergistic effect on pepper yield and quality.

Correlation studies carried out in the field experiment showed that the soil pH was positively correlated with organic carbon and phosphorus but negatively correlated with micronutrient Fe (Table 41). The organic carbon in soil showed positive correlation with most of the soil attributes and also with yield of black pepper. The bulk density showed negative correlation with most of the soil attributes. The other physical properties *viz.*, water holding capacity and hydraulic conductivity showed positive correlation with organic carbon in soil. The build up of soil organic carbon due to the addition of organic fertilizers would have improved the soil physical properties. Pradhan and Mishra (1999) have also reported similar findings.

The increase in soil pH as a result of the application of organics would have increased the micronutrient availability as shown by the significant positive correlation between soil pH and soil Mn and Zn. The hydrophilic humic acid –micronutrient complex formed as suggested by the E_4/E_6 ratio would have increased the soil availability of micronutrients. Similar result was obtained by Hamza (2000), which was attributed to the increase in all cations and hence zinc availability due to increase in pH. A significant correlation of organic carbon with available Zn was obtained similar to the result reported by Singh *et al.* (1999). Soil zinc correlated significantly and negatively with soil copper. According to Saha *et al.* (1996) and Hamza (2000), the high level of

copper in the soil would have produced antagonistic effect and hindered Zn^{2+} ion in soil solution by a shift in equilibrium in opposite direction.

The highly significant and positive correlation of soil physical attributes, water holding capacity and hydraulic conductivity with black pepper yield shows that water holding capacity can be considered as an index for determining soil fertility and crop productivity (Table 41). Positive and significant interrelationship ($r = 0.48^*$) between organic carbon content of soil and water holding capacity was observed by Singh *et al.* (1997). The soil bulk density was negatively and significantly correlated with the black pepper yield, which denotes that a decrease in soil bulk density improves soil physical property and crop productivity.

The correlation studies carried out from the data generated in the incubation experiment showed that there was significant and positive correlation between soil organic carbon and dehydrogenase and acid phosphatase activities indicating that higher organic carbon levels stimulate microbial activity and provide a favourable environment for enzyme synthesis and accumulation in soil (Table 42). The high status of phosphatase activity in the soil might be due to the increased production of microorganisms on addition of organic fertilizers. Similar results have been reported by Dinesh *et al.* (2000). Similarly, positive relationship between total N content and enzyme activity indicates higher C turnover in soils amended with organic manures compared to control (Saviozzi *et al.* 1997).

The increase in dehydrogenase activity indicates that larger biomass of microbes was accompanied by an increase in their activity (Dhillon *et al.* 1996; Sneh Goyal *et al.* 1999; Martin and Stefan 2000). The high significant correlation between soil pH and the enzyme activities were similar to the findings reported by Aoyama and Nagumo (1996). The organic fertilizers increased the soil pH and enriched the soil with soil organic matter thus stimulating soil biological activity (Marinari *et al.* 2000).

The enzyme activities were highly correlated with the soil organic carbon, total N and microbial biomass carbon in the pepper growing soils of Wayanad and Peruvannamuzhi (Tables 42 and 43). Hence soil enzyme activity can be used as an index to characterize the status of soil fertility as suggested by Abdel Maksoud *et al.* (1997).

5.8. Economics

The impact of the organic treatments over control is well reflected in the economics worked out. Application of FYM incurred an additional cost of Rs. 10,000 and Rs. 30,000 for vermicompost application. The NPK fertilizer application resulted in an additional cost of Rs. 2,500 (Table 44). But, the additional net return per hectare was highest for vermicompost treatment, Rs. 1,16,200 and Rs. 84,000 for FYM treatment. The additional net return for NPK treatment was Rs. 18,200. The benefit/cost ratio was highest for FYM treatment, followed by vermicompost treatment. The cost of cultivation using vermicompost is higher than FYM treatment, so in spite of higher yield in vermicompost treatment the benefit/cost ratio was comparatively lower than that of FYM treatment. The benefit/cost ratio underlines the significance of the organic treatments over control.

From the foregoing discussion of results of the present study the following broad conclusions may be drawn.

SUMMARY AND CONCLUSIONS

Rubina M R “The effect of organic fertilizers on soil quality, nutrient availability and quality of black pepper ” Thesis. Department of Chemistry, University of Calicut, 2005

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SUMMARY AND CONCLUSION

Chapter 6

SUMMARY AND CONCLUSIONS

The objectives of the present study were-

- To study the influence of different organic fertilizers on soil physical parameters.
- To study the soil availability and uptake pattern of nutrients due to the application of organic fertilizer.
- To investigate and characterize the organic matter build up of black pepper growing soil.
- To study biological index of soil fertility based on soil dehydrogenase and acid phosphatase activities.
- To study the effect of organic fertilizers on P- adsorption.
- To study soil quality as influenced by organic fertilization and
- To assess the effect of treatments on crop growth, yield and quality of black pepper.

To achieve the above objectives the following studies were made-

1. A greenhouse experiment to assess the effect of different organic fertilizers on organic fractions of soil, transformation, and availability of nutrients, growth and yield of black pepper. The experiment was laid out in Peruvannamuzhi Experimental Farm of the Indian Institute of Spices research (IISR), Calicut, Kerala.
2. An incubation experiment to evaluate the relative efficiency of organic fertilizers in enhancing microbial biomass in typical black pepper growing soils Ustic Humitropept of Peruvannamuzhi and Tropic Argiustoll of Wayanad. The study was conducted at IISR laboratory.
3. A laboratory incubation experiment to study the effect of organic fertilizers on P-adsorption in typical black pepper growing Ustic Humitropept soils was laid.

4. A field experiment to study the relative efficiency of organic fertilizers on physico-chemical properties of soil, organic fractions of soil, on soil quality and yield and quality of black pepper. The field experiment was laid out at Ashoka Plantations, Boikeri, Coorg, Karnataka state.

The following salient findings were obtained from the above investigations-

1. A decrease in bulk density with an increase in water holding capacity of soil was obtained with the addition of organic fertilizers to soil. The incorporation of organic fertilizers ameliorated the soil physical properties of major pepper growing soil. Among the organic sources, FYM was significantly superior, followed by vermicompost.
2. Annual application of organic fertilizers over the years significantly increased the soil pH. The humic acid contributed by the soil organic matter on the application of organic fertilizers not only complexed Al^{3+} and Fe^{2+} ions in the acidic soil but also increased the soil pH.
3. The organic carbon build up in the soil was significantly enhanced due to the application of organic sources. Among the organic fertilizers vermicompost through addition of organic matter from the source was superior.
4. Application of vermicompost in the soil enhanced the soil availability of micronutrients due to the solubilizing effect of complexes formed by organic fractions in vermicompost.
5. The microbial biomass of soil was enhanced due to the application of organic fertilizers. Among the organic sources vermicompost was superior. The increased levels of accumulated enzymes in the soil and more importantly, direct contribution of enzymes by the organic manures themselves, might be responsible for greater soil enzyme activity.
6. Adsorption of P was significantly reduced with the increased levels of P- application in the presence of FYM, compared to other organic sources. This may be due to the formation of phospho-humic complexes and coating of sesquioxide surface humus to form protective cover that reduces P fixing capacity of an acid soil. Among

the soils, the higher adsorption in Peruvannamuzhi soil as compared to that of Pulpally may be attributed to the higher clay content in the soil of Peruvannamuzhi.

7. Humic acid fraction had high E_4/E_6 value which indicates the release of hydrophilic organic fraction from soil organic matter. The humic acid content was high in the high altitude pepper growing ultisol soil of Coorg.
8. The soil enzyme activities were highly correlated with the soil organic carbon, total N and microbial biomass carbon.
9. The yield of black pepper was significantly increased due to application of organic fertilizers. Among the sources, vermicompost and FYM treatments were superior. The improved physico-chemical and biological properties of soil resulted in higher yield.
10. Application of organic sources significantly enhanced the quality of black pepper. The piperine and oleoresin content was enhanced due to application of organic fertilizers.
11. Among the organic treatments, the benefit /cost ratio was highest on FYM application followed by vermicompost treatment.

From the results obtained in these experiments the following broad conclusions might be drawn-

1. Application of organic fertilizers decreases the bulk density and increases water holding capacity of the soil. The decrease in bulk density was significantly high in FYM treated plot. The other organic treatments also showed decrease in bulk density of the soil but at a subdued level. In the surface layers of soils organic matter bonded to clay particles through association with Al or Fe plays a major role in the formation of stable soil aggregates. The other possible organic binding agents are polysaccharides, waxes and other hydrophobic organics which help in binding soil particles. These hydrophobic materials resist biodegradation and chemical decomposition and have long residence

time in soils. Hence there has been further decrease in bulk density at crop maturity in the experimental plot.

2. The organic fertilizers have moderating effect on soil acidity. The rise in soil pH on addition of organic fertilizers might be due to microbial decomposition and decarboxylation of organic acid anions released from organic sources by microbial action. Another possible mechanism for the organic fertilizer induced increase in pH is specific adsorption of humic material or organic acids on to Al and Fe hydrous oxides with the consequent release of OH⁻ ions.
3. The increase in the accumulation of humic acid in the high altitude pepper growing soil of Coorg is attributed to continuous addition of litter and subsequent humus synthesis. The high value of E₄/E₆ of humic acid shows low degree of aromatic condensation and large proportion of aliphatic groupings in the humus.
4. The adsorption of P reduced with the increased levels of P application in the presence of FYM. It is evident that in relation to P adsorption and P availability, the major effect of applying organic fertilizers to soils is the addition of P in the residues. The added P increases P availability directly and is specifically adsorbed onto soil colloid surfaces and as a result, the availability of subsequently added P is raised. Adsorption of organic compounds produced during residue decomposition also decreases adsorption of subsequently added P and increases its availability. Further, the increase in soil pH that occurs during the decomposition of organic fertilizers increases surface negative charge and decreases P adsorption.
5. Enzyme activity in the soil was enhanced due to application of organic fertilizers and was highest in vermicompost treatment. Greater dehydrogenase and acid phosphatase activity is an indicator of higher microbial biomass and enzyme activities. The improved soil physical properties and nutrient availability would have increased the enzyme activity.

6. The application of organic fertilizers which regulate soil microbial activity is ultimately reflected in the total nitrogen and organic carbon content of the soil. Soil organic carbon provides a measurement of soil's total inventory of organic matter, while microbial biomass and enzyme activity reflect forms of labile organic matter. These properties also have a functional role in soil and thus provide information relating to the magnitude of that function in nutrient storage, biological activity and soil structure. Soil quality assessment is therefore based on the relation between composition and functioning of soil.
7. The overall beneficial effect of organic agriculture is reflected in the Benefit/Cost analysis. For every rupee invested the net return was Rs.3.8 for FYM application and Rs. 3.2 for vermicompost application.

Cattle dung which is a near inexhaustible resource of organic matter can be potential organic fertilizer .On the whole the net return for vermicompost application is lower than that of FYM, but the piperine and oleoresin content of black pepper was highest on vermicompost incorporation in soil. Hence the use of vermicompost is attractive for practical application because of the unique way in which it is produced even right in the field and the nutrient rich extractant –vermiwash which improved the yield and quality of black pepper. But the higher production cost of vermicompost resulted in lower B/C ratio inspite of high yield obtained on application of vermicompost. Farmers can make vermicomposting cost effective if it is produced in their farms by recycling the farm wastes with initial investment which will fetch net return in the long run.

It is felt that there exists some information gap for which more work is considered necessary. These are indicated below -

Future research must concentrate on

1. The extent to which an increase in pH and adsorption of organic molecules results in increased P availability is still unclear and further research is

warranted. Knowledge of the extent to which application of organic residues can reduce fertilizer P requirement is needed so that integrated soil fertility management can be devised. Such programmes would greatly benefit resource poor farmers who are currently struggling to produce crops on acidic P-deficient soils.

2. The future endeavor to improve and maintain soil quality and resilience must address certain indicators, which are quantifiable as physical, chemical and biological parameters or functions of soils. Our approach in this respect must be holistic and realistic.
3. There is a great scope for future research both in relation to the mechanism that are involved and their relative importance and also the practical significance of using organic fertilizers in the management of black pepper growing acid soils.

A window of hope has finally opened in the form of a growing demand for organic food, world wide and this opportunity should not be missed and could be exploited for benefit of Indian farmers.

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Chapter 7

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