EFFECT OF HERBICIDES AND CHEMICAL CHARACTERISTICS OF SOIL ON MICROBIAL BIOMASS CARBON AND ENZYME ACTIVITY

by

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(2015 - 11 - 047)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

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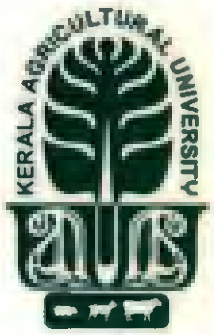

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF HORTICULTURE

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KERALA, INDIA

2017

DECLARATION

I, hereby declare that this thesis entitled **"EFFECT OF HERBICIDES AND CHEMICAL CHARACTERISTICS OF SOIL ON MICROBIAL BIOMASS CARBON AND ENZYME ACTIVITY"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"EFFECT OF HERBICIDES AND CHEMICAL CHARACTERISTICS OF SOIL ON MICROBIAL BIOMASS CARBON AND ENZYME ACTIVITY"** is a bonafide record of research work done independently by Ms. Amritha K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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31/08/2017

Amritha K.

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LIST OF ABBREVIATIONS

AICRP	All India Coordinated Research Programme
ARS	Agricultural Research Station
@	at the rate of
CD	Critical Difference
cm	centimetre
CRD	Completely Randomized Design
^0C	degree Celsius
dS m^{-1}	deci Siemen per metre
DAHA	Days after herbicide application
EC	Emulsifiable Concentrate
et al	and coworkers
Fig	Figure
g	gram
g L^{-1}	gram per Litre
g mol^{-1}	gram per mole
h	hour
IUPAC	International Union for Pure and Applied Chemistry
KAU	Kerala Agricultural University
kg	kilogram
kg a.i. ha^{-1}	kilogram active ingredient per hectare
kg ha^{-1}	kilogram per hectare
LD_{50}	Lethal Dose, 50 per cent or median lethal dose
Mg m^{-3}	Megagram per meter cube
m Pa	mega Pascal
m	metre

m^2	metre square
MBC	microbial biomass carbon
µg	microgram
µg C g^{-1} day^{-1}	micrograms of carbon produced per gram per day
µg N-NH_4 g^{-1} hr^{-1}	micrograms of ammoniacal nitrogen produced per gram of soil per hour
µg PNP g^{-1} hr^{-1}	micrograms of para nitro phenol produced per gram of soil per hour
µg TPF g^{-1} day^{-1}	micrograms of triphenyl formazon produced per gram soil per day
mg kg^{-1}	milligram per kilogram
mL	millilitre
mm	millimetre
M	Molar
MUB	Modified Universal Buffer
nm	nano metre
N	normal
NH_4OAc	Neutral normal ammonium acetate
No.	Number
O.M.	Organic matter
PNP	Para nitro phenol phosphate
%	Per cent
pH	Soil reaction
ppm	parts per million
r	Correlation coefficient
RSC	Royal Society of Chemistry
S_1	medium organic matter soils
S_2	high organic matter soils

SC	Soluble Concentrate
$t_{1/2}$	Half-life
THAM	Tris (hydroxymethyl) amino methane
TPF	Triphenyl formazon
TTC	2,3,5,-triphenyl tetrazolium chloride
USDA	United States Department of Agriculture
viz	namely

INTRODUCTION

1. INTRODUCTION

Rice as a staple food crop plays an important role in food as well as nutritional security particularly for Asian countries. India ranks second in rice production after China. It contributes 20 per cent of the world rice production (USDA, 2016). Demand for food grain is expected to increase with rise in world population. Area under rice in India is reported to be 44.1million ha, with a production of 106.64 million tones and productivity of 2416 kg ha^{-1}(Indiastat, 2015-16). To sustain and safegaurd food security in the country, the productivity of rice has to be enhanced under limited resources. Various biotic and abiotic stresses are the limiting factors in enhancing rice productivity. The major stress is imposed by competition due to weeds for water, nutrients, light, and space. Hence, weed management is indispensable in crop production.

Scarcity and high cost of labour for hand weeding has resulted in an increase in the use of herbicides in rice. Herbicidal weed control is efficient and less expensive compared to the other methods. Statistics on total world-wide consumption of pesticides revealed that, 47.5 per cent are in the form of herbicides followed by 29.5 per cent of insecticides and 17.5 per cent of fungicides. While in India, out of the total consumption of pesticides, herbicides (18 %) stands second position followed by insecticides. Sondhia (2014) studied the consumption of herbicides in different crops in India and found that rice ranks second position in herbicide consumption (31 %) followed by wheat (44 %).

Herbicides are used to enhance the productivity of crops by reducing weed competition but when entering into soil they directly affect soil microbes which ultimately deteriorate soil health. The soil microorganisms are monitoring to even minute changes in soil environment. Despite the beneficial impact of herbicides in improving and stabilizing rice productivity, these chemical residues are known to contaminate the soil and inhibit the enzyme activity (Aparna, 2011). Microbial

biomass carbon and activity of soil enzymes are considered to be two important biological parameters determining soil health

Soil microbial biomass, both source and sink of available nutrients plays an important role in nutrient transformation (Singh *et al.*, 1989). The direct and indirect effects of toxic chemicals on soil biology are reduction in population, and reduced mineralization of organic compounds. Urease, phosphatase and dehydrogenase are involved in transformation of nitrogen and phosphorus in soil and on microbial activity of soil (Rao *et al.*, 2015). Herbicide application had negative effect on soil microorganisms and soil enzymes like dehydrogenase, urease and phosphatase activity (Abbas *et al.*, 2015). Therefore, the estimation of biological parameters like microbial biomass carbon and activity of enzymes in the soil will be useful to understand the potential adverse effect of herbicide on soil quality and to predict the persistence of herbicide residues in the soil system under rice. Pendimethalin and oxyfluorfen are the most effective pre emergence herbicides for weed control in rice. Among the post emergence herbicides, bispyribac-sodium and cyhalofop-butyl are widely used in Kerala. These chemicals are recommended at the rate of 1.5 kg a. i. ha^{-1}, 0.15 kg a. i. ha^{-1}, 25g a. i. ha^{-1} and 0.08 kg a. i. ha^{-1} respectively (KAU, 2016).

Information on the above aspects are limited in the soils of Kerala. Therefore, the present research programme was formulated with the following objectives:

- To determine the impact of pre and post emergence herbicides on microbial biomass carbon and the soil enzymes namely dehydrogenase, phosphatase and urease.
- To study the influence of chemical characteristics of soil on these biotic components.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The literature pertaining to the present investigation on the "Effect of herbicides and chemical characteristics of soil on microbial biomass carbon and enzyme activity" is reviewed below under different headings.

2.1. TECHNICAL INFORMATION ON HERBICIDES

Herbicides emerged as an important weed management tool throughout the world. In India, herbicide use increased upto 30 per cent during the last 10 years. Soil is the repository of all types of chemical inputs including the herbicides. During the application of herbicides, a major portion of these chemicals accumulate in the surface layer of soil (0-15cm) where most of the microbiological activities occur (Das and Kole, 2006). Herbicides are toxic in nature; therefore their repeated use may cause residue problems and affect soil health. The fate of herbicide in soil depends on its adsorption, absorption, volatilization, leaching, runoff, photodecomposition, degradation by microbial and chemical process etc.

Persistence of herbicides in soil varies with the nature of chemicals, its solubility in water, molecular weight, complexity in chemical structure, and vapour pressure. The technical information of herbicides used in the study are given in Table 2.1 (RSC, 1987).

One of the most important factors determining fate of herbicide in the environment is its solubility in water. Water soluble herbicides generally have low adsorption capacities, and are consequently more mobile in the environment for microbial metabolism and other degradation processes. The hydrophobic character of a pesticide will increase by a decrease in its water solubility, thereby resulting in stronger adsorption on soil organic matter (Hance, 1965).

Table 2.1.1 Technical information on pendimethalin

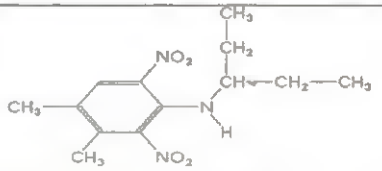
Common name	Pendimethalin
Chemical name	*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine (IUPAC) Coming under dinitroaniline group.
Chemical formula	$C_{13}H_{19}N_3O_4$
Chemical structure	
Molecular weight	281.31g mol^{-1}
Physical form	Orange yellow crystals
Melting point	54-58° C
Boiling point	330° C
Vapour pressure	4 m Pa (25° C)
Solubility	0.3mg L^{-1}(in water at 20° C), 700 g L^{-1} (in acetone) 628 g L^{-1} (in xylene)
Specific gravity	1.19 (25° C)
Trade names	Stomp, Panida
Stability	Very stable in storage, stable to acids and alkali, slowly decomposed by light.
Mammalian toxicity: Acute oral LD_{50} for rats	4050 mg kg^{-1}
Degradation and metabolism: In soil In plant	The 4- methyl group on the benzene ring is oxidised to carboxylic acid via the alcohol. The amino nitrogen is also oxidised. Half-life in soil is 3-4 months. The 4- methyl group on the benzene ring is oxidised to carboxylic acid via alcohol. The amino nitrogen is also oxidised. At harvest time, residues in crops are below the validated sensitivity of the analytical method (0.05ppm).

Table 2.1.2 Technical information on oxyfluorfen

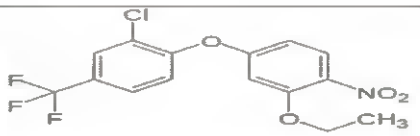
Common name	Oxyfluorfen
Chemical name	2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene (IUPAC) Coming under diphenyl ether group.
Chemical formula	$C_{15}H_{11}ClF_3NO_4$
Chemical structure	
Molecular weight	361.7 g mol^{-1}
Physical form	Orange crystalline solid
Melting point	84-85° C
Boiling point	358.2° C
Vapour pressure	0.026 m Pa (25° C)
Solubility	0.1 mg L^{-1} (in water at 25° C) Readily soluble in most of the organic solvents
Specific gravity	1.35 (73° C)
Trade names	Goal, Koltar
Stability	Stable in acid and alkaline media. Decomposed rapidly by ultra violet radiation.
Mammalian toxicity: Acute oral LD_{50} for rats	>5000 mg kg^{-1}
Degradation and metabolism: In soil	Strongly adsorbed on soil, not readily desorbed and shows negligible leaching. Photodecomposition in water is rapid and on soil is slow. Microbial degradation is not a major factor. Half - life in soil is approximately 30-56 days.
In plants	Oxyfluorfen is not readily metabolised in plants

Table 2.1.3 Technical information on bispyribac- sodium

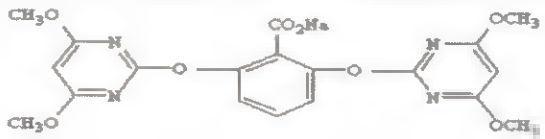
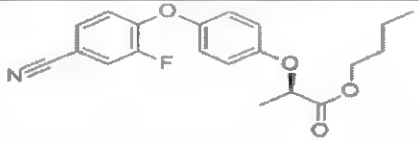
Common name	Bispyribac- sodium
Chemical name	Sodium 2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoate (IUPAC) Coming under pyrimidinyl-oxybenzoate family
Chemical formula	$C_{19}H_{17}N_4NaO_8$
Chemical structure	
Molecular weight	452.355 g mol^{-1}
Physical form	White powder
Melting point	223-224° C
Boiling point	-
Vapour pressure	5.05 $x10^{-6}$ m Pa (25° C)
Solubility	73.3 g L^{-1} at 20° C(in water) Slighly to moderately soluble in organic solvents
Specific gravity	1.29 (20° C)
Stability	Stable under normal conditions
Trade names	Nominee Gold, Taarak, Adora
Mammalian toxicity: Acute oral LD_{50} for rats	> 4111 mg kg^{-1}
Degradation and metabolism: In soil	Bispyribac-sodium will exist almost entirely in the anionic form at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important process for dissipation of chemical from the environment. Compound will exist in the anionic form in the environment and anions generally do not adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. Bispyribac-sodium is broken down by microbes and has a half-life (the time it takes for half of the active ingredient to degrade) of 42- 115 days.
In plant	Bispyribac sodium is a systemic herbicide that moves throughout the plant tissue and works by interfering with production of a plant enzyme necessary for growth, acetolactate synthase (ALS).

Table 2.1.4 Technical information on cyhalofop-butyl

Common name	Cyhalofop-butyl
Chemical name	Butyl (2R)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoate (IUPAC) Coming under aryloxyphenoxy-propionate herbicide
Chemical formula	$C_{20}H_{20}FNO_4$
Chemical structure	
Molecular weight	357.381 g mol^{-1}
Physical form	White crystalline solid
Melting point	49.5° C
Boiling point	>270° C
Vapour pressure	$5.3x10^{-2}$ m Pa
Specific gravity	1.172 (20° C)
Trade name	Clincher
Stability	Stable at pH 4, hydrolysed slowly at pH 7
Solubility	>250 (g L^{-1} at 20° C) in acetonitrile, dichloroethane, methanol, acetone, ethyl acetate; 6.06 in n-heptane; 16.0 in n-octanol. Solubility in water (mg L^{-1} at 20° C): 0.44
Mammalian toxicity: Acute oral LD_{50} for rats	>5000 mg kg^{-1}
Degradation and metabolism: In soil In plant	Half-lives of 3-11 hours in soil indicate microbial metabolism is rapid. Compound has low mobility in soil. The key metabolic transformation of cyhalofop-butyl involves hydrolysis of the butyl ester side-chain to give cyhalofop acid, and subsequent oxidation of cyano group to a carboxylic acid. It controls the weeds by inhibiting acetyl coenzyme-A carboxylase. The enzyme is responsible for the biosynthesis of fatty acids in selected grass species. This blockage of fatty acid production results in the loss of lipids and eventual death of the dividing cells in the growing point or tip of the grass.

Bailey *et al.* (1968) reported that within a chemical family the magnitude of pesticide adsorption is directly related to and governed by the degree of water solubility.

Abiotic chemical reactions rarely lead to appreciable changes in pesticide structure, but enzymatic reactions are responsible for major structural changes. Soil microorganisms are the major sources of such degradative enzymes, therefore microbial degradation is an important factor affecting the persistence of pesticides in soil (Kaufman *et al.*, 1985). Herbicides with low molecular weight and simple chemical structures are easily degraded and their persistence in soil is comparatively less.

The vapour pressure of herbicides determines its volatility. Herbicides with higher vapour pressure dissipate more rapidly than herbicides with lower vapour pressure (Curran, 2016).

Besides herbicide structure, soil properties and climatic conditions prevailing during and after the application of herbicides influence the fate of herbicides in the soil (Sondhia, 2009).

2.2 ECOLOGICAL SIGNIFICANCE OF SOIL MICROBIAL BIOMASS CARBON

Microbes constitute about one quarter of all living biomass on earth and are responsible for significant nutrient cycling of both macro and micro nutrients (Alexander, 1977) and therefore influencing nutrient availability and ultimately soil health and quality.

Microbial biomass carbon is a measure of the carbon (C) contained within the living component of soil organic matter. Microbes decompose soil organic matter releasing carbon dioxide and plant available nutrients. Soil microbial

biomass carbon comprises 1-5 per cent of total organic carbon (Zhang and Zhang, 2003). Because of its high turn-over rate, microbial biomass carbon responds more rapidly to changes in soil microclimate than soil organic matter (Powlson *et al.*, 1987). Microbial biomass carbon was the labile pools in soil and therefore, the nutrient availability and productivity of agro ecosystems mainly depends on their size and activity (Friedel *et al.*, 1996).

Most of the enzymatic transformations in soil are accomplished by microbial biomass due to which part of the organic materials are stabilized as humus and the remaining carbon and the other nutrients are utilized by micro-organisms for their growth and multiplication (Anderson and Domsch, 1980).

2.2.1. Effect of soil characteristics on microbial biomass carbon

Seasonal changes in soil moisture, temperature, and available residue had a strong influence on activity of soil microbial biomass (Diaz-ravina *et al.*, 1995).

Brookes (1995) mentioned that the soil microbial biomass is the main driving force in the decomposition of organic materials and is frequently used as an early indicator of changes in soil chemical and physical properties resulting from soil management practices and environment stress.

Soil microbial biomass carbon is closely associated with soil moisture (Patel *et al.*, 2010). Microbial biomass is a sensitive parameter and can be used as an early warning of changes in ecosystem before they are detectable in other ways.

As organic matter is the preferred energy source for the microorganisms, ecosystems with high organic substances tend to have higher microbial biomass content. Usually the highest microbial activity in soil takes place in the surface horizon compared to deeper horizons (Januszek, 2011). Ravindran and Yang

(2015) claimed that microbial biomass carbon is highest in the surface soil and declined with soil depth.

2.2.2. Effect of herbicide application on soil microbial biomass carbon

Hart (1995) concluded that the application of chemicals exhibit adverse impact on microbial biomass, and the severity was high in soil with low clay content, cation exchange capacity, organic carbon and organic matter content. Because of reduced sorptive capacity of soil, large portion of the applied chemical would be biologically available in soil.

The active soil microorganisms utilize the herbicides as well as their degraded products for their growth and metabolism (Das and Debnath, 2006). Das *et al.* (2012) reported that application of herbicides at their recommended rates significantly stimulated the activities of microorgainsms in soil resulting in greater accumulation of microbial biomass carbon.

Bera and Ghosh (2013) mentioned that microorganisms are able to degrade herbicides and utilize them as a source for their own physiological processes. However, before degradation, herbicides have toxic effects on microorganisms, reducing their abundance, activity and consequently, the diversity of their communities in soil. Dipika (2014) also reported the adverse effect of herbicides on soil microbial biomass carbon.

2.3 ECOLOGICAL SIGNIFICANCE OF SOIL ENZYMES

Soil enzymes are constantly being synthesised, accumulated, inactivated and decomposed in the soil, hence playing an important role in agriculture and particularly in nutrient transformations (Tabatabai and Bremner, 1969). Kiss *et al.* (1975) reported that enzymes accumulated in soil are present as free enzymes,

such as exoenzymes released from living cells, endoenzymes released from disintegrating cells, and enzymes bound to cell constituents such as disintegrating cells, in cell fragments, and in viable but non proliferating cells. Soil enzymes are catalysing several important reactions necessary for the life processes of microorganisms in soil.

Soil enzymes are a group of enzymes that are usual inhabitants in the soil playing a major role in maintaining soil ecology, physical and chemical properties, fertility, and soil health (Zornoza *et al.*, 2009). Soil is a living system where all biochemical activities proceed through enzymatic processes. Enzyme activity is a soil property that is chemical in nature, but has a direct biological origin. Soil enzymes may originate from plants, animals or microbes and can exist in bound or free form within the soil.

Soil enzymatic activities are recognised as more sensitive bio-indicators than plants and animals for any disturbance in soil (Hinojosa *et al.*, 2004). These enzymes have been suggested as suitable indicators of soil quality because they are strictly related to the nutrient cycles and transformations, and they have high sensitivity to the changes caused by both natural and anthropogenic factors (Gianfreda *et al.*, 2005).

Soil enzymes are strongly connected with soil organic matter and microbial activity. It is obvious that any action altering the life functions of soil organism could indirectly affect soil enzyme activities. Soil enzymes are specific with respect to the types of reactions they participate. Fornasier *et al.* (2014) opined that activity of soil enzymes can be used as an index of microbial growth and activity in soils.

Enzyme persistence in the soil depending on the location and soil conditions *viz.*, soil depth, temperature, acidity, particle size distribution and organic matter (Ekenler and Tabatabai, 2003).

Enzyme activities are usually greater in organically than in inorganically fertilized soils (Gianfreda and Bollaga, 1996). Enzyme activities in the coarse sand fraction were more sensitive to the application of fertilizers than those in finer fractions (Liang *et al.*, 2014).

The degraded pesticides interact with the soil and with its indigenous microorganisms, thus altering its microbial diversity, biochemical reactions and enzymatic activity and affect the overall soil health and quality (Hussain *et al.*, 2009). Most of the herbicides applied to the soil had inhibitory effect on soil enzyme activity (Sebiomo *et al.*, 2011).

Enzymes participate in the biodegradation of natural and anthropogenic organic compounds in soil, and are often used as indicators of changes that occurs in the soil environment in response to crop protection chemicals, including herbicides (Bacmaga *et al.*, 2012). Wahsha *et al.* (2016) observed that enzyme activities in soil varied widely depending on the sampling locations, and that the presence of contaminants is reflected in reduced enzymatic activity.

2.4 SOIL DEHYDROGENASE ENZYME

Dehydrogenases exist in soil as integral parts of intact cells and reflect the total range of oxidative activities of the soil micro flora. Dehydrogenase activity is a measure of the oxidation- reduction capability of the active soil microbial biomass (Shaw and Burns, 2006). According to Gomez *et al.* (2009), dehydrogenase activity is the most robust indicator of the physiological status of soil microbes because dehydrogenase is present in all living cells, unlike other enzymes which are mostly extracellular.

Dehydrogenase is involved in biological oxidation of soil organic matter, and is utilised for dehydrogenation of organic matter by transferring hydrogen and

electrons from substrates to acceptors (Maurya *et al.*, 2011). The enzyme activity is more in the surface layer of soil. Dehydrogenases, as oxidoreductase enzymes, play the major role in the energy production of organisms.

2.4.1. Effect of soil characteristics on dehydrogenase activity

Several environmental factors, including soil moisture, oxygen availability, oxidation- reduction potential, pH, organic matter content, depth of soil profile and temperature can significantly affect dehydrogenase activity in the soil environment.

The reduced water availability can inhibit the dehydrogenase activity by lowering intracellular water potential, and thereby lowering the hydration and enzyme activity (Wall and Heiskanen, 2003). Dehydrogenase activity is strongly affected by soil moisture. The metabolic activity and the survival of microorganisms are also strongly influenced by the availability of water content in soil (Uhlirova *et al.*, 2005). Gu *et al.* (2009) reported that dehydrogenase activity is higher in flooded soil than in non-flooded conditions. As soil becomes dry, the water potential increases, and as well microbial activity as intracellular enzyme activity reduces (Geisseler *et al.*, 2011).

Oxygen diffusion rate (ODR) is considered to be the most crucial regulator of microbial activities (Hutchinson, 1995). Brzezinska *et al.* (1998) indicated that among the all aeration parameters, redox potential (Eh) plays a vital role in determining the level of soil dehydrogenase activity. Increase of soil dehydrogenase activity is indirectly connected with decrease of redox potential values. Stepniewski *et al.* (2000) concluded that decrease of soil water content cause significant increase of ODR and redox potential. Low ODR level was favourable and congenial for soil dehydrogenase activity. Wolinska and Bennicelli (2010) also reported the importance of dehydrogenase and their dependency on soil aeration status.

Pascual *et al.* (2000) found that soils characterized with low microbial and biological activity, also display the lowest dehydrogenase activity. Soil organic matter is an indicator of soil quality, because of its role as nutrient sink and source that can enhance soil physical and chemical properties, and also promote biological activity (Salazar *et al.*, 2011). The activity of soil enzymes were strongly connected with the availability of organic matter in the soil. The presence of higher organic matter level in soil can provide enough substrate to support higher microbial biomass, hence higher enzyme production (Yuan and Yue, 2012). There was a positive correlation between dehydrogenase activity and organic matter content in the soil.

According to Frankenberger and Johanson (1982), the lowering of enzymatic activity in soil with the increase of soil acidity is the impact of destroying ion and hydrogen bonds in enzyme active centre. Generally, the soil enzyme activities tend to increase with soil pH (Blonska, 2010). Natywa and Selwet (2011) noted positive correlation between dehydrogenase activity and pH in soils under maize growth at pH ranged from 5.17 to 7.27.

pH affects soil enzyme activity level in three different ways *viz.*, by changing the ionic form of the active sites of the enzymes, which alter the enzyme activity and their reaction rate, by altering the three dimensional shape of enzymes and by affecting the affinity of the substrate to the enzyme. Thus, the pH factor is considered to be the predictor of dehydrogenase activity in the soil environment (Moeskops *et al.*, 2010).

Dehydrogenase is present inside the viable microbial cells and its activity must be highest at temperature between 20° C to 30° C, which was close to the optimum temperature for microbial growth, activity and development (Wolinska and Stepniewska, 2011). The rate of enzyme catalysis increases with increase in

temperature until the unfavourable temperature, at which enzyme becomes denaturized.

Depth of the soil profile is the environmental factor reducing soil dehydrogenase activity level. Xiang *et al.* (2008), who observed that dehydrogenase activity level was roughly four fold higher in surface than in subsurface soil. Microorganisms occurrence is higher in the surface layer of the soil profile. At the lowest part of the soil the number of microbial cells is limited, and consequently dehydrogenase activity level displayed a diminishing trend.

Soil dehydrogenase activity was negatively correlated with soil water potential, oxygen diffusion rate, and redox potential (Wolinska and Stepniewska, 2011). Dehydrogenase activity reached superior values at lower soil water potential, lower oxygen diffusion rate and lower redox potential conditions. Soil characteristics such as organic matter and soil texture are known to affect dehydrogenase activity. Organic carbon, soil moisture, available magnesium content, and pH were most closely correlated with dehydrogenase activity (Pereira *et al.*, 2013).

2.4.2. Effect of herbicide application on dehydrogenase activity

Strzelec (1986) reported the inhibitory effect of herbicides on the activity of dehydrogenase enzyme level in soil. Dehydrogenase activity was proved to be an important predictor of side effects that are associated with the use of sulfonyl urea herbicides (Pampulha and Oliveira, 2006). Fertilizer application and pesticide use can adversely affect the dehydrogenase activity at a significant level in the soil environment (Kumar *et al.*, 2013).

Dehydrogenase is negatively correlated to the herbicide concentrations. Dehydrogenase was least tolerant to the effect of the herbicides and even the recommended dose of herbicide affected the dehydrogenase activity (Singh,

2014). Application of post emergence herbicide cyhalofop-butyl reduces the dehydrogenase activity level in soil soon after its application (Dipika and Chowdhury, 2015). Mariora *et al.* (2015) found that application of metsulfuron methyl greatly inhibited the dehydrogenase activity.

2.5 SOIL PHOSPHATASE ENZYME

Soil phosphatase is a group of enzymes that involved in hydrolyses of the esters of phosphates and anhydrides to ortho phosphoric acid. The enzymes are classified as acid and alkaline phosphatases, because of their optimum activities in their respective pH ranges. Alkaline phosphatase activity is by microorganism alone, while acid phosphatase is contributed both by the plant roots as well as soil inhabiting microbes (Chhonkar *et al.*, 2007)

Phosphatase enzymes originating from microorganisms and as well as from the root exudates, cleaves the phosphate from organic substrates and is involved in the phosphorus cycle in soil (Huang *et al.*, 2011).

2.5.1 Effect of soil characteristics on phosphatase activity

There is a positive relationship between phosphatase and organic matter content in the soil environment since the enzyme was bound to humic- protein complex (Harrison, 1983).

Phosphatase activities were correlated with the content of organic matter and decreased with soil depth (Tabatabai and Dick, 1979).

Phosphatase showed the highest activities in coarse sand, and lowest in silt fraction. Mc Laren (1975) also reported a negative correlation with clay and silt content of soil and phosphatase level. Phosphatase activity was mainly associated

with larger soil fractions which containing plant debris and less humified organic matter.

Zibilske and Bradford (2003) have found significant correlation between phosphatase activity, extractable P and dissolved organic carbon in soil.

Phosphatase activity is also influenced by the available soil phosphorus status (Nowak *et al.*, 2006).

2.5.2 Effect of herbicide application on phosphatase activity

Voets *et al.* (1974) reported 61.8 per cent decrease in phosphatase activity due to atrazine. Tu (1981) observed a decrease in phosphatase activity level by using 2,4-D herbicide. Phosphatases are among those soil enzymes that respond negatively to herbicide application, the activity lowered by the impact of applied herbicides (Wyszkowska and Kucharski, 2004).

Dipika and Chowdhury (2015) reported that the application of cyhalofop-butyl significantly reduced the acid phosphatase activity at highest level soon after its application.

Abbas *et al.* (2015) reported that buctril super (bromoxynil) herbicide is injurious for soil microorganisms and phosphatase enzyme activity. Maximum phosphatase activity was reported in soil without herbicide application.

2.6 SOIL UREASE ENZYME

Urease plays a major role in catalyses the hydrolysis of urea to carbon dioxide and ammonia, and had an important role in the nitrogen cycling. Urease enzyme is widely distributed in nature and has been detected in plants, animals and microorganisms.

Bremner and Mulvaney (1978) found that urease is unique among soil enzymes and the enzyme extracted from the soil as enzyme- humus conjugate and it represents 20 to 40 per cent of total activity in soil.

2.6.1 Effect of soil characteristics on urease activity

Urease activity in soil is influenced by many factors including crop history, organic matter, heavy metals, soil temperature, and pH (Yang *et al.*, 2006).

Urease activity in soils was positively correlated with organic carbon and cation exchange capacity (Dalal, 1975). Hussain *et al.* (1990) also reported significant positive correlation of urease activity with total nitrogen and organic carbon content in soil and no profound correlation with clay, sand and silt content and concluded that organic matter content of the soil is the major factor controlling variation in urease activity level in soil. Soil enzymes appeared to be immobilized on soil organic matter and soil organic matter is considered as an index of both organic carbon and total nitrogen content. Significant correlation between enzyme activity and organic carbon and total nitrogen were also recorded by Frankenberger and Tabatabai (1991). Zelles *et al.* (1992) concluded that activity of urease existing as extracellular enzyme in soil may increase with organic carbon content.

Urease activity is preferably higher in paddy soil with abundant organic matter and higher levels of total nitrogen content (Zeng Lu- Sheng *et al.*, 2005).

Dash *et al.* (1981) observed a negative correlation between urease activity with soil pH and moisture content. Shahinrokhsar *et al.* (2008) studied the soil properties influencing urease enzyme activity and concluded that the urease enzyme activity in soil showed a negative correlation with soil pH. This is mainly

because urease activity decreases with increase in pH of the soils and has more suitability with acid soil conditions but extremely acidic condition affects the activity. Joa *et al.* (2010) found that the soil pH value was lowered with increasing dose of inorganic fertilizers. This is due to the development of soil acidity which caused compositional change in microbial community and hence, a decrease in soil urease activity.

The significant correlation between urease activity and clay content could be due to the fact that the enzymes are adsorbed on clays and are thus protected from denaturation, and are thus assayed for expression of their activities (Mortland, 1970). Urease activity was predominant in clay-size fractions of soil than other fractions like sand and silt, this mainly depending on mineral sorption processes rather than on substrate availability (Kandeler *et al.*, 1999). Jayan (2012) also found that soil urease activity was significantly and positively correlated with clay content and there was no significant correlation with silt content.

Zantua *et al.* (1977) reported that the urease activity in soil was positively correlated with total nitrogen content. Guan (1987) reported that more availability of nitrogen resource for soil microorganisms resulted in higher soil urease activity because of substrate availability.

Tabatabai (1977) reported a decrease in urease activity at lower depth in the soil profiles.

2.6.2 Effect of herbicide application on urease activity

The inhibition of soil urease activity is probably by the effect of herbicide. It could be due to competitive or non- competitive inhibition by the applied herbicides (Letherberg and Burns, 1976). Soil urease which was extracellular was

deactivated more quickly than the part which was an integral part of soil humus complex.

Urease was found to be most resistant and dehydrogenases were least resistant to soil contamination with a mixture of herbicides *viz.*, diflufenican + mesosulfuron-methyl + iodosulfuron-methyl-sodium (Bacmaga *et al.*, 2015).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The research programme entitled "Effect of herbicides and chemical characteristics of soil on microbial biomass carbon and enzyme activity" consisted of pot culture experiment and soil analysis. The present study was undertaken with the objective to determine the impact of pre and post emergence herbicides on microbial biomass carbon and the soil enzymes namely dehydrogenase, urease and phosphatase and also to study the influence of chemical characteristics of the soil on microbial biomass carbon and enzyme activity. Pot culture experiment was conducted with rice crop at net house attached to Radio Tracer Laboratory, College of Horticulture, Vellanikkara. The analysis of physical, chemical, and biological characteristics of soil samples were carried out at the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Vellanikkara. The materials used and the methods followed for the studies are summarized below:

3.1 POT CULTURE EXPERIMENT WITH RICE (Variety: Jyothi)

3.1.1 Collection and processing of soil samples

The soil samples for pot culture experiments were collected from four different locations in Thrissur district *viz.,* rice field of Agricultural Research Station, Mannuthy (soil with medium organic matter content with a history of herbicide application), non-cropped area of Agricultural Research Station, Mannuthy (soil with medium organic matter content without a history of herbicide application), rice field of Kole area, Alappad (soil with high organic matter content with a history of herbicide application), and non- cropped area of Kole land, Alappad (soil with high organic matter content without history of herbicide application).

Approximately 400 kg of representative soil samples (0-0.15m depth) were collected from four different locations in sacks. These soil samples were air dried, processed and sieved through 2mm sieve and kept for pot culture

experiment and also for soil analysis. Soil samples for preliminary analysis were preserved in polythene covers. Notations used for the soil samples for preliminary analysis are furnished in Table 3.1

3.1.1.1 Details of the four sampling locations

1) Rice field of Agricultural Research Station, Mannuthy

The soil sampling site at the Agricultural Research Station Mannuthy was a double cropped paddy land which was under the long term herbicide trial for fourteen years from 2001 to 2014. The entire area under the experiment was ploughed well after the harvest of second crop of rice of 2014, so as to homogenise the experimental area for conducting other weed control experiments in rice. The first crop of rice was taken during April-May to August-September and second crop during September-October to December-January every year. The land is usually left fallow during the summer season. Cyhalofop-butyl (Clincher®), chlorimuron ethyl 10 % + metsulfuron methyl 10 % (Almix®), butachlor (Butachlor®), pretilachlor (Refit®), 2,4-D (Fernoxone®), and bispyribac-sodium (Nominee Gold®) were the major herbicides used in rice fields. *Ludwigia perennis* (neer-karayambu), *Cyperus rotundus* (muthanga), *Gomphrena decumbens* (neervadamalli), *Paspalum conjugatum* (sour grass) and *Echinochloa* spp.were the major weeds observed in the rice field (Plate 1).

2) Non-cropped area of Agricultural Research Station, Mannuthy

This site was located near the jack gene sanctuary of Agricultural Research Station Mannuthy. The lands were protected from agrochemical use because it was attached to model organic farm. Major vegetation noticed in this site are *Fimbristylis miliacea* (mungai), *Cyperus* spp., *Sida acuta* (kurunthotti), *Aerva lanata* (balipoovu), *Cleome viscosa* (wild mustard), *Clerodendron infortunatum* (perukilam), *Ageratum conyzoides* (goat weed), and *Kyllinga monocephala* (white kyllinga) (Plate 2).

Plate 1. Rice field of Agricultural Research Station, Mannuthy

Plate 2. Non -cropped area of Agricultural Research Station, Mannuthy

3) Rice field of Kole land, Alappad

In the Alappad Kole, the soil sampling field was single cropped land, where rice was grown during September-October to February-March. The land is inundated with water during the rest of the year. Chemical weed control is indispensable for rice production in this area. Herbicides used in this area are cyhalofop-butyl (Clincher®), butachlor (Butachlor®), pretilachlor (Refit®), 2,4-D (Fernoxone®), and bispyribac-sodium (Nominee Gold®).The major weeds observed in this site are *Leptochloa chinensis* (kuthiravalli), *Ludwigia perennis* (neer-karayambu), *Echinochloa* spp. (kavada), *Cyperus iria* (manjakora), *Lindernia crustacea* (kakkapovu), and *Monochoria vaginalis* (karimkoovalam) (Plate 3).

4) Non-cropped area of Kole land, Alappad

Non-cropped area selected for soil sampling was from farmer's field (Mr. Kesavaraj, Purathur) in Alappad Kole lands of Thrissur. Major vegetation observed were *Ludwigia perennis* (neer-karayambu), *Lindernia crustacean* (kakkapovu), and *Fimbristylis miliacea* (mungai). Soils in this area were high in organic matter and there was no history of herbicide application (Plate 4).

Representative soil samples were taken from the above mentioned areas during March-April, 2016.

Plate 3. Rice field of Kole land, Alappad

Plate 4. Non- cropped area of Kole land, Alappad

Table 3.1 Details of soil samples/ soils taken for the preliminary analysis

Description of soil sample	**Notation**
Soil with medium Organic Matter content (Rice field of ARS, Mannuthy) with a history of herbicide application	Rice field, ARS (S_1): Control
Soil with medium Organic Matter content (Non cropped area of ARS, Mannuthy) without a history of herbicide application	Non-cropped area, ARS (S_1): Absolute control
Soil with high Organic Matter content (Rice field of Kole land, Alappad) with a history of herbicide application	Rice field, Kole (S_2): Control
Soil with high Organic Matter content (Non-cropped area of Kole land, Alappad) without a history of herbicide application	Non-cropped area, Kole (S_2): Absolute control

3.1.2 Experimental details of pot culture experiment

Pot culture experiment (Plate 5) consisted of following treatments

Treatments

A) Soil type (2)

1) Soil with medium organic matter content (Rice field of ARS, Mannuthy) with a history of herbicide application
2) Soil with high organic matter content (Rice field of Kole area, Alappad) with a history of herbicide application

B) Herbicides (4)

1) Pendimethalin @ 1.5 kg a. i. ha^{-1} } Pre emergence
2) Oxyflourfen @ 0.15 kg a. i. ha^{-1} }
3) Cyhalofop- butyl @ 0.08 kg a. i. ha^{-1} } Post emergence
4) Bispyribac-sodium @25g a. i. ha^{-1} }

C) Control (4)

1) Soil with medium organic matter content (Rice field of ARS, Mannuthy) with a history of herbicide application
2) Soil with medium organic matter content (Non-cropped area of ARS, Mannuthy) without a history of herbicide application
3) Soil with high organic matter content (Rice field of Kole area, Alappad) with a history of herbicide application
4) Soil with high organic matter content (Non- cropped area of Kole lands, Alappad) without a history of herbicide application

Design: Factorial experiment in CRD

Total number of treatments: 12(4x2+4) (Treatment details are given in Table 3.2)

Replications: 6

Total number of pots: 72

Mud pots of 25 cm diameter and 25 cm depth were taken for the study. The pots were filled with soil (5kg pot^{-1}).

Plate 5: General view of pot culture experiment

Table 3.2 Treatment details of pot culture experiment

Soil	**Treatment**	**Notation**
Medium O.M. (S_1) soil with a history of herbicide application (Rice field)	Pendimethalin	S_1 pendi
	Oxyfluorfen	S_1 oxy
	Cyhalofop butyl	S_1 cyhalo
	Bispyribac sodium	S_1 bispyri
	Control	S_1 control
Medium O.M. (S_1) soil without a history of herbicide application (non-cropped)	Absolute control	S_1 absolute control
High O.M. (S_2) soil with a history of herbicide application (Rice field)	Pendimethalin	S_2 pendi
	Oxyfluorfen	S_2 oxy
	Cyhalofop butyl	S_2 cyhalo
	Bispyribac sodium	S_2 bispyri
	Control	S_2 control
High O.M. (S_2) soil without a history of herbicide application (non-cropped)	Absolute control	S_2 absolute control

3.1.2.1 Variety

Short duration rice variety Jyothy (PTB 39) was used as test crop. The cultivar used was red kernelled, long bold type with 110-115 days duration, Moderately tolerant to BPH (Brown plant hopper- *Nilaparvata lugens*) and blast disease (Causal organism: *Pyricularia grisea*) and Moderately susceptible to sheath blight (Causal organism: *Rhizoctonia solani*). This cultivar was suitable for direct seeding, transplanting, and special systems of Kole and Kuttanad. Seeds for pot culture experiments were procured from Krishi Vigyan Kendra, Pattambi, Palakkad.

Seeds are soaked for 12 hours in a solution of *Pseudomonas fluorescenes* (10 g per litre of water per kg of seeds). Pre-germinated rice seeds were sown on the third day and nine plants were maintained in each pot (Plate 6).

The soil was kept under submerged condition by maintaining 3 cm water above the soil surface and the water level was maintained throughout the rice growing period except during the application of herbicides (moisture was maintained at saturation level during the application of herbicides for the homogenous distribution of treatments and same moisture is maintained three more days for the maximum adsorption of chemicals on soil particles).

3.1.2.2 Manures and Fertilizers

Organic manures in the form of farmyard manure were collected from local farmers (Mr.Devan) near the College campus, Vellanikkara. Liming materials were collected from local market. Fertilizers like urea, Rajphos and muriate of potash were procured from local market to supplement major nutrients like nitrogen, phosphorus and potassium respectively.

Farmyard manure was mixed with soil and first dose of lime was applied as basal dressing and second dose was applied as top dressing about one month after sowing. Organic manure, lime and fertilizers were applied according to the

(6 a)

(6 b)

(6 c)

Plate 6. Rice at different growth stages a) germination, b) seedling and c) flowering stage

Package of Practice Recommendations of Kerala Agricultural University for wet land direct seeded rice crop (KAU, 2016).

3.1.2.3 Herbicide application

Herbicides for the experiments were procured from M/S Kavungal agency, Mannuthy, Thrissur. Details of herbicides used for the pot culture experiments are summarized in Table 3.3.

Pre emergence herbicides like pendimethalin @ 1.5 kg a. i. ha^{-1} and oxyfluorfen @ 0.15 kg a. i. ha^{-1} were applied at six days after sowing (Plate 7) and post emergence herbicides cyhalofop- butyl @ 0.08 kg a. i. ha^{-1} and bispyribac-sodium @25g a. i. ha^{-1} at 16-18 days after sowing (Plate 8). Quantity of herbicide formulations (Panida® 30 % EC for pendimethalin, Goal® 23.5 % EC for oxyfluorfen, Clincher® 10 % EC for cyhalofop- butyl and Nominee Gold® 10 % SC for bispyribac-sodium) required for each pot was measured, mixed with water and applied using plastic vial with holes to each pots (2 m L per pot).

3.1.2.4 Plant protection

According to the requirement, plant protection measures were carried out as per the Package of Practices Recommendations (KAU, 2016).

3.1.2.5 Harvesting

Harvesting was done at 110 days after sowing. The crop harvested from each pot was threshed and weight was recorded for the separated grains and straw. Yield was worked out on per pot basis.

3.3 Details of herbicides used for the study

Name of the chemical	**Trade name**	**Active ingredient content and formulation**	**Colour code**	**Manufacturer**
Pendimethalin	Panida	30 % EC	Blue	Rallis India Limited, Nariman Bhavan, Mumbai.
Oxyfluorfen	Goal	23.5 % EC	Green	Dow Agro Sciences India Private Limited, Mumbai.
Cyhalofop-butyl	Clincher	10 % EC	Green	Dow Agro Sciences India Private Limited, Mumbai.
Bispyribac-sodium	Nominee Gold	10 % SC	Blue	Kumiai Chemical Industry Co. Limited, Japan.

Plate 7. Application of pre emergence herbicides (oxyfluorfen and pendimethalin)

Plate 8. Application of post emergence herbicides (cyhalofop- butyl and bispyribac- sodium)

3.2 SOIL ANALYSIS

Laboratory analysis includes analysis of soil before experimentation and during the period of experimentation.

3.2.1 Analysis of soil before experimentation

3.2.1.1 Physical characteristics of soil

Major physical characteristics of the soil *viz.*, soil texture, water holding capacity, bulk density, particle density, porosity, and volume of expansion were analysed before starting the experiments and the methods adopted for the analysis are given in Table 3.4

3.2.1.2 Chemical characteristics of soil

Chemical characteristics of the soil *viz.*, pH, electrical conductivity, organic carbon, cation exchange capacity, available N, P, K, Ca, Mg, and micro nutrients like Fe, Mn, Cu, and Zn were analysed before starting the experiments and the methods adopted for the analysis are given in Table 3.5.

Table 3.4 Methods used for estimation of physical characteristics of soil

Characteristic	Method	Reference
Soil texture	International pipette method	Robinson, 1922
Water holding capacity	Keen- Raczkowski brass cup method	Piper, 1942
Bulk density		
Particle density		
Porosity		
Volume of expansion		

Table 3.5 Methods used for estimation of chemical characteristics of soil

Characteristic	Method		Reference
	Extraction	Estimation	
pH	1:2.5 Soil Water suspension	Potentiometry	Jackson, 1958
Electrical Conductivity		Conductometry	
Organic carbon	Wet digestion		Walkely and Black, 1934
Cation Exchange Capacity	Summation method		Hendershot and Duquette,1986
Available N	Alkaline permanganometry		Subbiah and Asija, 1956
Available P	Bray No. 1	Colorimetry	Bray and Kurtz, 1945
Available K	1N NH_4OAc	Flame photometry	Jackson, 1958
Available Ca		ICP OES (Model: Optima® 8x00 series)	
Available Mg			
Available micronutrients (Fe, Mn, Cu, and Zn)	0.1 N HCl		Sims and Johnson, 1991

3.2.1.3 Biological characteristics of soil

Microbial biomass carbon, activity of enzymes *viz.*, dehydrogenase, urease and phosphatase were analysed before starting the experiments and the methods adopted for the analysis are given below:

1. Microbial biomass carbon

Microbial biomass carbon was determined by following a procedure described by Jenkinson and Powlson (1976).

Five sets of 10 g soil were weighed for each sample and taken in 25 ml beakers. Moisture content was determined gravimetrically for one set of sample. Of the remaining four sets of soil, two sets were subjected to chloroform fumigation, which causes cell walls of microbes to lysis and denature the cellular contents. While another two sets of sample were un-fumigated.

For the fumigation distilled chloroform was prepared. Chloroform was taken in a separating funnel and washed two times with concentrated sulphuric acid (each with half the volume of chloroform) and discarded the acid bottom phase carefully after phase separation. Precaution was taken to open the stop-cock after each shaking to release the pressure inside. It was washed twice with the same volume of distilled water and collected the bottom white coloured phase. These washings were given to make the chloroform free of ethanol. This ethanol free chloroform was kept in 250 ml porcelain dishes placed at bottom portion of vacuum desiccator (Plate 9). Few glass beads were kept in the dishes to reduce the bumping. All the beakers containing soil were kept in a vacuum desiccator. Inner surface of the desiccator was lined with moistened filter paper.

Vacuum pump was connected to the desiccator until the chloroform boils. After that outlet was closed and vacuum pump was switched off and kept the desiccator in dark for 24 h. Next day the vacuum was released and the beaker containing chloroform and inner paper lining was taken out. Back suction was

Plate 9: a) Vacuum desiccator b) inside of the desiccator where soil samples kept in beakers c) bottom portion where distilled chloroform kept in porcelain dishes

performed for five to six times to ensure removal of excess adhered chloroform vapour.

Both the fumigated and un-fumigated soils were extracted with 0.5M K_2SO_4. To each soil sample, 25 ml of 0.5M K_2SO_4 was added and shaken for 30 minutes. The soil suspension was filtered through Whatmann No.1 filter paper. Filtrate (10 ml) was transferred to 500 ml round bottom flask. Reagents *viz.*, 0.2N $K_2Cr_2O_7$ (4 ml), concentrated H_2SO_4 (10 ml) and orthophosphoric acid (5 ml) were added to each flasks. Distilled water (10 ml) was used as blank. The flasks were kept on hot plate at 100° C for 30 minutes under reflux. Then 250 ml distilled water was added immediately to stop the reaction. The contents were allowed to cool to room temperature. Two to three drops of ferroin indicator was added and titrated the contents against 0.05 N ferrous ammonium sulphate to get a brick-red end point.

Microbial biomass carbon (MBC) in the soil was calculated using the following formula:

$$\mathbf{MBC\ (\mu g\ g^{-1}\ soil) = EC_F - EC_{UF} / K_{EC}}$$

Where EC_F: Extractable C in the fumigated samples

EC_{UF}: Extractable C in the un-fumigated samples

$K_{EC} = 0.25 \pm 0.05$ and K value was derived based on the efficiency of extraction of microbial biomass carbon.

2. Dehydrogenase activity

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

About 1g of air dried soil was weighed to and taken in an air tight screw capped test tube of 15 ml capacity, to which 0.2 ml of 3% triphenyl tetrazolium chloride solution was added to saturate the soil. Then 0.5 ml of 1% glucose solution was added in each tube. Gently tapered the bottom of the tube to drive

out all trapped oxygen, and thus a water seal was formed above the soil and ensured that no air bubbles were formed in tube. Tube was incubated at 28±0.5° C for 24 h. After incubation, 10 ml methanol was added and the contents were vigorously shaken for proper mixing. Samples were allowed to stand for six hour. Clear pink coloured supernatant was removed and the readings were taken with a spectrophotometer at a wave length of 485 nm.

A series of standards were used for preparing the calibration curve. The results were expressed in terms of Triphenyl formazan hydrolysed g^{-1} of soil 24 hrs^{-1} in micrograms.

3. Urease activity

The urease activity of the soil samples were estimated according to a method suggested by Tabatabai and Bremner, 1972.

To 5g soil in a 50 ml volumetric flask, 0.2 ml toluene, and 9 ml of THAM buffer (Tris (hydroxymethyl) amino methane) were added and shaken for few seconds to mix the contents properly. Then, 0.2M urea (1 ml) solution was added and again swirled for few seconds and kept for incubation at 37° C for 2h. After 2 hours, approximately 35 ml of KCl-Ag_2SO_4 solution was added. Then the contents were mixed properly and allowed to stand until the contents have cooled to room temperature. Final volume was made up to 50 ml by the addition of KCl-Ag_2SO_4 solution. Stoppered the flask and inverted several times to mix the contents. To determine ammoniacal nitrogen in the resulting suspension, 20 ml of the aliquot of suspension was pipetted into a 100 ml distillation flask and determined the ammoniacal nitrogen released by steam distillation of this aliquot with 0.2 g of MgO for four minutes.

Controls were performed as per the same procedure described for assay of urease activity. But 1ml of 0.2M urea solution was added after the addition of 35 ml of KCl-Ag_2SO_4 solution.

4. Phosphatase activity

Phosphatase activity was estimated as per the procedure defined by Tabatabai and Bremner, 1969.

For each sample, four sets of 1 g soil were weighed in 50 ml volumetric flasks. Two sets were taken for the assay of acid phosphatase and remaining two sets for alkaline phosphatase. Among the two, one was kept as control. Toluene (0.2 ml) and MUB buffer (4 ml) (for the acid phosphatase MUB buffer at pH 6.5 and for alkaline phosphatase pH 11) were added to samples. After that for one set of samples, 1 ml of p- nitro phenyl phosphate solution (p-nitro phenyl phosphate was prepared with MUB buffer 6.5 for acid phosphatase and MUB buffer 11 for alkaline phosphatase) was added. Contents were mixed properly and kept for incubation for 1h. After incubation, 1 ml $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The contents were swirled for few seconds for mixing. Para nitro phenyl phosphate solution prepared using the respective buffer was added to the remaining set (control) of samples.

All the suspensions were filtered quickly through Whatmann No. 2 filter paper. The yellow colour intensity of the filtrate was measured using spectrophotometer at a wave length of 440 nm.

3.2.2 Analysis of soil during the period of experimentation

During the pot culture studies, soil samples were collected at six intervals (on the day but two hour before application of herbicides, 7, 15, 30, 60 days after application and at the day of harvest).

About 50 g soil samples were taken from three sites in each pot at a depth of 15 cm and the pooled samples were used for analysis.

3.2.2.1 Chemical characteristics of soil

Chemical characteristics of the soil *viz*., pH, electrical conductivity and organic carbon, were analysed at six intervals (on the day but two hour before application, 7, 15, 30, 60 days after application and at the day of harvest) during the period of experimentation and the methods adopted for the analysis were same as given in Table 3.5.

3.2.2.2 Biological characteristics of soil

Microbial biomass carbon, activity of dehydrogenase, urease and phosphatase were analysed on the day but two hour before application, 7, 15, 30, 60 days after application and at the day of harvest. The methods adopted for the analysis were same as given in section 3.2.1.3.

3.2.2.3 Plant characters

1. Biometric observations on rice

Height of the plant and productive tillers were recorded at 60 days after sowing.

2. Yield and yield attributes

Grain and straw yield were estimated after the completion of crop. Yield attributes like number of panicles per pot, number of grains per panicle and 1000 grain weight were recorded.

3. Plant analysis at harvest for major nutrients

Two plant samples were selected from each pot for the elemental analysis. Samples were washed and air dried and packed in brown paper cover. These samples were oven dried at 70° C for 48 hours. Dried samples were powdered, sieved and stored in polythene bags. The standard analytical procedures followed for plant analysis were given in Table 3.6.

Table 3.6 Standard analytical procedures followed for the estimation of major nutrients in plants

Characteristic	Method		Reference
	Extraction	Estimation	
Total Nitrogen	Microkjeldahl digestion	Distillation	Piper, 1942
Total phosphorus	Micro wave digestion system(HNO_3)	Colorimetry	Watanabe and Olsen, 1965
Total potassium		Flame photometry	Piper, 1942

3.2.3 STATISTICAL ANALYSIS

Data on various chemical and biological characteristics of soil during the pot culture experiment were statistically analysed using OPSTAT software (two factorial CRD (2x6) with first factor (soil types) at two levels and second factor (herbicides+ control+ absolute control) at six levels).

Correlation analysis was carried out for studying the influence of chemical characteristics on biological characteristics of the soil during the period of pot culture experiment.

RESULTS

4. RESULTS

The results pertaining to the study on the "Effect of herbicides and chemical characteristics of soil on microbial biomass carbon and enzyme activity" are presented in this section.

Representative soil samples collected from four sites *viz.*, rice field of Agricultural Research Station, Mannuthy (Medium organic matter soil with a history of herbicide application:S_1 Control), non-cropped area of Agricultural Research Station, Mannuthy (Medium organic matter soil without a history of herbicide application: S_1 Absolute control), rice field of Kole area, Alappad (high organic matter soil with a history of herbicide application: S_2 Control), and non-cropped area of Kole land, Alappad (high organic matter soil without history of herbicide application: S_2 Absolute control) were analysed to study their physical, chemical and biological properties before the pot culture experiment.

4.1 PHYSICAL CHARACTERISTICS OF SOILS TAKEN FOR THE STUDY

The major physical characteristics of the soil *viz.*, soil texture, bulk density, particle density, porosity, water holding capacity, and volume of expansion were analysed at the beginning of the experiment to have basic information of the soils to be experimented.

Texture of the soil samples varied from sandy clay loam to clay (Table 4.1). The clay content was more (58.31 %) in the rice field of Kole land, Alappad with a history of herbicide application (S_2 Control) whereas the sand fraction was highest (55.40 %) for soils of non- cropped area of Kole land without a history of herbicide application (S_2 Absolute control). Lower values for clay were recorded (22.30 to 28.30 %) in three locations *viz.*, non- cropped area of ARS Mannuthy without a history of herbicide application (S_1 Absolute control) followed by soil collected from rice field of ARS Mannuthy with a history of herbicide application (S_1 Control) and soils from non-cropped area of Kole land Alappad without a history of herbicide application (S_2 Absolute control). The silt content of soils taken for the study varied from 16.30 to 23.90 per cent.

Table 4.1 Mechanical composition of soil samples taken for the study

Soil / soil sampling site		Soil separates (%)			Texture
		Sand	Silt	Clay	
Medium O. M* (S_1) ARS, Mannuthy	Control (rice field)	51.00	22.80	26.20	Sandy clay loam
	Absolute control (non-cropped area)	53.80	23.90	22.30	Sandy clay loam
High O.M* (S_2) Kole, Alappad	Control (rice field)	20.15	21.54	58.31	Clay
	Absolute control (non-cropped area)	55.40	16.30	28.30	Sandy clay loam

*O.M: Organic matter

Control: Rice field with a history of herbicide application

Absolute control: Non-cropped area without a history of herbicide application

Bulk density of the soils ranged from 1.12 to 1.45 Mg m^{-3}(Table 4.2). Soil samples from non- cropped area of ARS Mannuthy (S_1 Absolute control) recorded the highest bulk density (1.45 Mg m^{-3}) and the lowest bulk density value (1.12 Mg m^{-3}) was recorded by soil samples of rice field of Kole land, Alappad (S_2 Control).

Particle density of the soil samples ranged from 2.07 to 2.36 Mg m^{-3}(Table 4.2). Lowest particle density (2.07 Mg m^{-3}) was recorded by S_2 Control (rice field of Alappad, Kole). Soils from non-cropped area of ARS Mannuthy (without a history of herbicide application) showed the highest particle density of 2.36 Mg m^{-3}. Particle density was 2.33 and 2.16 Mg $m^{-3}$for the samples of S_1 Control and S_2 Absolute control respectively. Soil samples with a history of herbicide application recorded lower particle density in both the soil types compared to the soils without a history of herbicide application.

Porosity of the soil samples varied from 38.55 to 46.85 per cent (Table 4.2). Soil samples of non-cropped area of ARS Mannuthy (S_1 Absolute control) recorded lowest porosity (38.55 %) as compared to soils from rice field of ARS Mannuthy (S_1 Control) which recorded a value of 42.48 per cent. Similar trend was observed in the samples collected from Kole lands, Alappad (40.20 % for S_2 Absolute control and 46.85 % for S_2 Control). Among the four different types of soil samples collected for the study, highest porosity (46.85 %) was recorded in soil with high organic matter content which was collected from rice field of Kole land, Alappad (with a history of herbicide application).

Maximum water holding capacity (43.60 %) was observed in the soil samples collected from rice field of Kole land, Alappad (S_2 Control) (Table 4.2). Soil samples collected from non- cropped area of ARS Mannuthy without the history of herbicide application (S_1 Absolute control) recorded the lowest water holding capacity (34.74 %). Soil samples from rice field of ARS Mannuthy and non-cropped area of Kole land, Alappad recorded water holding capacity of 38.58 and 38.92 per cent respectively.

Table 4.2 Physical characteristics of soil samples taken for the study

Soil / soil sampling site		Bulk density ($Mg\ m^{-3}$)	Particle density ($Mg\ m^{-3}$)	Porosity (%)	Water holding capacity (%)	Volume of expansion (%)
Medium O. M.* (S_1) ARS, Mannuthy	Control (rice field)	1.34	2.33	42.48	38.58	15.62
	Absolute control (non-cropped area)	1.45	2.36	38.55	34.74	14.17
High O. M.* (S_2) Kole, Alappad	Control (rice field)	1.12	2.07	46.85	43.60	26.25
	Absolute control (non-cropped area)	1.29	2.16	40.20	38.92	16.19

*O.M: Organic matter

Control: Rice field with a history of herbicide application

Absolute control: Non-cropped area without a history of herbicide application

Highest volume of expansion (26.25 %) was recorded in the soil samples of rice field Alappad, Kole (S_2 Control). Soil samples collected from the non-cropped area of ARS Mannuthy (S_1 Absolute control) recorded the lowest volume of expansion (14.17 %). Soil samples of rice field, ARS Mannuthy (S_1 Control) had a volume of expansion 15.62 per cent. Volume of expansion of 16.19 per cent was recorded by soil samples of non-cropped area of Kole land, Alappad (S_2 Absolute control).

4.2 CHEMICAL CHARACTERISTICS OF SOILS TAKEN FOR THE STUDY

The major chemical characteristics of the soil *viz.*, pH, electrical conductivity, organic carbon, cation exchange capacity, available N, P, K, Ca, Mg, and micro nutrients like Fe, Mn, Cu, and Zn were also analysed before starting the experiment.

Soil samples collected from the four different locations were acidic in nature. pH of the soils varied from 5.01 to 5.68 (Table 4.3). Soil samples collected from non-cropped area of ARS Mannuthy (S_1 Absolute control) recorded highest pH (5.68) and lowest pH (5.01) was observed in soil samples from rice field of Kole land, Alappad (S_2 Control). Rice soils of ARS Mannuthy (S_1 Control) had a pH of 5.36. Soil sample collected from non-cropped area of Kole land, Alappad (S_2 Absolute control) had a pH of 5.20.

Electrical conductivity varied from 0.087 to 0.122 dS m^{-1}(Table 4.3). Soil samples from rice field, ARS Mannuthy and non-cropped area of ARS Mannuthy (S_1 Control and S_1 Absolute control respectively) had electrical conductivity 0.087 dS m^{-1} and 0.096 dS m^{-1}respectively. Highest electrical conductivity (0.122 dS m^{-1}) was observed in the samples of non-cropped area of Kole land, Alappad (S_2 Absolute control). Samples from rice field of Kole land, Alappad had electrical conductivity of 0.113 dS m^{-1}.

Organic carbon content of the soil samples showed a variation in the range of 0.85 to 2.47 per cent (Table 4.3). Rice field of Kole land with a history of herbicide application (S_2 Control) had highest organic carbon content (2.47 %)

Table 4.3 Status of pH, EC, organic carbon, cation exchange capacity and available nutrients in the soil samples

Soil / soil sampling site		pH	EC ($dS\ m^{-1}$)	Organic carbon (%)	Cation exchange capacity (c mol (+) kg^{-1})	Available N ($kg\ ha^{-1}$)	Available P ($kg\ ha^{-1}$)	Available K ($kg\ ha^{-1}$)
Medium O.M.* (S_1) ARS, Mannuthy	Control (rice field)	5.36	0.087	0.85	9.53	237.98	21.62	124.80
	Absolute control (non-cropped area)	5.68	0.096	1.08	10.40	258.40	23.35	199.36
High O.M.* (S_2) Kole, Alappad	Control (rice field)	5.01	0.113	2.47	15.43	296.30	27.90	325.61
	Absolute control (non-cropped area)	5.20	0.122	1.92	13.69	269.69	24.76	260.96

*O.M: Organic matter

Control: Rice field with a history of herbicide application

Absolute control: Non-cropped area without a history of herbicide application

and the lowest value (0.85 %) was recorded by rice field of ARS Mannuthy (S_1 Control). Soil samples of non- cropped area of ARS Mannuthy (S_1 Absolute control) and Kole land of Alappad (S_2 Absolute control) had organic carbon content of 1.08 per cent and 1.92 per cent respectively.

Cation exchange capacity of soils varied from 9.53 to 15.43 c mol (+) kg^{-1} (Table 4.3). Soils collected from rice field as well as non-cropped area of Kole land, Alappad recorded higher cation exchange capacity (15.43 and 13.69 c mol (+) kg^{-1}respectively). Soils of rice field and non-cropped area of ARS Mannuthy registered cation exchange capacity of 9.53 and 10.40 c mol (+) kg^{-1} respectively.

Available nitrogen content of soil samples were 237.98 kg ha^{-1}, 258.40 kg ha^{-1}, 296.30 kg ha^{-1}, and 269.69 kg ha^{-1} for S_1 Control, S_1 Absolute control, S_2 Control, S_2 Absolute control respectively (Table 4.3).

Available phosphorus content of the soil samples ranged from 21.62 to 27.90 kg ha^{-1}. Soil samples collected from rice field, ARS Mannuthy (S_1 Control) registered the lowest value of 21.62 kg ha^{-1} which was followed by the samples from non-cropped (23.35 kg ha^{-1}) area of ARS Mannuthy (S_1 Absolute control). Highest value (27.90 kg ha^{-1}) for available phosphorus was registered by the soil samples collected from rice field of Kole land, Alappad (S_2 Control). Soil samples of Kole land, Alappad (S_2 Absolute control) had an available phosphorus content of 24.76 kg ha^{-1}(Table 4.3).

The data on status of available potassium in soils are presented in Table 4.3. Potassium content varied from 124.81 kg ha^{-1} to 325.61 kg ha^{-1}. Rice field of Kole land, Alappad (S_2 Control) had highest available potassium (325.61 kg ha^{-1}) and the lowest value was recorded by soil collected from rice field (S_1 Control) of ARS Mannuthy (124.81 kg ha^{-1}). Available potassium status of samples from non-cropped area of ARS Mannuthy (S_1 Absolute control) was 199.36 kg ha^{-1}. Soil samples collected from non-cropped area of Kole land, Alappad recorded available potassium content of 260.96 kg ha^{-1}.

Available secondary nutrients like Ca and Mg varied from 728.50 to 1753.00 mg kg^{-1} and 167.62 to 414.31 mg kg^{-1}respectively (Table 4.4). Rice field of Kole land Alappad recorded highest available Ca (1753.00 mg kg^{-1}) and Mg (414.31 mg kg^{-1}). Lowest available Ca (728.50 mg kg^{-1}) was registered by rice field ARS Mannuthy and lowest value for available Mg (167.62 mg kg^{-1}) was recorded by non-cropped area of Kole land, Alappad. Available Ca content of 1194.01 mg $kg^{-1}$and Mg content of 215.90 mg kg^{-1}were recorded by soil samples of non-cropped area of ARS Mannuthy. Non-cropped area of Kole land registered available Ca and Mg content of 1575.01 and 167.62 mg kg^{-1} respectively in their soil samples.

Available micronutrients like Fe, Mn, Zn, and Cu varied from 19.22 to 127.82 mg kg^{-1}, 32.55 to 122.51 mg kg^{-1}, 1.67 to 6.43 mg kg^{-1}, and 2.47 to 7.37 mg kg^{-1}respectively (Table 4.4).

Iron content (mg kg^{-1}) of the four selected soils followed the order, rice field of Kole (127.82 mg kg^{-1}) > non-cropped area of Kole (69.53 mg kg^{-1}) > non-cropped area of ARS Mannuthy (46.99 mg kg^{-1}) > rice field of ARS Mannuthy (19.22 mg kg^{-1}). Manganese content were in the order of rice field of Kole (122.51 mg kg^{-1}) > non-cropped area of Kole (76.42 mg kg^{-1}) > rice field of ARS Mannuthy (36.40 mg kg^{-1}) > non-cropped area of ARS Mannuthy (32.55 mg kg^{-1}). Highest available Zn was recorded by non-cropped area of Kole land (6.43 mg kg^{-1}) which was followed by rice field of Kole (3.03 mg kg^{-1}), non-cropped area of ARS Mannuthy (1.77 mg kg^{-1}), rice field of ARS Mannuthy (1.67 mg kg^{-1}). Available copper content was higher in the rice field of Kole (7.37 mg kg^{-1}) followed by rice field of ARS Mannuthy (4.84 mg kg^{-1}), non-cropped area of ARS Mannuthy (2.91 mg kg^{-1}) and non-cropped area of Kole (2.47 mg kg^{-1}) respectively.

Table 4.4 Status of secondary and micronutrients in the soil samples used for the study

Soil / soil sampling site		Secondary nutrients (mg kg^{-1})		Micronutrients (mg kg^{-1})			
		Available Calcium	Available Magnesium	Available Iron	Available Manganese	Available Zinc	Available Copper
Medium O.M.* (S_1) ARS, Mannuthy	Control (rice field)	728.50	197.80	19.22	36.40	1.67	4.84
	Absolute control (non-cropped area)	1194.01	215.90	46.99	32.55	1.77	2.91
High O.M.* (S_2) Kole, Alappad	Control (rice field)	1753.00	414.31	127.82	122.51	3.03	7.37
	Absolute control (non-cropped area)	1575.01	167.62	69.53	76.42	6.43	2.47

*O.M: Organic matter

Control: Rice field with a history of herbicide application

Absolute control: Non-cropped area without a history of herbicide application

4.3 BIOLOGICAL CHARACTERISTICS OF SOILS TAKEN FOR THE STUDY

The data on biological characteristics of soil samples collected for the study are presented in Table 4.5.

Microbial biomass carbon content of soils varied from 153.31 to 421.10 μg C $g^{-1}day^{-1}$. Highest Microbial biomass carbon content of 421.10 μg C g^{-1} day^{-1} was observed in rice field of Kole land (S_2 Control) followed by non- cropped area (S_2 Absolute control) of Kole land (322.01 μg C $g^{-1}day^{-1}$), non- cropped area of ARS Mannuthy (192.12 μg C $g^{-1}day^{-1}$) (S_1 Absolute control) , and rice field of ARS Mannuthy (153.31 μg C $g^{-1}day^{-1}$) (S_1 Control).

Soil enzymes *viz.*, dehydrogenase, urease and phosphatase varied significantly among soil types taken for the study. Dehydrogenase activity was highest (85.37 μg TPF $g^{-1}day^{-1}$) in rice field of Kole land (S_2 Control) followed by non-cropped area of Kole land (78.43 μg TPF $g^{-1}day^{-1}$) (S_2 Absolute control), non-cropped area of ARS Mannuthy (61.21 μg TPF $g^{-1}day^{-1}$) (S_1 Absolute control), and rice field of ARS Mannuthy (57.12 μg TPF $g^{-1}day^{-1}$) (S_1 Control).

Urease activity varied from 50.62 to 79.11 μg N-NH_4 g^{-1} hr^{-1}. Rice soils of Kole land (S_2 Control) had urease activity 79.11 μg N-NH_4 g^{-1} hr^{-1} and for non-cropped area of Kole land (S_2 Absolute control) had urease activity 63.28 μg N-NH_4 g^{-1} hr^{-1}. Urease activity was 56.95 μg N-NH_4 g^{-1} hr^{-1} in non- cropped area of ARS Mannuthy (S_1 Absolute control) and 50.62 μg N-NH_4 g^{-1} hr^{-1} for rice soils of ARS Mannuthy (S_1 Control).

Highest phosphatase activity (98.02 μg PNP g^{-1} hr^{-1} for acid phosphatase and 72.21 μg PNP g^{-1} hr^{-1} for alkaline phosphatase) was observed in the rice field of Kole land (S_2 Control) and lowest activity for acid phosphatase (58.14 μg PNP g^{-1} hr^{-1}) and alkaline phosphatase (22.83 μg PNP g^{-1} hr^{-1}) was noticed in the soil samples from rice field of ARS Mannuthy (S_1 Control). Soil samples of non-cropped area of ARS Mannuthy (S_1 Absolute control) had acid phosphatase

Table 4.5 Biological characteristics of soil samples taken for the study

Soil / soil sampling site		Microbial biomass carbon (µg C $g^{-1}day^{-1}$)	Dehydrogenase activity (µg TPF $g^{-1}day^{-1}$)	Urease activity (µg N-NH_4 g^{-1} hr^{-1})	Acid phosphatase activity (µg PNP g^{-1} hr^{-1})	Alkaline phosphatase activity (µg PNP g^{-1} hr^{-1})
Medium O.M.* (S_1) ARS, Mannuthy	Control (rice field)	153.31	57.12	50.62	58.14	22.83
	Absolute control (non-cropped area)	192.12	61.21	56.95	70.43	36.75
High O.M.* (S_2) Kole, Alappad	Control (rice field)	421.10	85.37	79.11	98.02	72.21
	Absolute control (non-cropped area)	322.01	78.43	63.28	78.63	64.96

*O.M: Organic matter

Control: Rice field with a history of herbicide application

Absolute control: Non-cropped area without a history of herbicide application

activity 70.43 µg PNP g^{-1} $hr^{-1}$and alkaline phosphatase activity 36.75 µg PNP g^{-1} hr^{-1}. Acid phosphatase activity was 78.63µg PNP g^{-1} hr^{-1} and alkaline phosphatase activity was 64.96 µg PNP g^{-1} $hr^{-1}$for soil samples of non-cropped area of Kole land (S_2 Absolute control). Acid phosphatase activity was comparatively higher than alkaline phosphatase activity in all the soils taken for the study.

4.4 CHEMICAL CHARACTERISTICS OF THE SOIL DURING THE PERIOD OF POT CULTURE EXPERIMENT

The major chemical characteristics of the soil *viz*., pH, electrical conductivity, and organic carbon were analysed at different intervals i.e. on the day but two hour before the herbicide application, 7, 15, 30, and 60 days after herbicide application (0 DAHA, 7 DAHA, 15 DAHA, 30 DAHA, and 60 DAHA) and at harvest.

4.4.1 pH

The data on pH of soil at different intervals showed significant difference among soil types, treatments and interaction between soil types and treatments (Table 4.6). Among the soil types highest pH was observed in medium O.M soils (rice field and non-cropped area of ARS Mannuthy) compared to high O.M. soils (rice field and non-cropped area of Kole land, Alappad) throughout the intervals. In both the soil types, pH value showed a decreasing trend towards the harvest. Among the treatments, absolute control recorded higher pH compared to all other treatments throughout the experiment and lowest pH by pendimethalin treatment.

Soil samples just before herbicide application showed pH in the range of 5.17 to 5.73. In both the soil types, absolute control treatment registered significantly higher pH (5.73 for S_1 and 5.26 for S_2).

Table 4.6 pH of soil samples taken at different days after herbicide application (DAHA) in the pot culture experiment

Treatment	pH																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	5.38	5.17	5.28	5.35	5.15	5.25	5.33	5.14	5.24	5.28	5.11	5.19	5.22	5.07	5.15	5.10	4.97	5.04
Oxyfluorfen	5.39	5.17	5.28	5.36	5.16	5.26	5.35	5.15	5.25	5.32	5.13	5.22	5.28	5.10	5.19	5.18	5.06	5.12
Cyhalofop-butyl	5.38	5.17	5.28	5.35	5.16	5.25	5.34	5.15	5.25	5.32	5.14	5.23	5.29	5.12	5.20	5.24	5.08	5.16
Bispyribac-sodium	5.39	5.17	5.28	5.36	5.16	5.26	5.35	5.15	5.25	5.31	5.13	5.22	5.25	5.08	5.17	5.15	5.02	5.09
Control	5.39	5.19	5.29	5.36	5.18	5.27	5.34	5.17	5.25	5.33	5.16	5.24	5.30	5.15	5.22	5.25	5.11	5.18
Absolute control	5.73	5.26	5.49	5.72	5.24	5.48	5.71	5.23	5.47	5.69	5.21	5.45	5.67	5.19	5.43	5.62	5.14	5.38
Mean	5.44	5.19		5.42	5.18		5.40	5.17		5.38	5.15		5.34	5.12		5.26	5.06	
CD (0.05)	0.01*		0.02**	0.01		0.02	0.02		0.04	0.01		0.02	0.01		0.01	0.01		0.02
	0.02***			0.02			0.05			0.02			0.02			0.03		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

Seven days after the application of herbicides, pH of the soil decreased and the values ranged from 5.15 to 5.72. The highest pH (5.72) was noticed in S_1 absolute control and lowest was observed in S_2 pendimethalin with a pH of 5.15. The pH of S_1control (5.36) was found to be statistically on par with S_1 oxy (5.36), S_1 bispyri (5.36), S_1 cyhalo (5.35), and S_1 pendi (5.35). Absolute control of high organic matter soil without history of herbicide application (S_2 absolute control) recorded a pH of 5.24. In S_2 control, pH was 5.18 which was statistically on par with S_2 oxy (5.16), S_2 cyhalo (5.16), and S_2 bispyri (5.16) and significant difference was observed with S_2 pendi (5.15).

Fifteen days after the application of herbicides, pH of all the interactions was found to be decreased and S_1 absolute control recorded highest pH (5.71). The lowest pH was recorded in S_2 pendi (5.14) which was on par with S_2 oxy (5.15), S_2 cyhalo (5.15), S_2 bispyri (5.15), and S_2 control (5.17) and varied significantly with S_2 absolute control (5.23).

By 30 DAHA, pH of the soils were again decreased and they varied from 5.11 (S_2 pendi) to 5.69 (S_1 absolute control). S_1 control (5.33) was statistically on par with S_1 oxy (5.32), S_1 cyhalo (5.32), and S_1 bispyri (5.31) and varied significantly with S_1 pendi (5.28). S_2 control (5.16) and S_2 cyhalo (5.14) was statistically on par while differed significantly with S_2 oxy (5.13), S_2 bispyri (5.13) and S_2 pendi (5.11).

The pH of the soils varied from 5.07 to 5.67 after 60 days of herbicide application. The lowest pH was recorded by S_2 pendi (5.07) which was statistically on par with S_2 bispyri (5.08) and differed significantly with all other interactions.

After the harvest of rice, pH of the soils varied from 4.97 to 5.62. The value of pH was decreased and highest pH was observed in S_1 absolute control (5.62) followed by S_1 control (5.25), S_1 cyhalo (5.24), S_1 oxy (5.18), S_1 bispyri (5.15), S_2 absolute control (5.14), S_2 control (5.11), S_1 pendi (5.10), S_2 cyhalo (5.08), S_2 oxy (5.06), S_2 bispyri (5.02) and S_2 pendi (4.97) respectively.

4.4.2 Electrical conductivity

Data on electrical conductivity at different days after herbicide application are given in Table 4.7.

Significant variation was observed in electrical conductivity among the soil types (S_1 and S_2). The highest electrical conductivity was observed in high O. M. soils (S_2) compared to medium O.M. soils (S_1) throughout the intervals and the values showed a decreasing trend towards the harvest.

At all the intervals studied, the treatment mean values differed significantly. Absolute control recorded highest electrical conductivity compared to all other treatments at all the intervals. However, herbicide application did not bring any variation in the electrical conductivity of the samples tested. On the day just 2 h before the application of herbicides, electrical conductivity (mean value of treatments) varied from 0.098 to 0.109 dS $m^{-1}$and after seven days of herbicide application, electrical conductivity was decreased and it varied from 0.096 to 0.107 dS m^{-1}. At 15, 30, 60 days after herbicide application and at harvest electrical conductivity ranged from 0.094 to 0.104 dS m^{-1}, 0.091 to 0.102 dS m^{-1}, 0.090 to 0.101 dS m^{-1}, 0.088 to 0.100 dS m^{-1} respectively.

There was no significant difference in electrical conductivity among the interactions of soil types and treatments throughout the intervals studied.

Table 4.7 Electrical conductivity of soil samples taken at different days after herbicide application (DAHA) in the pot culture experiment

Treatment	Electrical conductivity (dS m^{-1})																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	0.086	0.110	0.098	0.084	0.108	0.096	0.080	0.107	0.094	0.078	0.104	0.091	0.077	0.103	0.090	0.075	0.100	0.088
Oxyfluorfen	0.086	0.111	0.098	0.083	0.109	0.096	0.080	0.107	0.094	0.078	0.104	0.091	0.077	0.104	0.090	0.075	0.101	0.088
Cyhalofop-butyl	0.086	0.109	0.098	0.083	0.108	0.096	0.080	0.107	0.094	0.078	0.105	0.092	0.077	0.103	0.090	0.075	0.101	0.088
Bispyribac-sodium	0.086	0.111	0.098	0.083	0.109	0.096	0.081	0.107	0.094	0.078	0.105	0.092	0.077	0.103	0.090	0.075	0.103	0.089
Control	0.088	0.111	0.099	0.088	0.109	0.099	0.085	0.106	0.095	0.082	0.105	0.094	0.080	0.104	0.092	0.080	0.104	0.092
Absolute control	0.096	0.122	0.109	0.094	0.119	0.107	0.091	0.117	0.104	0.089	0.115	0.102	0.088	0.114	0.101	0.087	0.114	0.100
Mean	0.088	0.112		0.086	0.110		0.083	0.108		0.081	0.106		0.079	0.105		0.078	0.103	
CD (0.05)	0.001*		0.002**	0.001		0.002	0.002		0.003	0.001		0.002	0.001		0.002	0.001		0.002
	NS***			NS			NS			NS			NS			NS		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

4.4.3 Organic carbon

Organic carbon content of soil at different days after herbicide application are given in Table 4.8. Significant variation was observed among the soil types, treatments, and interaction between soil types and treatments throughout the intervals studied.

Among the soil types (S_1 and S_2), highest organic carbon content was observed in S_2 at all the intervals compared to S_1 and the content was found to be decreased throughout the period of experimentation when it reached towards harvest (2.40 % for S_2 and 0.95 % for S_1 on the day (2 h before) of herbicide application, 2.37 % for S_2 and 0.92 % for S_1 at 2.34 % for S_2 and 0.90 % for S_1 at 15 DAHA, 2.32 % for S_2 and 0.87 % for S_1 at 30 DAHA, 2.30 % for S_2 and 0.82 % for S_1 at 60 DAHA, 2.27 % for S_2 and 0.78 % for S_1 at harvest).

Treatments (mean value of treatments) differed significantly for organic carbon content at different intervals studied. Organic carbon content was found to be decreased at different days after herbicide application in all the treatments studied. Before the application of herbicides, highest organic carbon content with 1.70 per cent was observed in control which was on par with cyhalofop-butyl, bispyribac-sodium, oxyfluorfen, and pendimethalin and lowest carbon content was observed in absolute control (1.55 %). Similar trend was followed upto 60 DAHA in all the treatments. At harvest, control differed significantly with all other treatments and lowest carbon content was observed in absolute control (1.46 %). Treatments like, pendimethalin, oxyfluorfen, bispyribac-sodium, cyhalofop-butyl was found to be statistically on par at all the intervals studied.

Analysis of interaction between soil types and treatments showed that organic carbon content varied from 0.91 to 2.48 per cent, 0.88 to 2.46 per cent, 0.85 to 2.43 per cent, 0.82 to 2.42 per cent, 0.76 to 2.39 per cent and 0.70 to 2.38 per cent on the day of herbicide application (just 2 h before the application), 7, 15, 30, and 60 days after herbicide application and at harvest respectively. S_2 control showed maximum organic carbon content among the interactions between

Table 4.8 Organic carbon content of soil samples at different days after herbicide application (DAHA) in the pot culture experiment

Treatment	Organic carbon (%)																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	0.91^{d}	2.48^{a}	1.70^{a}	0.88^{d}	2.46^{a}	1.67^{a}	0.85^{d}	2.43^{a}	1.64^{a}	0.82^{d}	2.41^{a}	1.61^{a}	0.76^{d}	2.38^{a}	1.57^{a}	0.70^{c}	2.34^{a}	1.52^{b}
Oxyfluorfen	0.91^{d}	2.48^{a}	1.70^{a}	0.88^{d}	2.46^{a}	1.67^{a}	0.85^{d}	2.43^{a}	1.64^{a}	0.82^{d}	2.41^{a}	1.62^{a}	0.76^{d}	2.38^{a}	1.57^{a}	0.73^{dc}	2.36^{a}	1.54^{b}
Cyhalofop butyl	0.91^{d}	2.48^{a}	1.70^{a}	0.88^{d}	2.46^{a}	1.67^{a}	0.85^{d}	2.43^{a}	1.64^{a}	0.82^{d}	2.41^{a}	1.62^{a}	0.77^{d}	2.39^{a}	1.58^{a}	0.74^{dc}	2.35^{a}	1.54^{b}
Bispyribac sodium	0.91^{d}	2.48^{a}	1.70^{a}	0.88^{d}	2.46^{a}	1.67^{a}	0.85^{d}	2.43^{a}	1.64^{a}	0.82^{d}	2.41^{a}	1.62^{a}	0.76^{d}	2.38^{a}	1.57^{a}	0.72^{dc}	2.34^{a}	1.53^{b}
Control	0.91^{d}	2.48^{a}	1.70^{a}	0.88^{d}	2.46^{a}	1.67^{a}	0.87^{d}	2.43^{a}	1.65^{a}	0.85^{d}	2.42^{a}	1.63^{a}	0.78^{d}	2.39^{a}	1.59^{a}	0.75^{d}	2.38^{a}	1.57^{a}
Absolute control	1.13^{c}	1.97^{b}	1.55^{b}	1.12^{c}	1.95^{b}	1.53^{b}	1.10^{c}	1.91^{b}	1.51^{b}	1.09^{c}	1.89^{a}	1.49^{b}	1.08^{c}	1.87^{b}	1.47^{b}	1.06^{c}	1.86^{b}	1.46^{c}
Mean	0.95^{b}	2.40^{a}		0.92^{b}	2.37^{a}		0.90^{b}	2.34^{a}		0.87^{b}	2.32^{a}		0.82^{b}	2.30^{a}		0.78^{b}	2.27^{a}	
CD (0.05)	0.02*		0.04**	0.02		0.03	0.01		0.02	0.01		0.02	0.01		0.02	0.02		0.03
	0.06***			0.04			0.03			0.03			0.03			0.04		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

the treatments and soil types at all the intervals studied and it was on par with S_2 cyhalo, S_2 oxy, S_2 bispyri, and S_2 pendi throughout the intervals studied. Significantly lower organic carbon content was shown by S_1 pendi (0.70 %) at harvest compared to S_1 control (0.75 %). However, upto 60 DAHA, S_1 pendimethalin was statistically on par with other treatments except absolute control. Among the interactions, significant variation was found in case of control and absolute control with S_1 and S_2 and it followed the same order *viz.*, S_2 control > S_2 absolute control > S_1 absolute control > S_1 control.

4.5 BIOLOGICAL CHARACTERISTICS OF SOIL DURING THE PERIOD OF POT CULTURE EXPERIMENT

Microbial biomass carbon, dehydrogenase activity, urease activity, acid phosphatase and alkaline phosphatase activity were analysed at six intervals (on the day of herbicide application (but 2 h before the application), 7, 15, 30, and 60 DAHA and at harvest) during the period of pot culture experiment.

4.5.1 Microbial biomass carbon

Analysis of data on the microbial biomass carbon showed significant difference among the soil types, treatments, and interaction between soil type and treatments at different intervals studied (Table 4.9).

Among the soil types, highest microbial biomass carbon (MBC) was observed in S_2 at all the intervals when compared to S_1. Decline in the microbial biomass carbon was observed at 7, 15 and 30 DAHA and a sudden increase at 60 DAHA followed by drastic decrease at harvest in both S_1 (samples from ARS Mannuthy) and S_2 (Kole land, Alappad).

Table 4.9 Effect of herbicide application on microbial biomass carbon at different days after herbicide application (DAHA)

Treatment	Microbial biomass carbon(µg C $g^{-1}day^{-1}$)																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	168.10^{d}	453.10^{a}	310.60^{a}	131.00^{h}	422.38^{c}	276.69^{c}	128.00^{f}	425.28^{b}	276.64^{b}	107.45^{g}	412.18^{b}	259.82^{cd}	171.40^{fg}	454.35^{c}	312.88^{d}	91.30^{i}	356.43^{c}	223.87^{d}
Oxyfluorfen	168.10^{d}	453.83^{a}	310.97^{a}	143.03^{g}	436.07^{b}	289.55^{b}	129.33^{f}	429.40^{ab}	279.37^{b}	115.62^{fg}	417.75^{ab}	266.68^{b}	180.37^{gh}	458.47^{c}	319.42^{c}	109.30^{gh}	379.23^{b}	244.27^{b}
Cyhalofop butyl	168.83^{d}	454.58^{a}	311.71^{a}	153.50^{f}	441.32^{ab}	297.41^{a}	132.00^{f}	430.20^{ab}	281.10^{b}	118.28^{f}	420.85^{ab}	269.56^{b}	185.98^{g}	467.00^{b}	326.49^{b}	117.43^{g}	383.33^{b}	250.38^{b}
Bispyribac sodium	168.10^{d}	454.40^{a}	311.25^{a}	142.50^{g}	434.18^{b}	288.34^{b}	129.14^{f}	426.63^{b}	277.88^{b}	115.37^{fg}	414.58^{b}	264.98^{bc}	177.83^{h}	457.98^{c}	317.91^{cd}	99.44^{hi}	361.37^{c}	230.40^{cd}
Control	168.83^{d}	455.32^{a}	312.08^{a}	155.58^{f}	447.88^{a}	301.73^{a}	147.92^{e}	433.83^{a}	290.88^{a}	136.40^{e}	424.42^{a}	280.41^{a}	197.33^{f}	502.73^{a}	350.03^{a}	135.43^{f}	418.68^{a}	277.06^{a}
Absolute control	218.28^{c}	358.28^{b}	288.28^{b}	202.97^{c}	348.83^{d}	275.90^{c}	191.53^{d}	334.20^{c}	262.87^{c}	186.67^{d}	322.18^{c}	254.42^{d}	234.47^{c}	369.53^{d}	302.00^{c}	175.10^{e}	293.07^{d}	234.09^{c}
Mean	176.71^{b}	438.25^{a}		154.76^{b}	421.78^{a}		142.99^{b}	413.26^{a}		129.96^{b}	401.99^{a}		191.23^{b}	451.68^{a}		121.33^{b}	365.35^{a}	
CD (0.05)	4.23*		7.33**	2.97		5.14	2.72		4.71	3.92		6.79	3.29		5.70	5.03		8.71
	10.37***			7.27			6.67			9.60			8.06			12.32		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

Among the treatments, mean value of control recorded highest value of MBC at all the intervals and it was maximum at 60 DAHA (350.03 µg C $g^{-1}day^{-1}$) and it decreased towards harvest with a minimum value of 277.06 µg C $g^{-1}day^{-1}$. On the day (but 2 h before) of herbicide application, mean values of control (312.08 µg C g^{-1} day^{-1}), pendimethalin (310.60 µg C g^{-1} day^{-1}), oxyfluorfen (310.97 µg C g^{-1} day^{-1}), bispyribac-sodium (311.25 µg C g^{-1} day^{-1}) and cyhalofop-butyl (311.71 µg C g^{-1} day^{-1}) were statistically on par and differed significantly with absolute control (288.28 µg C g^{-1} day^{-1}). At 7 DAHA, control recorded highest MBC (301.73 µg C $g^{-1}day^{-1}$) and it was statistically on par with cyhalofop-butyl (297.41 µg C $g^{-1}day^{-1}$). Lowest MBC was shown by pendimethalin (276.69 µg C $g^{-1}day^{-1}$) which was closely on par with absolute control (275.90 µg C $g^{-1}day^{-1}$). By 15 DAHA, microbial biomass carbon again found to be decreased in all the treatments. By 30 DAHA, highest MBC was observed in control (280.41) and lowest by absolute control (254.42 µg C $g^{-1}day^{-1}$). Similarly, microbial biomass carbon was highest in control (350.03 µg C $g^{-1}day^{-1}$) and lowest in absolute control (302.00 µg C $g^{-1}day^{-1}$) at 60 DAHA but at harvest pendimethalin recorded lowest value of microbial biomass carbon (223.87µg C g^{-1} day^{-1}).

Among interaction between soil types and treatments, S_2 control recorded highest microbial biomass carbon and lowest by S_1 pendi at all the intervals studied. At 7 DAHA, S_2 control (447.88 µg C $g^{-1}day^{-1}$) was statistically on par with S_2 cyhalo (441.32 µg C $g^{-1}day^{-1}$) and differed significantly with all other interactions. Microbial biomass carbon varied from 128.00 to 433.83 µg C $g^{-1}day^{-1}$, 107.45 to 424.42 µg C $g^{-1}day^{-1}$, 171.40 to 502.73 µg C $g^{-1}day^{-1}$, 91.30 to 418.68 µg C $g^{-1}day^{-1}$ at 15, 30, 60 DAHA and at harvest respectively.

At all the intervals, all the interaction between soil types and treatments were followed the same order *viz.*, S_2 control > S_2 cyhalo > S_2 oxy > S_2 bispyri > S_2 pendi > S_2 absolute control > S_1 absolute control > S_1 control > S_1cyhalo > S_1 oxy > S_1 bispyri > S_1 pendi. Microbial biomass carbon was found to be declined

upto 30 DAHA, and there was drastic increase in MBC at 60 DAHA followed by a sudden decrease at harvest in all the interactions studied.

4.5.2 Dehydrogenase activity

Data on dehydrogenase activity at different days after herbicide applications in the pot culture experiment are shown in Table 4.10. Significant variation was observed in dehydrogenase activity among the soil types and interaction between soil types and treatments at all the intervals. Treatments differed significantly in all the intervals except at seven days after herbicide application and at harvest.

Among the soil types, S_2 showed highest dehydrogenase activity at all the intervals and lowest activity by S_1. Dehydrogenase activity was found to be increased upto 60 DAHA and thereafter it decreased in all the treatments except in herbicide treatments at 15 DAHA. Maximum mean dehydrogenase activity was observed at 60 DAHA in both S_1 (88.51 µg TPF $g^{-1}day^{-1}$) and S_2 (126.38 µg TPF $g^{-1}day^{-1}$).

On the day but 2 h before the herbicide application, dehydrogenase activity in all the treatments except absolute control were statistically on par. At seven days after herbicide application and at harvest no significant difference was observed among the mean value of dehydrogenase activity of all the treatments. Absolute control recorded highest mean dehydrogenase activity at 15 (86.39 µg TPF $g^{-1}day^{-1}$), 30 (87.97 µg TPF $g^{-1}day^{-1}$), and 60 (108.75 µg TPF $g^{-1}day^{-1}$) days after herbicide application and lowest activity by pendimethalin treatment (77.97 µg TPF $g^{-1}day^{-1}$, 84.94 µg TPF $g^{-1}day^{-1}$, and 104.38 µg TPF $g^{-1}day^{-1}$ at 15, 30, and 60 DAHA respectively). Maximum dehydrogenase activity was observed at 60 DAHA in all the treatments and thereafter it was decreased drastically.

On the day but 2 h before the application of herbicides, dehydrogenase activity of the interactions (between soil types and treatments) varied from 61.65

to 89.68 µg TPF $g^{-1}day^{-1}$. Highest dehydrogenase activity was observed in S_2 control (89.68 µg TPF $g^{-1}day^{-1}$) which was on par with S_2 oxy (89.67 µg TPF $g^{-1}day^{-1}$), S_2 bispyri (89.67 µg TPF $g^{-1}day^{-1}$), S_2 cyhalo (89.66 µg TPF $g^{-1}day^{-1}$) and S_2 pendi (89.65 µg TPF $g^{-1}day^{-1}$) followed by S_2 absolute control (82.31 µg TPF $g^{-1}day^{-1}$), S_1 absolute control (65.74 µg TPF $g^{-1}day^{-1}$) and lowest by S_1 control (61.65 µg TPF $g^{-1}day^{-1}$) and S_1 oxy (61.65 µg TPF $g^{-1}day^{-1}$) which was closely on par with S_1 bispyri (61.66 µg TPF $g^{-1}day^{-1}$) and S_1 pendi (61.66 µg TPF $g^{-1}day^{-1}$).

At seven days after herbicide application, dehydrogenase activity varied from 63.38 to 95.78 µg TPF $g^{-1}day^{-1}$. Highest activity was recorded by S_2 control (95.78 µg TPF $g^{-1}day^{-1}$) which was on par with S_2 cyhalo (95.61 µg TPF $g^{-1}day^{-1}$), S_2 oxy (95.58 µg TPF $g^{-1}day^{-1}$), S_2 bispyri (95.57 µg TPF $g^{-1}day^{-1}$) and S_2 pendi (95.27 µg TPF $g^{-1}day^{-1}$). Lowest dehydrogenase activity was observed in S_1 pendi (63.38 µg TPF $g^{-1}day^{-1}$) which was on par with S_1 bispyri (64.21 µg TPF $g^{-1}day^{-1}$) and S_1oxy (64.53 µg TPF $g^{-1}day^{-1}$).

At 15 DAHA, highest dehydrogenase activity was observed in S_2 control (98.97 µg TPF $g^{-1}day^{-1}$) followed by S_2 absolute control (96.59 µg TPF $g^{-1}day^{-1}$), S_2 cyhalo (95.10 µg TPF $g^{-1}day^{-1}$), S_2 oxy (94.48 µg TPF $g^{-1}day^{-1}$), S_2 bispyri (94.37 µg TPF $g^{-1}day^{-1}$), S_2 pendi (94.21 µg TPF $g^{-1}day^{-1}$), S_1 absolute control (76.18 µg TPF $g^{-1}day^{-1}$), S_1 control (67.86 µg TPF $g^{-1}day^{-1}$), S_1 cyhalo (63.05 µg TPF $g^{-1}day^{-1}$), S_1 oxy (62.54 µg TPF $g^{-1}day^{-1}$), S_1 bispyri (62.00 µg TPF $g^{-1}day^{-1}$) and S_1 pendi (61.73 µg TPF $g^{-1}day^{-1}$).

By 30 DAHA, activity increased in all the interactions and highest dehydrogenase activity was observed in S_2 control (103.53 µg TPF $g^{-1}day^{-1}$) which was on par with S_2 cyhalo (103.41 µg TPF $g^{-1}day^{-1}$), S_2 oxy (103.36 µg TPF $g^{-1}day^{-1}$), S_2 bispyri (103.33 µg TPF $g^{-1}day^{-1}$), and S_2 pendi (102.56 µg TPF $g^{-1}day^{-1}$). Lowest dehydrogenase activity was recorded by S_1 pendi (67.32 µg TPF $g^{-1}day^{-1}$) which was on par with S_1 bispyri (68. 04 µg TPF $g^{-1}day^{-1}$), and S_1oxy (68.15 µg TPF $g^{-1}day^{-1}$).

Table 4.10 Effect of herbicide application on dehydrogenase activity at different days after herbicide application (DAHA)

Treatment	Dehydrogenase activity (µg TPF $g^{-1}day^{-1}$)																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	61.66^{d}	89.65^{a}	75.66^{a}	63.38^{e}	95.27^{a}	79.33	61.73^{f}	94.21^{c}	77.97^{c}	67.32^{e}	102.56^{a}	84.94^{c}	84.07^{f}	124.69^{b}	104.38^{c}	60.52^{d}	89.72^{a}	75.12
Oxyfluorfen	61.65^{d}	89.67^{a}	75.66^{a}	64.53^{de}	95.58^{a}	80.06	62.54^{f}	94.48^{bc}	78.51^{c}	68.15^{e}	103.36^{a}	85.76^{bc}	87.82^{e}	128.14^{a}	107.98^{ab}	61.81^{d}	90.09^{a}	75.95
Cyhalofop butyl	61.67^{d}	89.66^{a}	75.67^{a}	65.37^{d}	95.61^{a}	80.49	63.05^{f}	95.10^{bc}	79.08^{c}	69.82^{d}	103.41^{a}	86.62^{b}	88.01^{e}	128.53^{a}	108.27^{a}	62.34^{d}	90.89^{a}	76.62
Bispyribac sodium	61.66^{d}	89.67^{a}	75.67^{a}	64.21^{de}	95.57^{a}	79.89	62.00^{f}	94.37^{c}	78.19^{c}	68.04^{e}	103.33^{a}	85.68^{c}	86.53^{e}	126.84^{a}	106.69^{b}	61.65^{d}	89.83^{a}	75.74
Control	61.65^{d}	89.68^{a}	75.67^{a}	65.48^{d}	95.78^{a}	80.63	67.86^{e}	98.97^{a}	83.42^{b}	70.85^{d}	103.53^{a}	87.19^{ab}	88.36^{e}	128.82^{a}	108.59^{a}	63.06^{d}	91.83^{a}	77.45
Absolute control	65.74^{c}	82.31^{b}	74.03^{b}	69.26^{c}	90.12^{b}	79.69	76.18^{d}	96.59^{b}	86.39^{a}	78.01^{c}	97.92^{b}	87.97^{a}	96.27^{d}	121.23^{c}	108.75^{a}	67.31^{c}	85.43^{b}	76.37
Mean	62.34^{b}	88.44^{a}		65.37^{b}	94.65^{a}		65.56^{b}	95.62^{a}		70.36^{b}	102.35^{a}		88.51^{b}	126.38^{a}		62.78^{b}	89.63^{a}	
CD (0.05)	0.31*		0.53**	0.60		NS	0.90		1.55	0.54		0.93	0.83		1.45	1.30		NS
	0.75***			1.47			2.20			1.32			2.04			3.18		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

Dehydrogenase activity varied from 84.07 to 128.82 µg TPF $g^{-1}day^{-1}$ at 60 DAHA. Activity was found to be highest at this stage and thereafter activity decreased. At 60 DAHA, highest dehydrogenase activity was recorded in S_2 control (128.82 µg TPF $g^{-1}day^{-1}$) which was statistically on par with S_2 cyhalo (128.53 µg TPF $g^{-1}day^{-1}$), S_2 oxy (128.14 µg TPF $g^{-1}day^{-1}$), and S_2 bispyri (126.84 µg TPF $g^{-1}day^{-1}$). S_1 pendi recorded lowest dehydrogenase activity of 84.07 µg TPF $g^{-1}day^{-1}$. Dehydrogenase activity was 88.36 µg TPF $g^{-1}day^{-1}$ for S_1 control which was statistically on par with S_1 cyhalo (88.01 µg TPF $g^{-1}day^{-1}$), S_1 oxy (87.82 µg TPF $g^{-1}day^{-1}$), and S_1 absolute control (86.53 µg TPF $g^{-1}day^{-1}$).

At harvest, dehydrogenase activity varied from 60.52 to 91.83 µg TPF $g^{-1}day^{-1}$ and all the interactions were followed the same order as in case of sixty days after herbicide application. Highest activity was noticed in S_2 control (91.83 µg TPF $g^{-1}day^{-1}$) which was statistically on par with S_2 cyhalo (90.89 µg TPF $g^{-1}day^{-1}$), S_2 oxy (90.09 µg TPF $g^{-1}day^{-1}$), S_2 bispyri (89.83 µg TPF $g^{-1}day^{-1}$), and S_2 pendi (89.72 µg TPF $g^{-1}day^{-1}$). Dehydrogenase activity was 85.43 µg TPF $g^{-1}day^{-1}$ and 67.31 µg TPF g^{-1} day^{-1} for S_2 and S_1 absolute control respectively. Lowest dehydrogenase activity was observed as 60.52 µg TPF $g^{-1}day^{-1}$ for S_1 pendi and which was closely on par with S_1 bispyri (61.65 µg TPF $g^{-1}day^{-1}$), S_1 oxy (61.81 µg TPF $g^{-1}day^{-1}$), and S_1 cyhalo (62.34 µg TPF $g^{-1}day^{-1}$).

4.5.3 Urease activity

Urease activity at different days after herbicide application is given in Table 4.11. Significant variation was observed in urease activity among soil types, treatments, and interactions between treatments and soil types at all the intervals of pot culture experiment studied.

Urease activity was found to be increased upto 60 DAHA in both soil types (S_1 and S_2), treatments (pendimethalin, oxyfluorfen, cyhalofop-butyl, bispyribac-sodium, control and absolute control), and interaction between soil

Table 4.11 Effect of herbicide application on urease activity at different days after herbicide application (DAHA)

Treatment	Urease activity(μg N-NH_4 g^{-1} hr^{-1})																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	72.70^{d}	98.08^{a}	85.39^{b}	79.10^{i}	120.09^{d}	99.60^{e}	101.61^{j}	187.20^{e}	144.41^{f}	126.34^{h}	208.02^{b}	167.18^{d}	187.63^{j}	367.81^{d}	277.72^{e}	72.95^{g}	117.92^{c}	95.43^{d}
Oxyfluorfen	72.70^{d}	98.05^{a}	85.38^{b}	83.86^{h}	123.66^{bc}	103.76^{d}	108.44^{h}	196.00^{c}	152.22^{d}	130.87^{fg}	208.63^{b}	169.75^{bc}	196.41^{h}	376.57^{c}	286.49^{c}	81.85^{f}	124.03^{ab}	102.94^{b}
Cyhalofop butyl	72.70^{d}	98.10^{a}	85.40^{b}	86.54^{g}	125.72^{ab}	106.13^{c}	110.17^{gh}	199.50^{b}	154.84^{c}	132.62^{ef}	210.54^{ab}	171.58^{b}	198.52^{h}	384.98^{b}	291.75^{b}	80.90^{f}	124.25^{a}	102.57^{bc}
Bispyribac sodium	72.70^{d}	98.08^{a}	85.39^{b}	82.71^{h}	122.89^{c}	102.80^{d}	104.48^{i}	192.50^{d}	148.49^{e}	128.94^{gh}	208.25^{b}	168.60^{cd}	192.30^{i}	370.27^{d}	281.29^{d}	81.64^{f}	120.64^{bc}	101.14^{bc}
Control	72.70^{d}	98.05^{a}	85.38^{b}	88.59^{g}	127.06^{a}	107.83^{b}	111.20^{h}	204.80^{a}	158.00^{b}	134.54^{e}	212.52^{a}	173.53^{b}	204.50^{g}	398.98^{a}	301.74^{a}	83.62^{f}	124.85^{a}	104.23^{ab}
Absolute control	85.42^{c}	91.80^{b}	88.61^{a}	104.41^{f}	117.06^{e}	110.74^{a}	136.50^{f}	187.20^{e}	161.85^{a}	168.00^{d}	191.30^{c}	179.65^{a}	262.51^{f}	290.10^{e}	276.30^{e}	99.03^{e}	114.09^{d}	106.56^{a}
Mean	74.82^{b}	97.03^{a}		87.54^{b}	122.75^{a}		112.07^{b}	194.53^{a}		136.89^{b}	206.54^{a}		206.98^{b}	364.78^{a}		83.33^{b}	120.96^{a}	
CD (0.05)	0.39*		0.67**	1.02		1.77	0.94		1.63	1.22		2.11	1.27		2.20	1.45		2.51
	0.95***			2.50			2.30			2.98			3.12			3.54		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

types and treatments. Among the soil types, S_2 (high O. M. soil) recorded highest urease activity compared to S_1 (medium O.M. soil) at all the intervals.

Among the treatments, mean urease activity was highest in absolute control at all the intervals except at 60 DAHA where, control recorded highest urease activity (301.74 µg N-NH_4 g^{-1} hr^{-1}). Lowest urease activity was observed in pendimethalin treatment at all the intervals studied. Significant variation was observed between pendimethalin and all other treatments. On the day but two hour before the application of herbicides, mean urease activity of the treatments varied from 85.38 to 88.61 µg N-NH_4 g^{-1} hr^{-1}. At seven, 15, 30, 60 DAHA and at harvest mean urease activity varied from 99.60 to 110.74 µg N-NH_4 g^{-1} hr^{-1}, 144.41 to 161.85 µg N-NH_4 g^{-1} hr^{-1}, 167.18 to 179.65 µg N-NH_4 g^{-1} hr^{-1}, 276.30 to 301.74 µg N-NH_4 g^{-1} hr^{-1}, and 95.43 to 106.56 µg N-NH_4 g^{-1} hr^{-1} respectively.

On the day but two hour before the application of herbicides, urease activity varied from 72.70 to 98.10 µg N-NH_4 g^{-1} hr^{-1} for the interactions. Interaction between S_1 and all the treatments as well as S_2 and all treatments were found to be statistically on par. Urease activity varied from 79.10 to 127.06 µg N-NH_4 g^{-1} hr^{-1}, 101.61 to 204.80 µg N-NH_4 g^{-1} hr^{-1}, 126.34 to 212.52 µg N-NH_4 g^{-1} hr^{-1}, 187.63 to 398.98 µg N-NH_4 g^{-1} hr^{-1}, 72.95 to 124.85 µg N-NH_4 g^{-1} hr^{-1} at 7, 15, 30, 60 DAHA and at harvest respectively. At seven, 15, 30, 60 DAHA, and at harvest S_2 control recorded highest activity and lowest urease activity by S_1 pendi.

Urease activity of all interactions were followed the same order upto 60 DAHA *viz.*, S_2 control followed by S_2 cyhalo, S_2 oxy, S_2 bispyri, S_2 pendi, S_2 absolute control, S_1 absolute control, S_1 control, S_1 cyhalo, S_1 oxy, S_1 bispyri and S_1 pendi. Urease activity of all the interactions was decreased at harvest.

4.5.4 Acid phosphatase activity

Effects of herbicide application on acid phosphatase activity are given in Table 4.12. Acid phosphatase activity showed significant difference among the

soil type, treatments, and interaction between soil type and treatments throughout the intervals studied.

Comparison of acid phosphatase activity in medium and high O.M. soils revealed that the activity was significantly higher in high organic matter soils compared to medium O.M. soils in all the treatments throughout the experiment. On the day but two hour before the herbicide application, mean acid phosphatase activity was 99.67 µg PNP $g^{-1}hr^{-1}$ in S_2 (high O.M. soils) and 68.63 µg PNP $g^{-1}hr^{-1}$ in S_1 (medium O. M. soils). There was significant increase in mean acid phosphatase activity at seven, 15, 30 and 60 days after herbicide application (114.60 µg PNP $g^{-1}hr^{-1}$for S_2 and 76.54 µg PNP $g^{-1}hr^{-1}$for S_1 at seven days after herbicide application, 117.70 µg PNP $g^{-1}hr^{-1}$ for S_2 and 78.68 µg PNP $g^{-1}hr^{-1}$ for S_1 at 15 DAHA, 125.18 µg PNP $g^{-1}hr^{-1}$ for S_2 and 84.84 µg PNP $g^{-1}hr^{-1}$ for S_1 at 30 DAHA, and 150.40 µg PNP $g^{-1}hr^{-1}$ for S_2 and 100.04 µg PNP $g^{-1}hr^{-1}$ for S_1 at 60 DAHA. Acid phosphatase activity decreased at harvest in both the soil types (123.66 µg PNP g^{-1} hr^{-1} in S_2 and 74.58 µg PNP $g^{-1}hr^{-1}$ in S_1).

Treatment mean values differed significantly for acid phosphatase activity. Among the treatments, control had highest activity compared to all other treatments throughout the intervals. On the day but two hour before the application of herbicides, the highest mean activity was shown by control (84.69 µg PNP g^{-1} hr^{-1}) which was statistically on par with all other treatments except absolute control (81.49 µg PNP g^{-1} hr^{-1}) which recorded the lowest mean acid phosphatase activity. At 7 DAHA, control recorded highest mean acid phosphatase activity (96.62 µg PNP g^{-1} hr^{-1}) which was statistically on par with oxyfluorfen (96.22 µg PNP $g^{-1}hr^{-1}$), cyhalofop-butyl (96.51 µg PNP $g^{-1}hr^{-1}$) and bispyribac-sodium (95.74 µg PNP $g^{-1}hr^{-1}$ followed by pendimethalin (94.62 µg PNP $g^{-1}hr^{-1}$) and absolute control (93.71 µg PNP $g^{-1}hr^{-1}$). Acid phosphatase activity in pendimethalin and absolute control were statistically on par.

At 15 DAHA, mean acid phosphatase activity was highest in control (100.20 µg PNP $g^{-1}hr^{-1}$) which was statistically on par with cyhalofop-butyl

Table 4.12 Effect of herbicide application on acid phosphatase activity at different days after herbicide application (DAHA)

Treatment	Acid phosphatase activity (µg PNP g^{-1} hr^{-1})																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	67.07^{d}	102.30^{a}	84.69^{a}	73.06^{f}	116.17^{b}	94.62^{bc}	75.37^{f}	118.37^{b}	96.87^{c}	79.05^{i}	124.62^{d}	101.84^{d}	92.14^{j}	150.36^{d}	121.25^{d}	63.02^{i}	119.53^{b}	91.28^{e}
Oxyfluorfen	67.07^{d}	102.29^{a}	84.68^{a}	74.58^{cf}	117.86^{a}	96.22^{a}	75.64^{f}	120.63^{ab}	98.14^{bc}	82.41^{h}	127.92^{bc}	105.17^{bc}	97.05^{hi}	152.81^{bc}	124.93^{bc}	69.09^{g}	126.92^{a}	98.00^{d}
Cyhalofop butyl	67.07^{d}	102.30^{a}	84.69^{a}	74.96^{e}	118.06^{a}	96.51^{a}	76.6^{cf}	120.96^{a}	98.78^{ab}	82.86^{gh}	128.86^{ab}	105.86^{b}	98.68^{gh}	154.40^{ab}	126.54^{b}	72.78^{f}	128.01^{a}	100.40^{c}
Bispyribac sodium	67.07^{d}	102.30^{a}	84.68^{a}	74.24^{cf}	117.24^{ab}	95.74^{ab}	75.49^{f}	120.02^{ab}	97.76^{bc}	81.53^{h}	127.08^{c}	104.31^{c}	96.05^{i}	151.76^{cd}	123.91^{c}	67.08^{h}	126.84^{a}	96.96^{d}
Control	67.07^{d}	102.30^{a}	84.69^{a}	75.07^{e}	118.18^{a}	96.62^{a}	78.82^{e}	121.59^{a}	100.20^{a}	84.12^{g}	130.25^{a}	107.19^{a}	100.01^{g}	156.46^{a}	128.24^{a}	82.56^{e}	128.32^{a}	105.44^{a}
Absolute control	76.43^{c}	86.54^{b}	81.49^{b}	87.36^{d}	100.05^{c}	93.71^{c}	90.18^{d}	104.51^{c}	97.35^{bc}	99.07^{f}	112.34^{e}	105.71^{b}	116.28^{f}	136.61^{e}	126.45^{b}	92.95^{d}	112.37^{c}	102.66^{b}
Mean	68.63^{b}	99.67^{a}		76.54^{b}	114.60^{a}		78.68^{b}	117.70^{a}		84.84^{b}	125.18^{a}		100.04^{b}	150.40^{a}		74.58^{b}	123.66^{a}	
CD (0.05)	0.32*		0.55**	0.68		1.18	1.02		1.76	0.58		1.00	0.94		1.64	0.74		1.29
	0.78***			1.67			2.49			1.41			2.31			1.82		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

(98.78 µg PNP $g^{-1}hr^{-1}$) and differed significantly with all other treatments. Acid phosphatase activity was highest in control and lowest in pendimethalin at 30 DAHA, 60 DAHA and at harvest.

Acid phosphatase activity at 30 and 60 DAHA were followed the same order *viz*., control (107.19 µg PNP $g^{-1}hr^{-1}$ at 30 and 128.24 µg PNP $g^{-1}hr^{-1}$ at 60 DAHA) > cyhalofop-butyl (105.86 µg PNP $g^{-1}hr^{-1}$ at 30 and 126.54 µg PNP $g^{-1}hr^{-1}$ at 60 DAHA) > absolute control (105.71 µg PNP $g^{-1}hr^{-1}$ at 30 DAHA and 126.45 µg PNP $g^{-1}hr^{-1}$ at 60 DAHA) > oxyfluorfen (105.17 µg PNP $g^{-1}hr^{-1}$ at 30 and 124.93 µg PNP $g^{-1}hr^{-1}$ at 60 DAHA) > bispyribac -sodium (104.31 µg PNP $g^{-1}hr^{-1}$ at 30 and 123.91 µg PNP $g^{-1}hr^{-1}$ at 60 DAHA) > pendimethalin (101.84 µg PNP $g^{-1}hr^{-1}$ at 30 and 121.25 µg PNP $g^{-1}hr^{-1}$ at 60 DAHA). Significant variation observed at harvest in the all treatments and highest mean acid phosphatase activity was in control (105.44 µg PNP $g^{-1}hr^{-1}$) followed by absolute control (102.66 µg PNP $g^{-1}hr^{-1}$), cyhalofop- butyl (100.40 µg PNP $g^{-1}hr^{-1}$), oxyfluorfen (98.00 µg PNP $g^{-1}hr^{-1}$), bispyribac-sodium (96.96 µg PNP $g^{-1}hr^{-1}$) and pendimethalin (91.28 µg PNP $g^{-1}hr^{-1}$). Acid phosphatase activity was statistically on par in soils treated with oxyfluorfen and bispyribac- sodium. There was a drastic decrease in acid phosphatase activity in all the treatments at harvest compared to 60 DAHA.

Interaction between soil types and treatments also differed significantly in case of acid phosphatase activity at all the intervals during the pot culture experiment. Acid phosphatase activity was highest in S_2 control and lowest in S_1 pendi throughout the intervals. On the day but two hour before the application of herbicides, highest activity was noted in S_2 control (102.30 µg PNP $g^{-1}hr^{-1}$) which was statistically on par with S_2 pendi, S_2 bispyri, S_2 cyhalo, and S_2 pendi with an activity ranged from 102.29 to 102.30 µg PNP $g^{-1}hr^{-1}$ followed by S_2 absolute control (86.54 µg PNP $g^{-1}hr^{-1}$), S_1 absolute control (76.43 µg PNP $g^{-1}hr^{-1}$), S_1 control (67.07 µg PNP $g^{-1}hr^{-1}$) which was on par with S_1 pendi, S_1 oxy, S_1 cyhalo and S_1 bispyri with same activity (67.07 µg PNP $g^{-1}hr^{-1}$) respectively. Interaction between each soil type and corresponding treatment combinations were

statistically on par in both S_1 and S_2 on the day but two hour before application of herbicides. There was an increase in acid phosphatase activity at all the intervals except a drastic decrease at harvest.

Acid phosphatase activity varied from 73.06 to 118.18 µg PNP $g^{-1}hr^{-1}$, 75.37 to 121.59 µg PNP $g^{-1}hr^{-1}$, 79.05 to 130.25 µg PNP $g^{-1}hr^{-1}$, 92.14 to 156.46 µg PNP $g^{-1}hr^{-1}$, and 63.02 to 128.32 µg PNP $g^{-1}hr^{-1}$ at 7, 15, 30, 60 DAHA and at harvest respectively. Acid phosphatase activity at all intervals followed the order *viz.*, S_2 control > S_2 cyhalo > S_2 oxy > S_2 bispyri > S_2 pendi > S_2 absolute control > S_1 absolute control > S_1 control > S_1cyhalo > S_1 oxy > S_1 bispyri> S_1 pendi.

4.5.5 Alkaline phosphatase activity

There was significant difference in alkaline phosphatase activity among soil types and interaction between soil types and treatments. Statistically, no significant difference recorded among the treatments at all intervals of pot culture experiment except on the day but two hour before the application of herbicides. The results are given in Table 4.13.

Among the soil types highest alkaline phosphatase activity was recorded in S_2 (high O. M. soils) compared to S_1 (medium O. M. soils) at all the intervals of pot culture experiment. Alkaline phosphatase activity followed an increasing trend upto 60 DAHA and declined at harvest. Maximum alkaline phosphatase activity (mean value of soil types) was observed at 60 DAHA in both the soil types (50.25 µg PNP $g^{-1}hr^{-1}$ for S_1 and 86.91 µg PNP $g^{-1}hr^{-1}$ for S_2).

Treatment mean values differed significantly only on the day but two hour before the herbicide application. The highest mean alkaline phosphatase activity of 56.01 µg PNP $g^{-1}hr^{-1}$ was observed in control, pendimethalin, oxyfluorfen, bispyribac-sodium, and cyhalofop-butyl. Lowest enzyme activity was noticed in absolute control (53.76 µg PNP $g^{-1}hr^{-1}$). Alkaline phosphatase activity varied from 58.44 to 60.02 µg PNP $g^{-1}hr^{-1}$, 59.68 to 61.05 µg PNP $g^{-1}hr^{-1}$, 61.13 to 62.93 µg

Table 4.13 Effect of herbicide application on alkaline phosphatase activity at different days after herbicide application (DAHA)

Treatment	Alkaline phosphatase activity (µg PNP g^{-1} hr^{-1})																	
	0 DAHA			7 DAHA			15 DAHA			30 DAHA			60 DAHA			Harvest		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	39.28^{d}	72.73^{a}	56.01^{a}	40.25^{d}	76.63^{a}	58.44	41.40^{d}	77.97^{a}	59.68	42.26^{d}	80.00^{a}	61.13	46.04^{d}	88.39^{a}	67.21	38.26^{e}	78.34^{a}	58.30
Oxyfluorfen	39.28^{d}	72.73^{a}	56.01^{a}	41.56^{d}	76.98^{a}	59.27	42.36^{d}	78.71^{a}	60.53	43.25^{d}	81.08^{a}	62.17	47.18^{d}	89.19^{a}	68.18	40.29^{de}	80.75^{a}	60.52
Cyhalofop-butyl	39.28^{d}	72.73^{a}	56.01^{a}	41.72^{d}	77.30^{a}	59.51	42.42^{d}	78.81^{a}	60.61	43.63^{d}	81.16^{a}	62.40	47.86^{d}	89.69^{a}	68.78	42.12^{de}	81.11^{a}	61.62
Bispyribac-sodium	39.28^{d}	72.73^{a}	56.01^{a}	41.44^{d}	76.97^{a}	59.20	41.47^{d}	78.51^{a}	59.99	43.16^{d}	80.81^{a}	61.98	46.84^{d}	88.85^{a}	67.84	39.29^{de}	80.03^{a}	59.66
Control	39.28^{d}	72.73^{a}	56.01^{a}	42.08^{d}	77.95^{a}	60.02	42.74^{d}	79.35^{a}	61.05	44.01^{d}	81.84^{a}	62.93	48.35^{d}	90.38^{a}	69.37	43.18^{d}	81.21^{a}	62.20
Absolute control	51.84^{c}	55.67^{b}	53.76^{b}	56.61^{c}	61.05^{b}	58.83	57.84^{c}	62.59^{b}	60.22	58.76^{c}	64.52^{b}	61.64	65.22^{c}	74.96^{b}	70.09	59.05^{c}	65.62^{b}	62.34
Mean	41.37^{b}	69.89^{a}		43.94^{a}	74.48^{a}		44.70^{b}	75.99^{a}		45.85^{b}	78.23^{a}		50.25^{b}	86.91^{a}		43.70^{b}	77.84^{a}	
CD (0.05)	0.78*		1.35**	1.20		NS	1.41		NS	1.10		NS	1.90		NS	1.76		NS
	1.90***			2.93			3.45			2.70			4.64			4.32		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

PNP $g^{-1}hr^{-1}$, 67.21 to 70.09 µg PNP $g^{-1}hr^{-1}$, and 58.30 to 62.34 µg PNP $g^{-1}hr^{-1}$ at 7, 15, 30, 60 days after herbicide application and at harvest respectively.

Alkaline phosphatase activity was highest in S_2 control and lowest in S_1 pendimethalin among the interactions at all the intervals studied. Alkaline phosphatase activity recorded maximum value at 60 DAHA for all the interactions. Enzyme activity increased upto 60 DAHA followed by decrease at harvest.

Enzyme activity varied from 39.28 to 72.73 µg PNP $g^{-1}hr^{-1}$, 40.25 to 77.95 µg PNP g^{-1} hr^{-1}, 41.40 to 79.35 µg PNP $g^{-1}hr^{-1}$, 42.26 to 81.84 µg PNP g^{-1} hr^{-1}, 46.04 to 90.38 µg PNP g^{-1} hr^{-1}, and 38.26 to 81.21 µg PNP g^{-1} hr^{-1} at the day but two hour before herbicide application, 7, 15, 30, 60 DAHA and at harvest respectively for the interactions.

The alkaline phosphatase activity were followed the same order for the interactions throughout the intervals *viz.*, S_2 control > S_2 cyhalo > S_2 oxy > S_2 bispyri > S_2 pendi > S_2 absolute control > S_1 absolute control > S_1 control > S_1 cyhalo > S_1 oxy > S_1 bispyri > S_1 pendi. Enzyme activity increased upto 60 DAHA followed by a decrease at harvest in all the interactions. Alkaline phosphatase activity was statistically on par at all the intervals for S_2 control, S_2 cyhalo, S_2 oxy, S_2 bispyri and S_2 pendi. Similar trend was observed in S_1 interactions (S_1 control on par with S_1 cyhalo, S_1 oxy, and S_1 bispyri) except a variation at harvest in the case of S_1 pendimethalin (38.26 µg PNP g^{-1} hr^{-1}).

4.6 PLANT CHARACTERS

4.6.1 Biometric observations on rice

Height of the plant and productive tillers were recorded at 60 days after sowing and the results obtained are given in Table 4.14.

4.6.1.1 Plant height

Plant height differed significantly between the soil types, treatments and interaction between soil type and treatments.

Among the soil types, S_2 recorded maximum height as compared to S_1 and among the treatments maximum height was observed in control (96.45 cm) and lowest in absolute control (96.04 cm). Plant height of absolute control plant was closely on par with pendimethalin (96.05 cm). All the other treatments (mean values) varied significantly in plant height.

Statistical analysis of the interaction between soil types and treatments showed significant difference in plant height. Among the interactions, S_2 control (97.95 cm) recorded maximum plant height which was followed by S_2 cyhalo (97.70 cm), S_2 oxy (97.56 cm) and S_2 bispyri (97.47 cm). Minimum plant height was observed in S_1 pendi (94.74 cm). Plant height of S_1 oxy (94.78 cm) and S_1 bispyri (94. 77 cm) are statistically on par.

4.6.1.2 Productive tillers

Number of productive tillers differed significantly between the soil type S_1 and S_2 and no significant difference was observed between the treatments and interactions between soil type and treatments.

Number of productive tillers varied from 16.83 (S_1 pendi and S_1 control) to 20 (S_2 pendi, S_2 oxy, S_2 cyhalo, S_2 bispyri, and S_2 control).

Table 4.14 Biometric observations of rice from pot culture experiment

Treatment	Biometric observations					
	Plant height (cm)			Productive tillers (No.)		
	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	94.74	97.36	96.05	16.83	20.00	18.42
Oxyfluorfen	94.78	97.56	96.17	17.00	20.00	18.50
Cyhalofop butyl	94.89	97.70	96.30	17.00	20.00	18.50
Bispyribac sodium	94.77	97.47	96.12	17.17	20.00	18.58
Control	94.94	97.95	96.45	16.83	20.00	18.42
Absolute control	94.95	97.12	96.04	17.83	18.33	18.08
CD (0.05)	0.01*		0.01**	0.56*		NS**
	0.02***			NS***		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

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4.6.2 Yield and yield attributes

Data on yield and yield attributes are given in Table 4.15.

4.6.2.1 Yield

Grain yield and straw yield was estimated after the completion of crop and it was given on per pot basis (Table 4.15)

Grain yield per pot differed significantly among soil types, treatments and interaction between soil type and treatments. Among the soil types, highest grain yield was recorded by high O.M soil (S_2) as compared to medium O.M. soil (S_1). Among the treatments, highest mean grain yield of 23.94 g/pot was obtained in control followed by absolute control (23.57 g/pot), cyhalofop-butyl (23.45 g/pot), bispyribac-sodium (23.33 g/pot), oxyfluorfen (22.52 g/pot) and finally lowest in pendimethalin (21.95 g/pot). Among the interactions, highest grain yield was 24.85 g/pot in S_2 control and lowest in S_1 pendimethalin with grain yield of 20.67 g/pot.

Significant variation was observed in straw yield (g/ pot) among soil types, treatments, and interaction between soil types and treatments. Highest straw yield was observed in S_2 as compared to S_1. Control recorded highest mean straw yield among the treatments (26.01g/pot) and straw yield was statistically on par with absolute control (25.65g/pot). Straw yield was found to be lowest in pendimethalin (23.53 g/pot). Among the interactions, S_2 control recorded highest straw yield (26.93 g/pot) and lowest by S_1 pendimethalin (21.75 g/pot) which was closely on par with S_1 oxyfluorfen (21.86 g/pot).

Table 4.15 Yield and yield attributes of rice from pot culture experiment

Treatment	Yield and yield attributes														
	Yield						Yield attributes								
	Grain yield (g/ pot)			Straw yield (g/ pot)			Panicles/ pot (No.)			Grains/ panicle (No.)			1000 grain weight (g)		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	20.67	23.23	21.95	21.75	25.31	23.53	9.67	10.67	10.17	84.33	85.15	84.74	25.35	25.57	25.46
Oxyfluorfen	20.78	24.26	22.52	21.86	26.34	24.10	9.67	11.00	10.33	84.67	86.15	85.41	25.38	25.60	25.49
Cyhalofop butyl	23.00	23.90	23.45	25.08	25.98	25.53	10.67	10.67	10.67	84.92	87.45	86.19	25.38	25.61	25.49
Bispyribac sodium	22.46	24.20	23.33	24.54	26.28	25.41	10.33	11.00	10.67	85.59	86.01	85.80	25.40	25.58	25.49
Control	23.02	24.85	23.94	25.10	26.93	26.01	10.67	11.00	10.67	84.93	88.23	86.58	25.40	25.60	25.50
Absolute control	23.26	23.87	23.57	25.34	25.95	25.65	10.67	10.67	10.67	85.47	87.45	86.46	25.50	25.58	25.54
CD (0.05)	0.04*		0.07**	0.21		0.36	NS		NS	NS		NS	0.01		0.01
	0.10***			0.50			NS			NS			0.02		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

4.6.2.2 Yield attributes

Yield attributes like number of panicles/pot, number of grains per panicle, and 1000 grain weight was given in Table 4.15. There was no significant difference in number of panicles/pot and number of grains per panicle among soil types, treatments and interaction between soil types and treatments.

Thousand grain weight was highest in high O.M. soils (S_2) than medium O.M. soils (S_1). Among the treatments, absolute control recorded highest mean grain weight (25.54 g) and lowest in pendimethalin (25.46 g) all other treatments was found to be statistically on par.

Among the interactions, S_2 cyhalofop-butyl recorded highest value (25.61g) which was closely on par with S_2 oxyfluorfen (25.60 g) and S_2 control (25.60 g). S_1 pendimethalin recorded lowest grain weight of 25.35g.

4.6.3 Nutrient content at harvest

Major nutrients like N, P, and K were analysed after the harvest of rice and the results are given in Table 4.16.

All the nutrients (N, P, and K) analysed were found to be highest in S_2 (high organic matter soils) compared to S_1 (medium organic matter soils).

Data on nitrogen content of plant samples revealed that there was no significant difference between the treatments and interaction between soil types and treatments. Nitrogen content differed significantly between two soil types S_1 and S_2.

Phosphorus content differed significantly between the soil types S_1 and S_2, treatments and interaction between soil types and treatments. Among the treatments, highest mean phosphorus content was observed in control (1.26 %)

Table 4.16 Content of major nutrients (N, P, and K) in rice at harvest

Treatment	Major nutrients (%)								
	N			P			K		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
Pendimethalin	2.19	2.67	2.43	0.90	1.34	1.12	1.96	2.39	2.18
Oxyfluorfen	2.20	2.73	2.46	0.95	1.36	1.16	2.05	2.55	2.30
Cyhalofop butyl	2.29	2.82	2.55	0.96	1.40	1.18	2.07	2.52	2.29
Bispyribac sodium	2.24	2.70	2.47	0.96	1.36	1.16	2.06	2.55	2.31
Control	2.36	2.81	2.59	1.09	1.42	1.26	2.10	2.63	2.37
Absolute control	2.57	2.69	2.63	1.16	1.22	1.19	2.22	2.56	2.39
CD (0.05)	0.01*		NS**	0.02		0.003	0.08		0.13
	NS***			0.01			NS		

CD (0.05)* : CD for comparing the soil types (S_1: Medium organic matter soil from ARS Mannuthy and S_2: High organic matter soil from Kole land, Alappad)
CD (0.05)** : CD for comparing the treatments
CD (0.05)***:CD for comparing the effect of treatments under each soil types (interaction)

and lowest in pendimethalin (1.12 %).Among the interactions, S_2 control recorded highest phosphorus content (1.42 %) and lowest by S_1 pendimethalin (0.90 %).

Potassium content of plant samples differed significantly among the soil types and treatments. There was no significant difference among the interaction between soil types and treatments. Potassium content was found to be higher in S_2 compared to S_1. The mean value of treatments varied from 2.18 to 2.39 per cent. Pendimethalin treatment recorded lowest potassium content of 2.18 per cent.

4.7 CORRELATION STUDIES

Data on soil characteristics *viz*., pH, organic carbon and biological activity in the soil namely microbial biomass carbon, dehydrogenase activity, urease activity, acid and alkaline phosphatase activity at different sampling intervals were subjected to correlation analysis. The correlation coefficients (r) between chemical and biological characteristics of the soil were estimated and presented in Table 4.17.

In the present study it was observed that soil acidity had negative correlation with microbial biomass carbon and enzyme activities *viz*., dehydrogenase, urease, acid and alkaline phosphatase at all the intervals studied.

Organic carbon had highly significant and positive correlation with microbial biomass carbon, dehydrogenase, urease, acid and alkaline phosphatase activity throughout the intervals.

Table 4.17 Coefficients of correlation (r) between chemical and biological characteristics of the soil during the period of pot culture experiment

Parameter	Correlation coefficient (r)					
	0 DAHA	7 DAHA	15 DAHA	30 DAHA	60 DAHA	Harvest
pH and microbial biomass carbon	-0.758**	-0.708*	-0.704*	-0.660*	-0.622*	-0.479
pH and dehydrogenase activity	-0.769**	-0.730**	-0.603*	-0.642*	-0.601*	-0.523
pH and urease activity	-0.620*	-0.564	-0.638*	-0.546	-0.535	-0.374
pH and acid phosphatase activity	-0.719**	-0.666*	-0.652*	-0.589*	-0.547	-0.386
pH and alkaline phosphatase activity	-0.669*	-0.607*	-0.595*	-0.574	-0.517	-0.387
OC and microbial biomass carbon	1.000**	0.997**	0.999**	0.999**	0.996**	0.993**
OC and dehydrogenase activity	0.999**	0.996**	0.964**	0.993**	0.993**	0.997**
OC and urease activity	0.977**	0.966**	0.981**	0.982**	0.985**	0.980**
OC and acid phosphatase activity	0.994**	0.994**	0.994**	0.990**	0.993**	0.979**
OC and alkaline phosphatase activity	0.983**	0.983**	0.984**	0.986**	0.990**	0.985**

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

DISCUSSION

5. DISCUSSION

The major findings obtained from the project entitled "Effect of herbicides and chemical characteristics of soil on microbial biomass carbon and enzyme activity" are discussed in this chapter.

Representative soil samples collected from four sites were analysed to study the physical, chemical and biological properties and the results are discussed in sections 5.1 to 5.3. Pot culture experiment was conducted with rice and the major findings are discussed in sections 5.4 to 5.6. Relationship between chemical properties and biological activity of the soil during pot culture experiment are discussed in section 5.7.

5.1 PHYSICAL CHARACTERISTICS OF SOILS TAKEN FOR THE STUDY

The most important physical property of the soil is its texture (relative proportion by weight percentage of sand, silt, and clay). Soil texture determines infiltration, hydraulic conductivity, water holding capacity, the ease of tilling the soil and the amount of aeration (which is vital to root growth) and also influence the soil fertility (Das, 2012). In this study, the samples collected from the four different locations were classified under sandy clay loam and clay texture (Fig.1). Clay content of the soil samples from the rice field of Kole land was very high (58.31 %) and hence these soils can be classified under the textural class clay. All the other three soils are coming under sandy clay loam. Results revealed that there was no difference in texture consequent to the continuous application of herbicides which was evidenced from the similarity in texture in the soils of non-cropped area and rice field of ARS Mannuthy. Texture is a basic property of a soil and it can- not be altered or changed (Brady, 1974).

Bulk density of a soil is a dynamic property that varies with the soil structural conditions and it is influenced by the amount of organic matter in soils. Medium O.M. soils (soil samples from ARS Mannuthy) recorded highest bulk

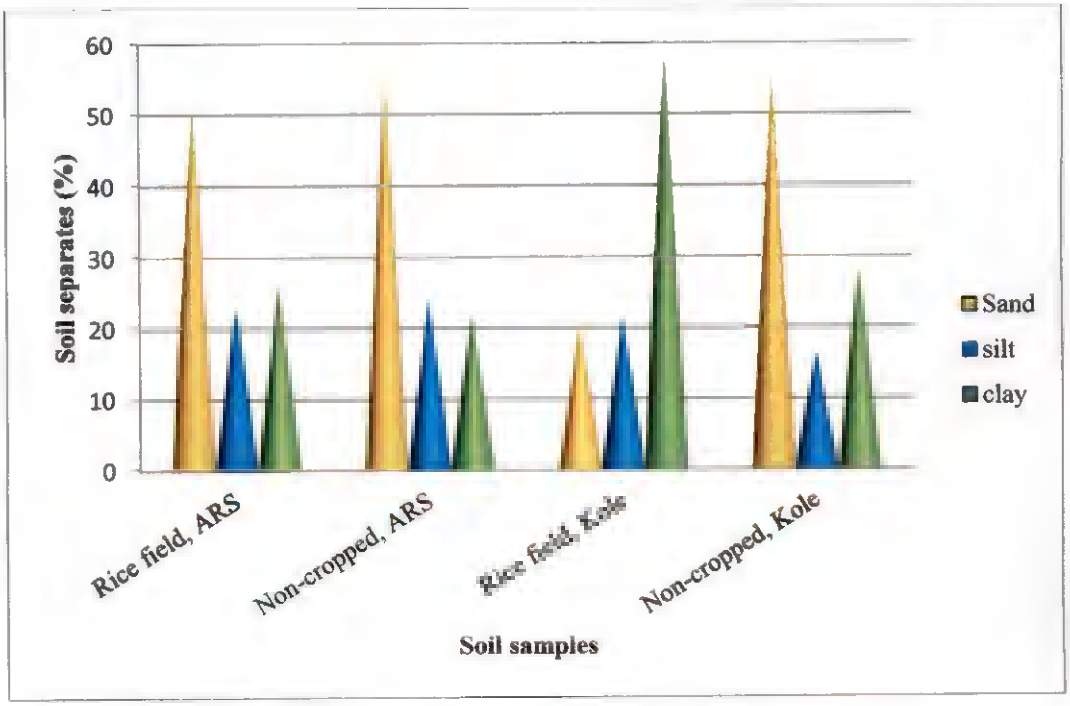

Fig. 1: Mechanical composition of soil samples taken for the study

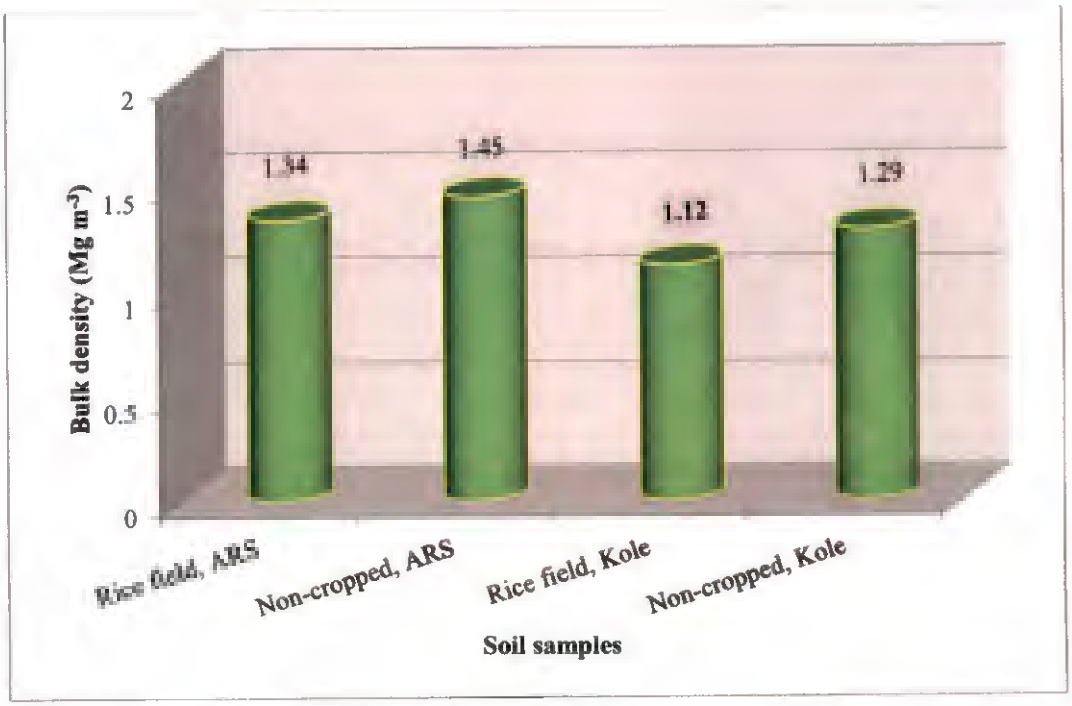

Fig. 2: Bulk density of soil samples taken for study

density value (1.34 to 1.45 Mg m^{-3}) as compared to high O.M soils from Kole land, Alappad (1.12 to 1.29 Mg m^{-3}). Rice soils have lower bulk density value due to the presence of clay content (Fig. 2).

Results indicated that particle density was higher in both the soils of ARS Mannuthy (Fig.3) compared to the soils of Kole land. This is because of comparatively lower organic matter content in these soils. Lower particle density was observed in samples taken from rice soils of Kole land as well as non-cropped area of Kole land due to high levels of organic matter.

Among the four different types of soil samples, highest porosity (46.85 %) was recorded in soil with high organic matter content which was collected from rice field of Kole land with a history of herbicide application (Fig.4). These soils also registered highest water holding capacity (43.60 %) and volume of expansion (26.25 %) (Fig.5 and 6).

Therefore, all the physical characteristics observed in the rice field of Kole land can be considered as favourable for the growth of microflora.

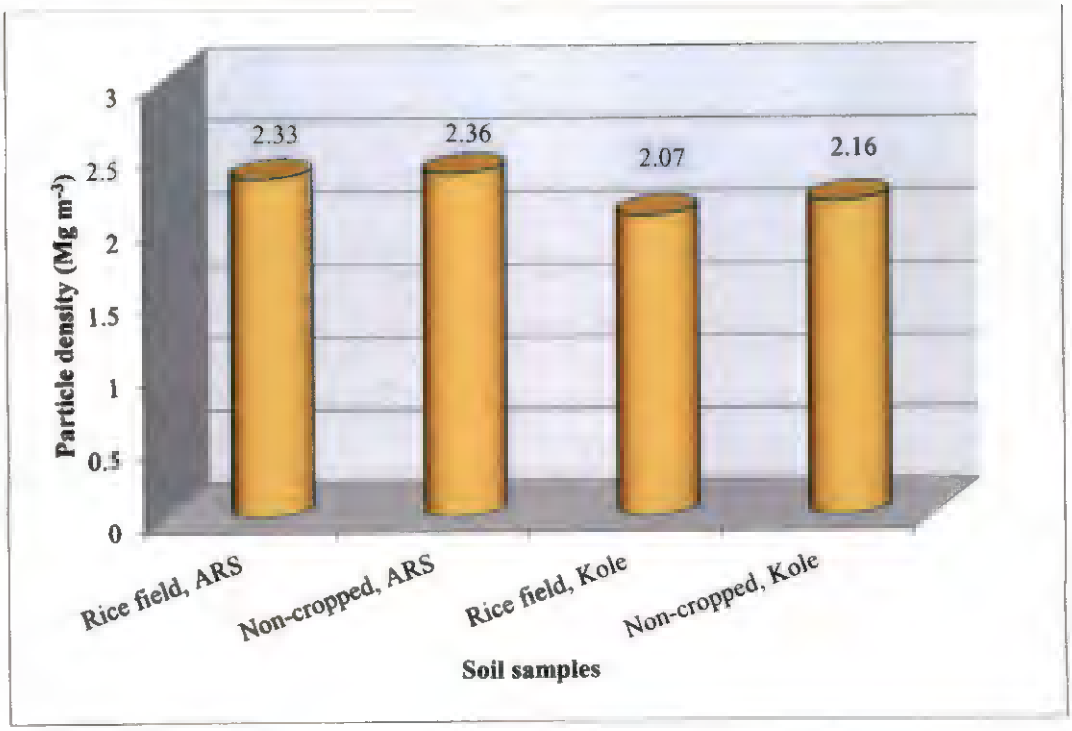

Fig. 3: Particle density of soil samples taken for study

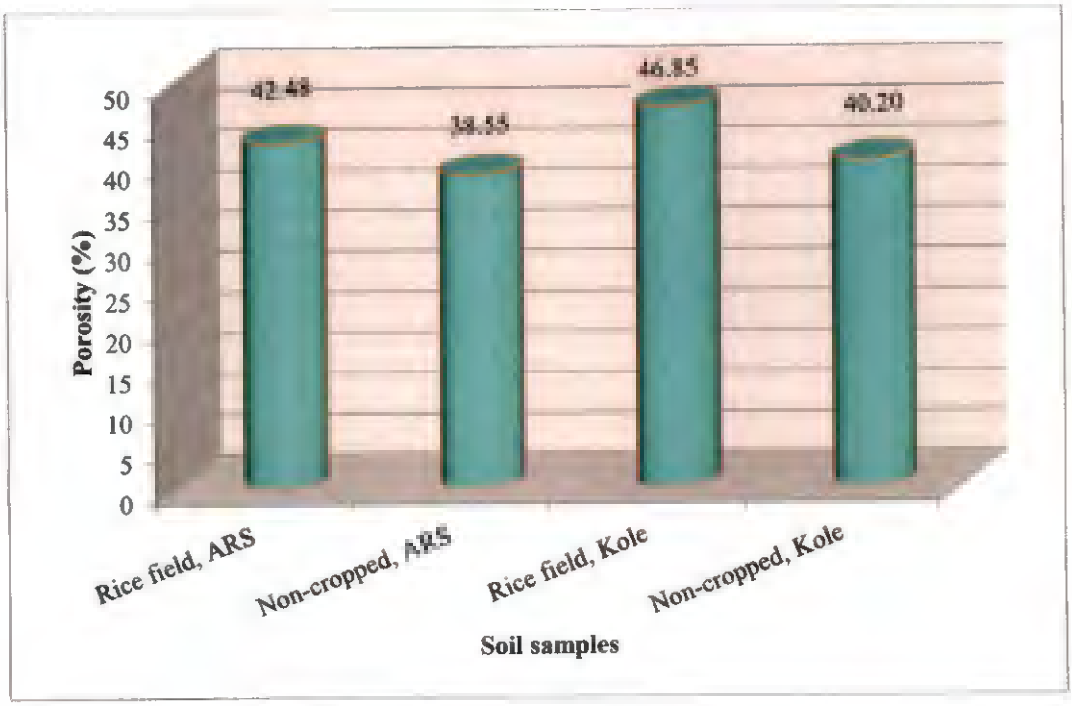

Fig. 4: Porosity of soil samples taken for study

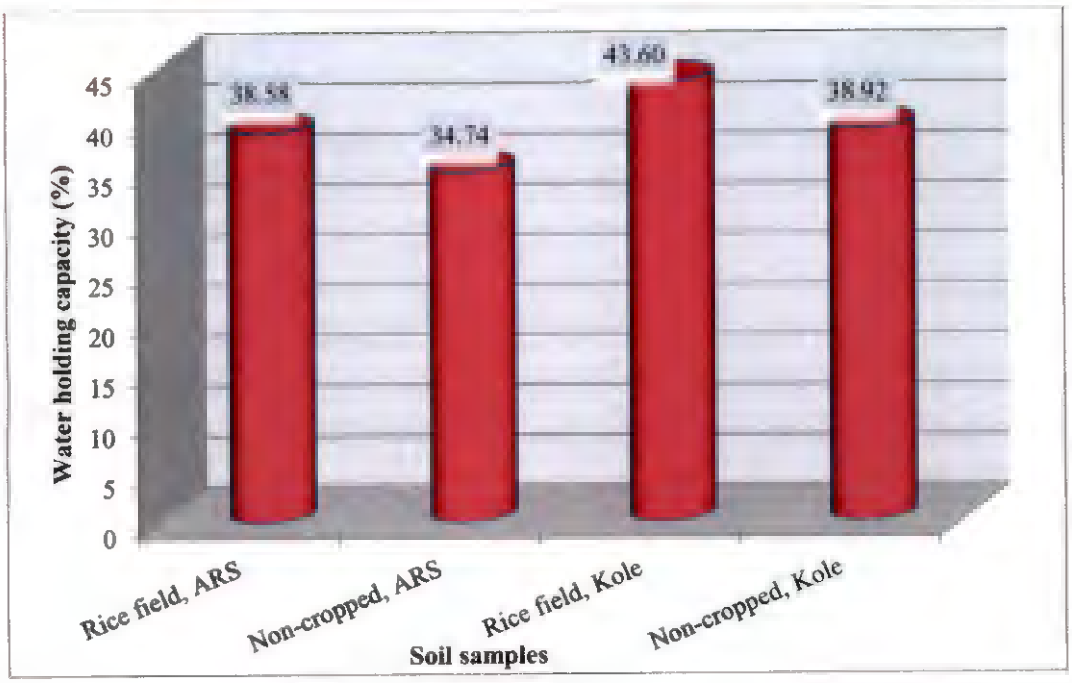

Fig. 5: Water holding capacity of soil samples taken for study

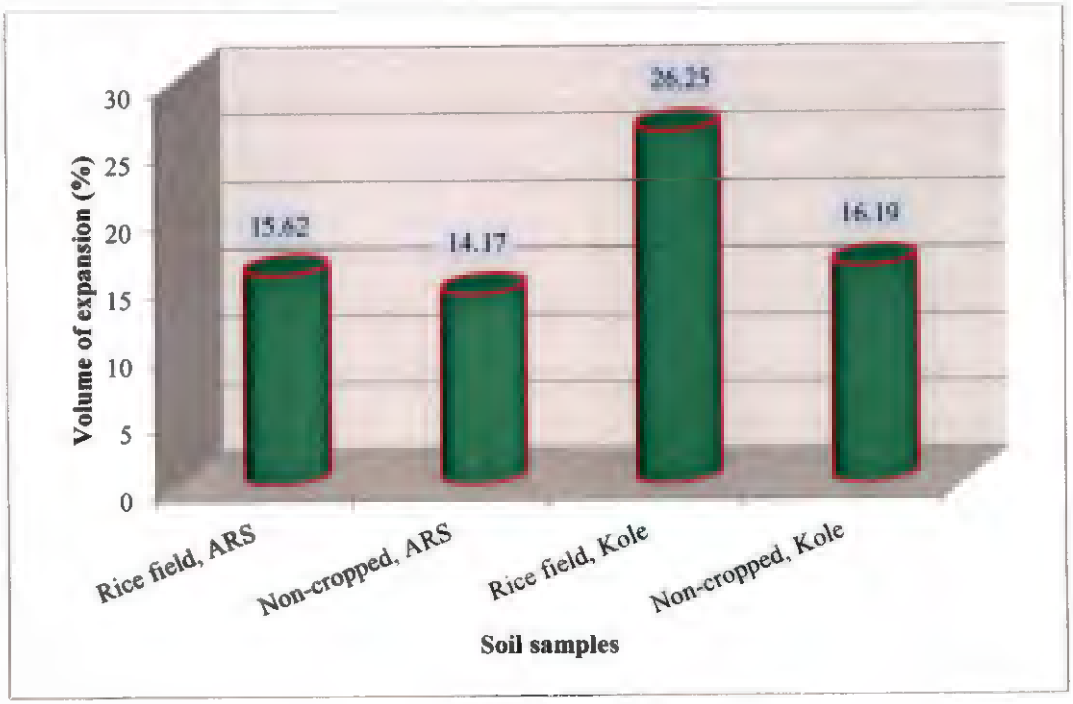

Fig. 6: Volume of expansion of soil samples taken for study

5.2 CHEMICAL CHARACTERISTICS OF SOILS TAKEN FOR THE STUDY

The soil samples differed in their pH in the range of 5.01 to 5.68. Rice fields registered lower pH in both the soil types (high O. M. and medium O.M. soils) which would have been the result of continuous application of fertilizers particularly nitrogenous fertilizers.

Electrical conductivity was highest in Kole land soils (rice field and non-cropped area) due to the presence of soluble salts. Rice soils of Kole lands have lower values compared to non-cropped area of Kole land mainly due to washing of soluble salts due to submergence during rainy season. Soil samples from all the sampling sites registered electrical conductivity within the safe limit.

Organic carbon content varied from 0.85 to 2.47 per cent. As per the soil fertility class, the soils collected from ARS Mannuthy was in medium range while Kole land soils were included in high fertility class. So the selected soils satisfied the criteria (based on organic matter content) for taking up pot culture experiment as in technical programme.

Cation exchange capacity was higher in Kole land soils (13.69 to 15.43 c mol (+) kg^{-1}) as compared to soils from ARS Mannuthy (9.53 to 10.40 c mol (+) kg^{-1}). The data followed the order of their organic matter content. Increase in cation exchange capacity with organic matter content is due the negative charges produced from the carboxyl group of organic matter (Parfitt *et al.*, 1995).

Available nitrogen, phosphorus and potassium content were also higher in Kole land soils due to high organic matter content. The soils under study can be ranked based on major nutrients as rice soils of Kole land > non-cropped, Kole > non-cropped, ARS > rice field, ARS which followed the same order as in organic carbon content. Available nutrients are functions of soil organic matter content (Brady, 1974).

As in the case of major nutrients N, P, and K, available secondary nutrients (Ca and Mg) and micronutrients (Fe, Mn, and Cu) were also higher in

rice soils of Kole land, Alappad except the zinc content which was highest in non-cropped area of Kole land, Alappad. All the soils recorded secondary and micronutrients in the sufficiency range.

Studies on the chemical characteristics of soil samples selected for the pot culture experiment indicated that organic carbon is the major factor determining the soil fertility. All the chemical characteristics were superior in high organic matter soils compared to medium organic matter soils, which favoured the growth of microflora.

5.3 BIOLOGICAL CHARACTERISTICS OF SOILS TAKEN FOR THE STUDY

The data on the initial analysis of biological characteristics of the soil samples *viz.*, microbial biomass carbon and enzyme activity (dehydrogenase, urease, acid and alkaline phosphatase activity) revealed that biological activity of the soil is dependent mainly on organic matter content.

All the biological parameters showed higher values in the rice soils of Kole land, Alappad, which recorded highest organic carbon content of 2.47 per cent. Microbial biomass carbon is related to soil organic carbon (Wardle, 1992). Biological parameters like microbial biomass carbon and enzyme activity increased with soil organic carbon content (Hojati and Nourbakhsh, 2006).

Acid phosphatase activity was predominant than alkaline phosphatase in the selected soils due to acidic pH of the soil. Srinivas (1993) reported the predominance of acid phosphatase in acid soils and alkaline phosphatase in alkaline soils.

5.4 CHEMICAL CHARACTERISTICS OF SOIL DURING THE PERIOD OF POT CULTURE EXPERIMENT

5.4.1 pH

The data revealed that within a particular soil type, the effect of herbicide treatment on the pH of the soil was not significant upto 30 DAHA when it was compared with control (Fig.7). After the harvest of rice, pH of soil samples decreased due to drying. Decrease in soil pH due to drying has been reported by Erich and Hoskins (2011).

The effect of herbicide treatment on soil pH was comparatively lower than that of soil type. The effect of treatment on soil pH was at lower magnitude in the cyhalofop-butyl treatment at 30 DAHA, 60 DAHA, and at harvest. Significant variation of pH in pendimethalin treatment at harvest might be due to the degradation of pendimethalin to carboxylic acid. The 4- methyl group on the benzene ring of pendimethalin is oxidised to the carboxylic acid (RSC, 1987). High organic matter soils registered lower pH at all intervals.

Significant variation was observed between control and absolute control at all the intervals. The two control treatments *viz.*, rice soils of ARS, Mannuthy (S_1 Control) and rice soils of Kole land, Alappad (S_2 Control) had lower pH values at 0 DAHA (initial samples), as both the soils received fertilizers continuously.

5.4.2 Electrical conductivity

Electrical conductivity showed a decreasing trend towards the harvest (Fig.8). At all the intervals, treatments differed significantly. However, herbicide application did not bring any variation in the electrical conductivity of the samples tested.

There was significant difference in electrical conductivity of the control (with history of herbicide application) and absolute control (without a history of herbicide application) of both soil types.

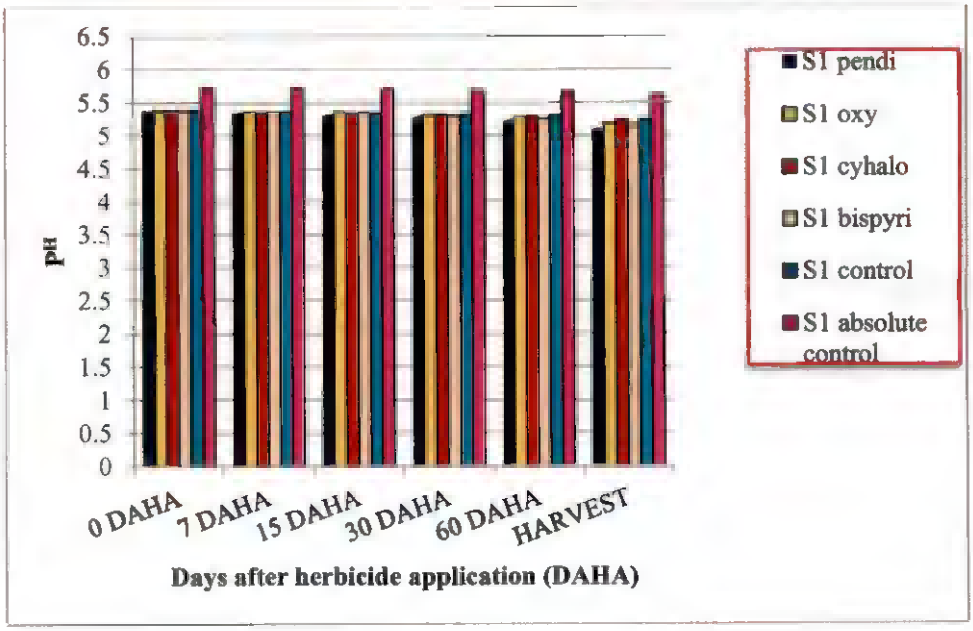

Fig. 7 a

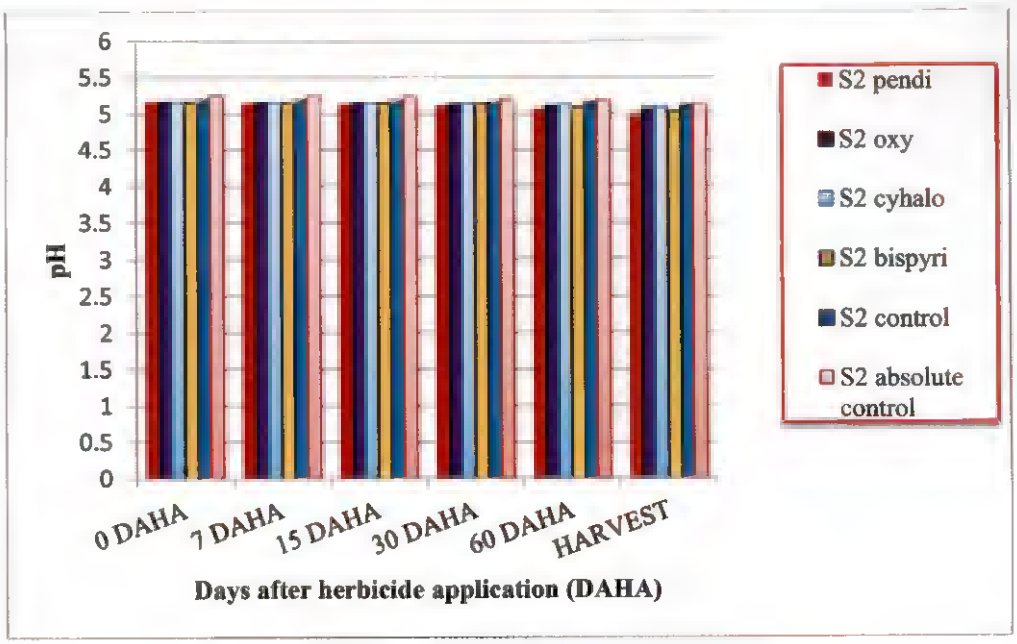

Fig. 7 b

Fig. 7. pH of a) medium and b) high organic matter soil samples at different days after herbicide application (DAHA)

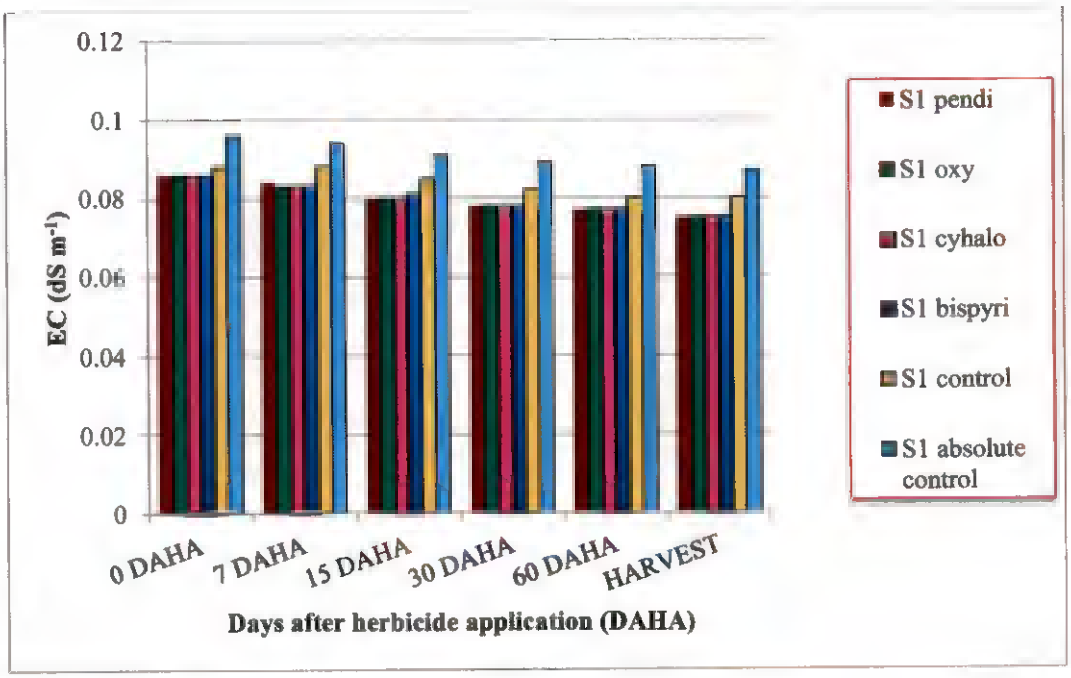

Fig. 8 a

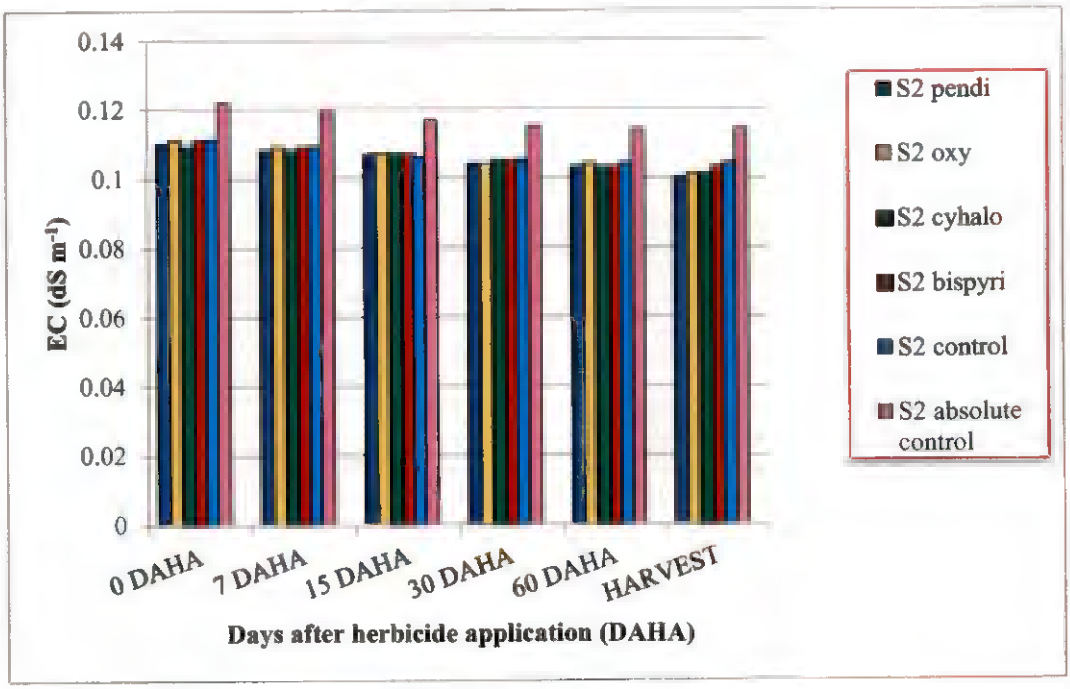

Fig. 8 b

Fig. 8 Electrical conductivity (EC) of a) medium and b) high organic matter soil samples at different days after herbicide application (DAHA)

There was no significant difference in electrical conductivity among the interactions of soil types and treatments throughout the intervals studied.

5.4.3 Organic carbon

Data on organic carbon at different intervals are presented in Figure.9. Organic carbon content showed a decreasing trend toward the harvest due to the degradation of organic matter and better utilisation of carbon during the active crop growth stages. Yoshikawa and Inubushi (1995) reported that organic carbon content of soil decreased during rice cultivation. The effect of treatments (except absolute control) within the soil type was not significant throughout the intervals except at harvest. At harvest, pendimethalin treatment recorded lower values in the medium organic matter soil which indicates that adverse effect of pendimethalin on soil microflora could be reduced by maintaining a higher organic matter content in the soil.

Significant variation in organic carbon content of soils from non-cropped areas with respect to rice soils throughout the intervals is mainly due to difference in initial characteristics of soil. Among the two soil types, high organic matter soils recorded highest organic carbon content throughout the intervals due to the contribution of organic matter for organic carbon.

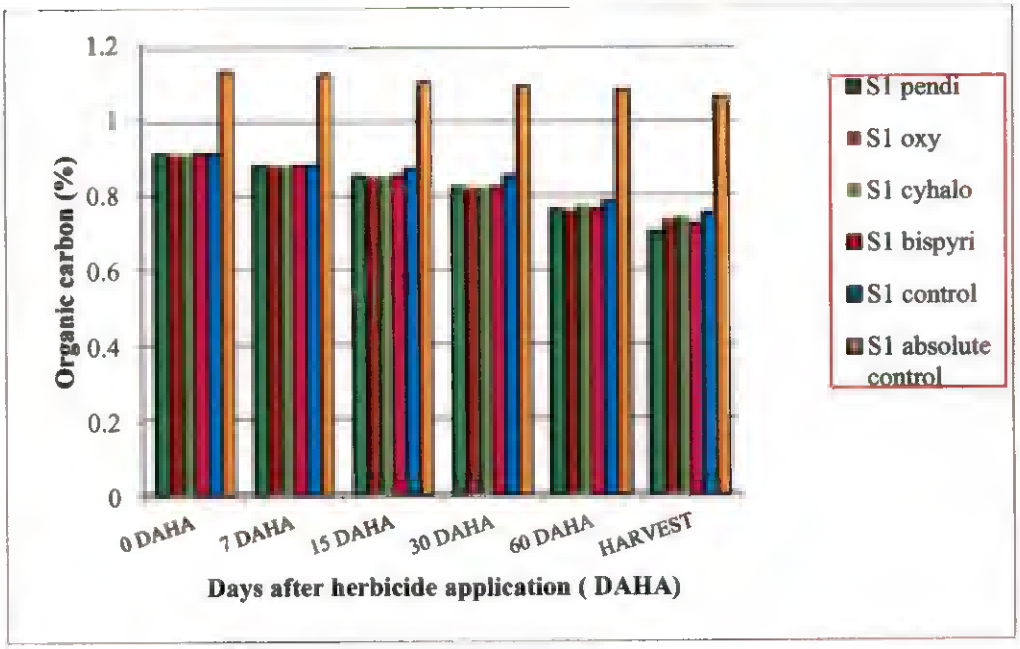

Fig. 9 a

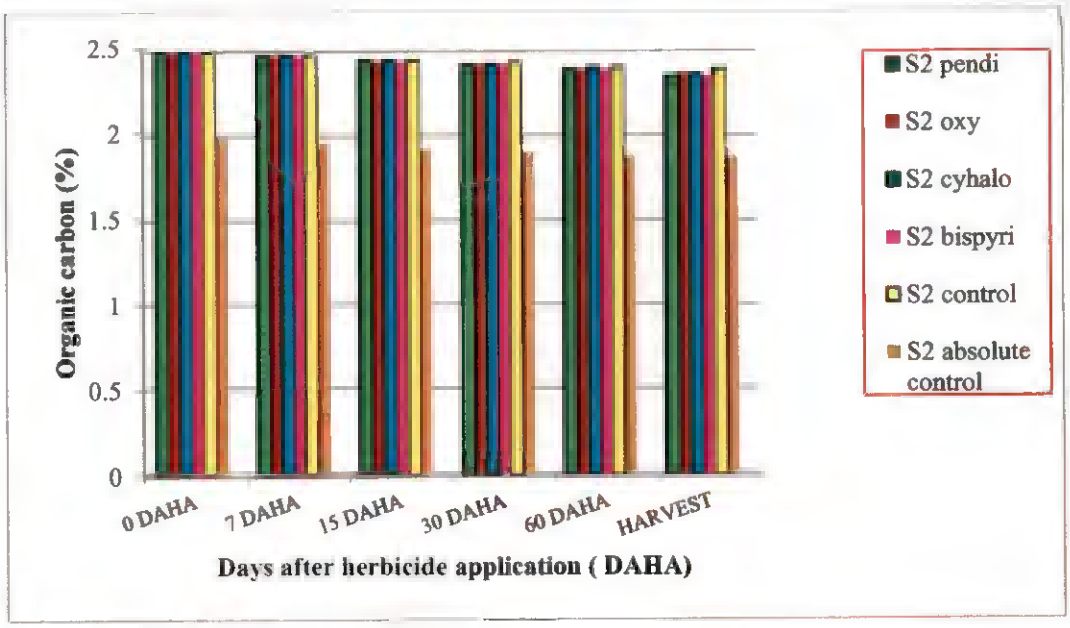

Fig. 9 b

Fig. 9: Organic carbon content in a) medium and b) high organic matter soils at different days after herbicide application (DAHA)

5.5 BIOLOGICAL CHARACTERISTICS OF SOIL DURING THE PERIOD OF POT CULTURE EXPERIMENT

5.5.1 Microbial biomass carbon

Effects of herbicide application on microbial biomass carbon are presented in Figure 10. High organic matter soils recorded higher microbial biomass carbon compared to medium organic matter soils due to enough substrate provided by high O.M. soil to support higher microbial biomass (Fig.11). Pendimethalin treated medium organic matter soils with history of herbicide application (S_1 pendimethalin) recorded lowest microbial biomass carbon throughout the intervals and highest value was observed in high organic matter soil with history of herbicide application (S_2 Control).

Microbial biomass carbon showed a decreasing trend upto 30 DAHA and thereafter drastic increase in MBC when it reaches 60 DAHA in all the interactions studied. This might be due to maximum rhizosphere effect of the root system which augmented the native microflora of the root system as 60 DAHA coincide with the panicle initiation stage. At harvest, microbial biomass carbon decreased and it was mainly due to the effect of moisture and decline in root activity at late maturity stage of rice. At the harvest stage of rice, a drastic decline in microbial biomass was also reported by Aparna (2000).

Percentage change in microbial biomass carbon in different treatments over zero day of application was worked out and given in Table 5.1. Microbial biomass carbon decreased throughout the study period except at 60 DAHA. The percentage decrease was higher in medium organic matter soils. The extent of decline in MBC was highest at harvest followed by 30 DAHA. At 30 DAHA and at harvest, reduction in microbial biomass carbon varied from 0.84 to 21.22 per cent and 8.44 to 32.59 per cent, respectively (Table 5.2). Among the herbicide treatments, maximum reduction in MBC was observed in pendimethailn followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl. Mammalian toxicity (oral LD_{50}) and persistence of the herbicides in the soil also followed the same

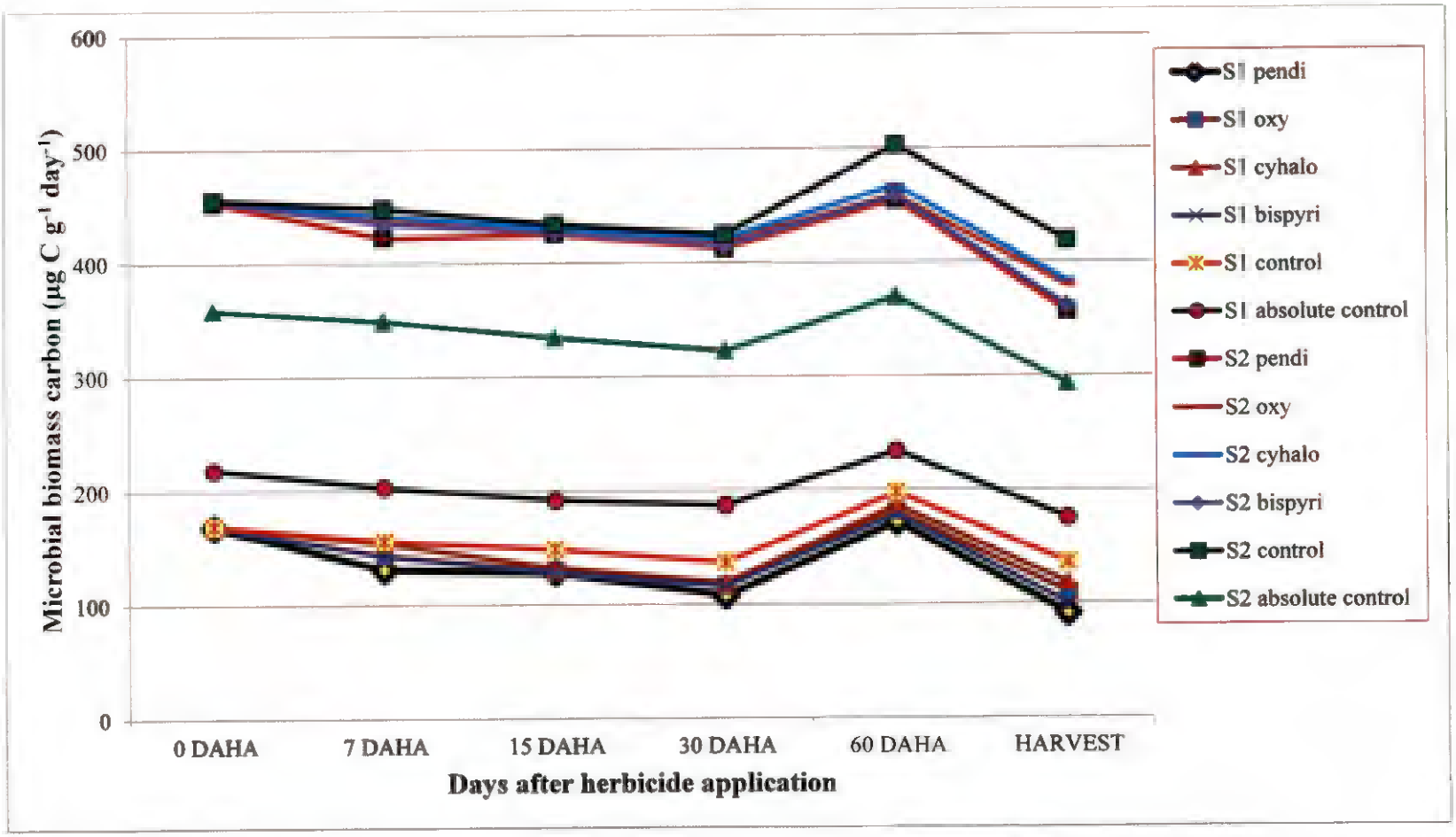

Fig. 10: Effect of herbicide application on microbial biomass carbon at different days after herbicide application (DAHA)

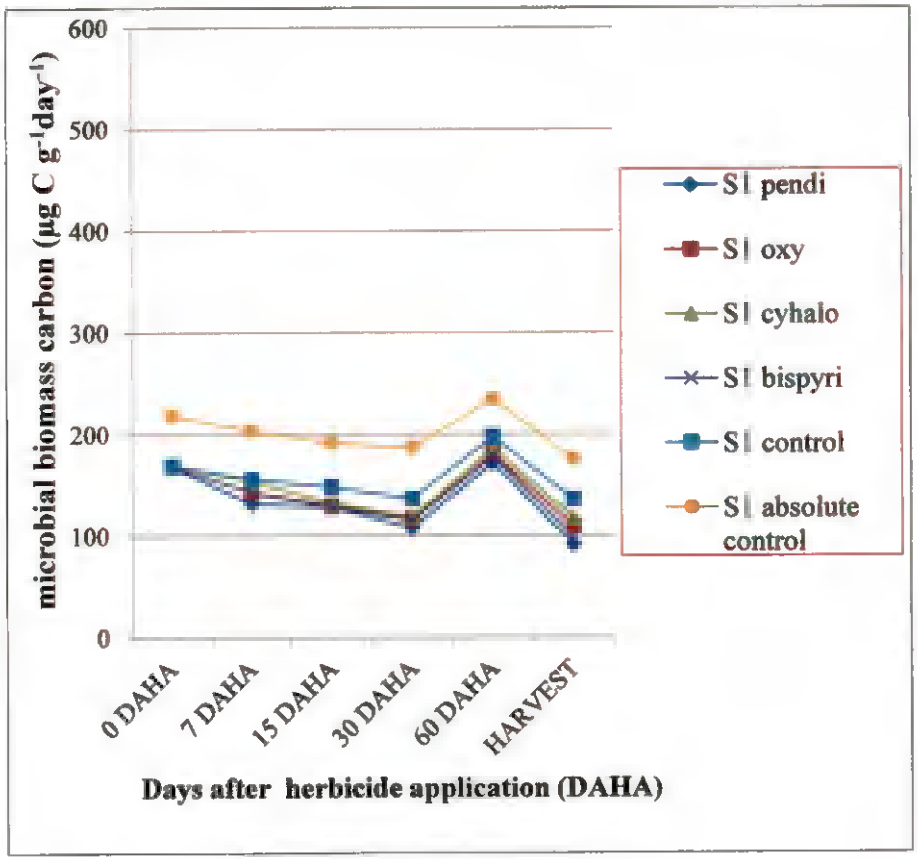

Fig. 11 a

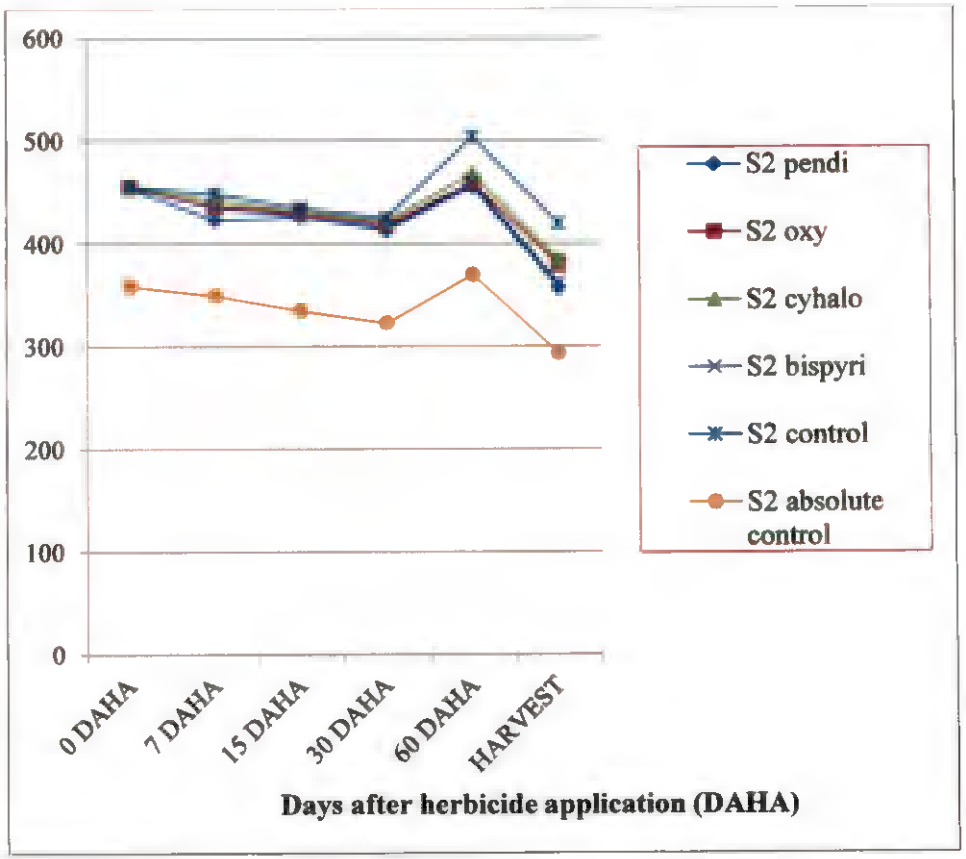

Fig. 11b

Fig.11: Effect of herbicide application on microbial biomass carbon in a) medium and b) high organic matter soils

Table 5.1 Percentage change in microbial biomass carbon with respect to zero day (2 h before herbicide application)

Treatment	Change in microbial biomass carbon (%)									
	7 DAHA		15DAHA		30 DAHA		60 DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	-26.83	-6.78	-23.85	-6.14	-36.08	-9.03	1.96	0.28	-45.69	-21.33
Oxyfluorfen	-14.91	-3.91	-23.06	-5.38	-31.22	-7.95	7.30	1.02	-34.98	-16.44
Cyhalofop- butyl	- 9.08	-2.92	-21.82	-5.36	-29.94	-7.42	10.16	2.73	-30.44	-15.67
Bispyribac- sodium	-15.23	-4.45	-23.18	-6.11	-31.37	-8.76	5.79	0.79	-40.84	20.47
Control	-7.85	-1.63	-12.39	-4.72	-19.21	-6.79	16.88	10.41	-19.78	-8.05
Absolute control	-7.02	-2.64	-12.25	-6.72	-14.48	-10.08	7.41	3.14	-19.78	-18.20

*(-) ve sign indicate decrease in microbial biomass carbon

Table 5.2 Percentage reduction in microbial biomass carbon with respect to control at different days after herbicide application

Treatment	Reduction in microbial biomass carbon (%)											
	0 DAHA		7 DAHA		15 DAHA		30 DAHA		60 DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	0.43	0.49	15.80	5.69	13.46	1.97	21.22	2.88	13.14	9.62	32.59	14. 87
Oxyfluorfen	0.43	0.33	8.07	2.64	12.56	1.02	15.23	1.57	8.60	8.80	19.30	9.42
Cyhalofop butyl	0.00	0.16	1.34	1.47	10.76	0.84	13.29	0.84	5.75	7.11	13.29	8.44
Bispyribac sodium	0.43	0.20	8.41	3.06	12.69	1.66	15.42	2.32	9.88	8.90	26.58	13.69

*S_1: medium O.M. soils S_2: high O.M. soils

order; pendimethalin > bispyribac-sodium > oxyfluorfen > cyhalofop-butyl (RSC, 1987). Percentage reduction was comparatively lower in high organic matter soils throughout the intervals and the same could be attributed to the buffering action of organic matter. Soils with high organic matter content reduced the adverse effects of applied herbicides (Rahman, 1976).

Among the pre emergence herbicides, maximum adverse effect on microbial biomass carbon was exerted by pendimethalin than oxyfluorfen. In the case of post emergence herbicides, bispyribac-sodium exerted adverse effect compared cyhalofop-butyl.

5.5.2 Dehydrogenase activity

Effects of herbicide application on dehydrogenase activity are depicted in Figure 12. Dehydrogenase activity at 15 DAHA was comparatively lower than activity at seven days after herbicide application in all herbicide treatments. This might be due to the effect of herbicide application on the activity of intracellular enzyme dehydrogenase. There was only gradual increase in dehydrogenase activity from 15 to 30 DAHA and thereafter sudden increase in activity was noticed at 60 DAHA and drastic decrease in activity towards the harvest in all the interactions. Lower activity of dehydrogenase at harvest is mainly due to dry condition prevailed at harvest of rice. Metabolism and survival of the soil microorganisms are affected by the soil moisture availability (Uhlirova *et al.*, 2005). As soils dry, the water potential increases, and as well microbial activity as intracellular enzyme activity slows down (Geisseler *et al.*, 2011).

High organic matter soils have highest dehydrogenase activity compared to medium organic matter soils (Fig.13). This might be due to enough substrate provided by high O.M. soil to support higher microbial biomass, and higher enzyme production. The findings are in close agreement with Yuan and Yue, 2012.

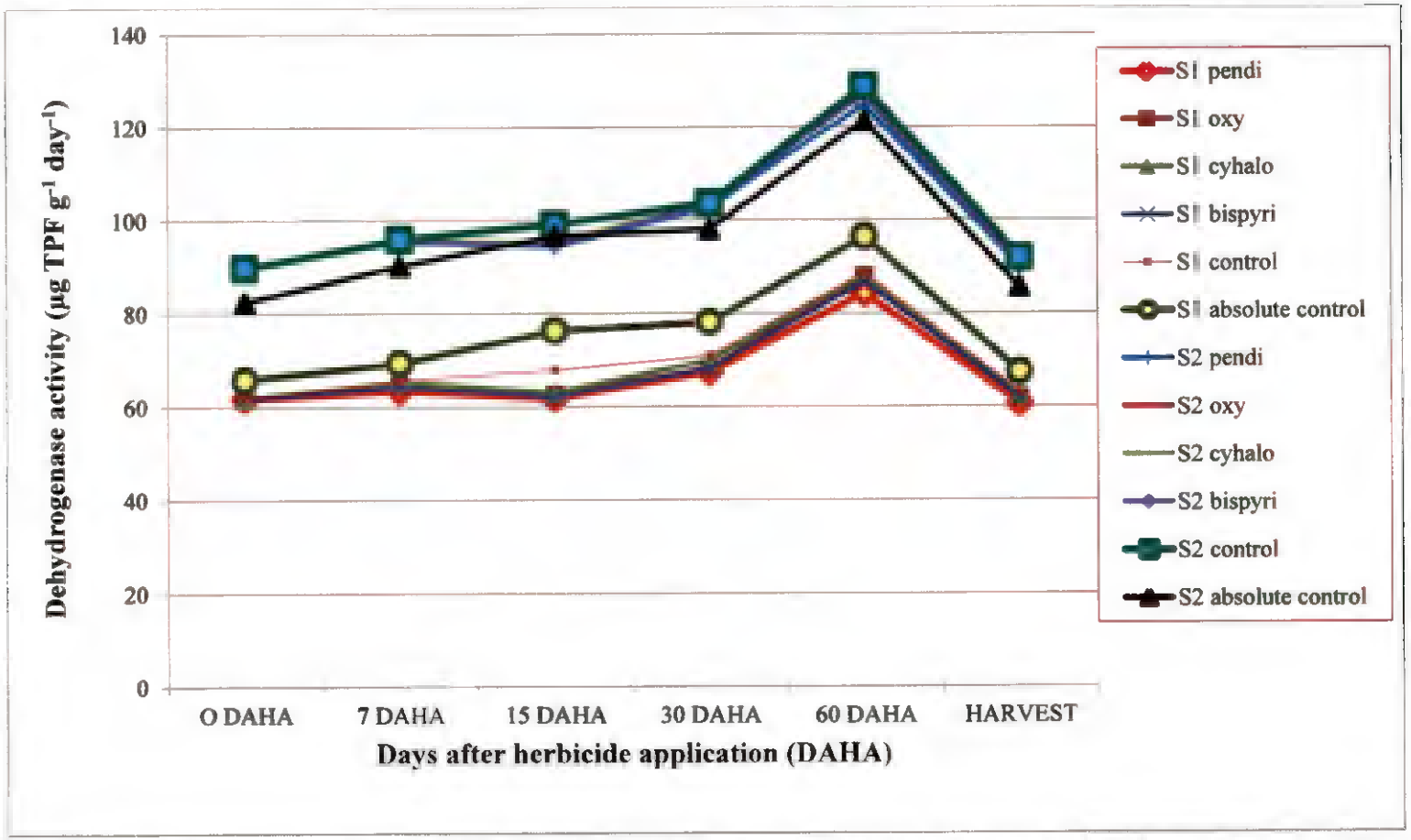

Fig. 12: Effect of herbicide application on dehydrogenase activity at different days after herbicide application (DAHA)

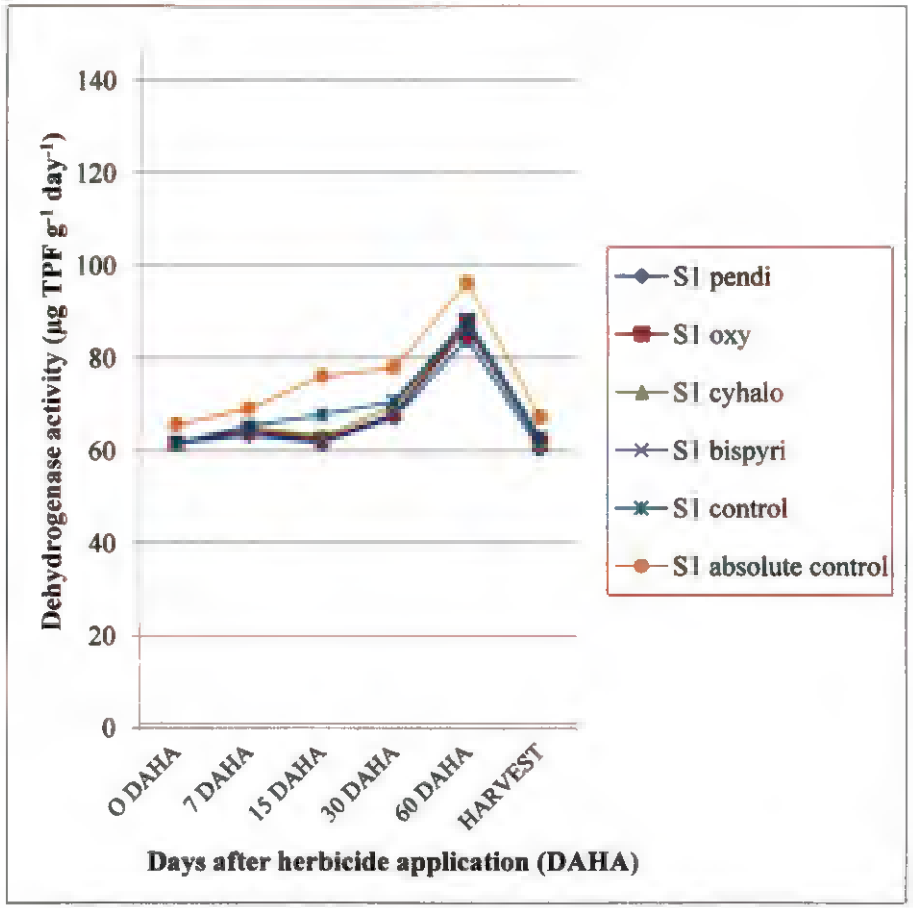

Fig. 13 a

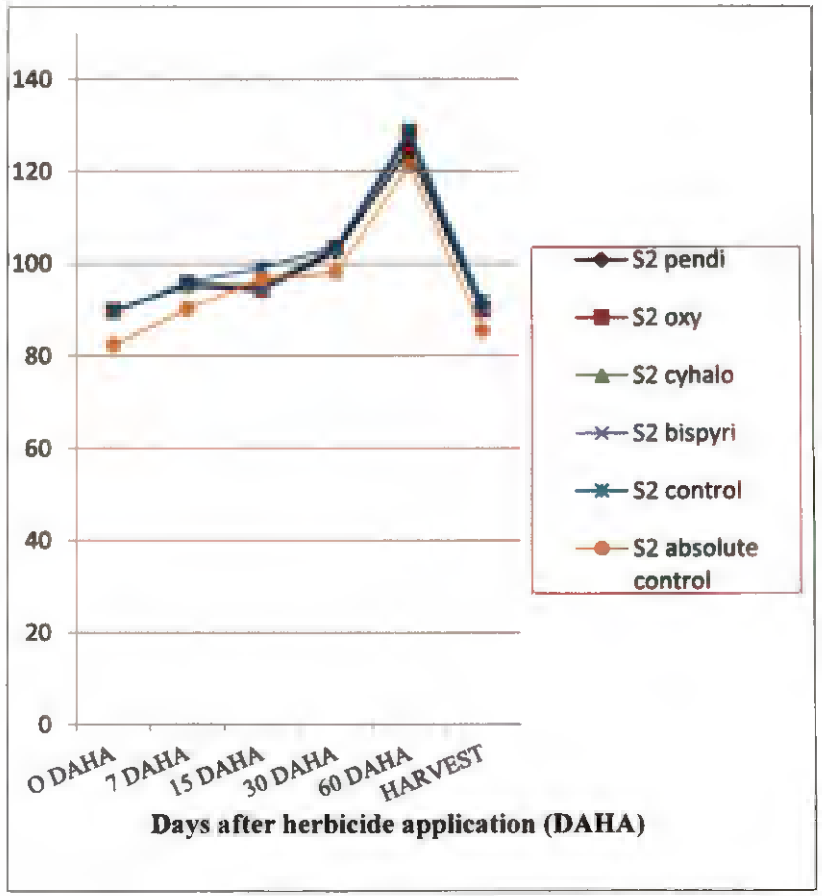

Fig. 13 b

Fig. 13: Effect of herbicide application on dehydrogenase activity in a) medium and b) high organic matter soils

Data on percentage increase in dehydrogenase activity with respect to zero day of herbicide application revealed that percentage increase in activity was comparatively lower at 15 DAHA and also at harvest in herbicide treatments (Table 5.3). At 15 DAHA, percentage increase in activity varied from 0.11 to 17.35 per cent. Percentage increase was lower in pendimethalin treated medium organic matter soil (0.11 %) and higher in non-cropped area of Kole land denoted as absolute control (17.35 %). Soils without history of herbicide application (absolute control) recorded comparatively higher percentage of enzyme activity than soil with history of herbicide application.

Among the herbicides, pendimethalin showed maximum reduction in dehydrogenase activity with respect to control followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl at all the intervals studied (Table 5.4). In the pendimethalin treatment, the percentage reduction in dehydrogenase activity at 15 DAHA was 9.03 and 4.81 per cent for S_1 and S_2 soils. In cyhalofop-butyl treatment, the corresponding changes being 7.09 and 3.91 per cent, respectively. At harvest, the extent of decline in pendimethalin treatment reduced to 4.03 and 2.30 per cent for S_1 and S_2 soils, respectively. Corresponding figures for cyhalofop-butyl treatment being 1.14 and 1.02 per cent, respectively. Pendimethalin showed maximum reduction in enzyme activity due to its acute toxicity. The effect of herbicides on microorganism activity was in accordance with their mammalian toxicity. Cyhalofop- butyl and oxyfluorfen belong to slightly toxic category in the pesticide classification based on LD_{50}.Whereas, pendimethalin and oxyfluorfen belong to the moderately toxic category as per oral LD_{50} value (RSC, 1987).

Results revealed that dehydrogenase activity was greatly affected by the pre emergence application of pendimethalin followed by post emergence application of bispyribac-sodium.

Table 5.3 Percentage increase in dehydrogenase activity with respect to zero day (2 h before herbicide application)

Treatment	Increase in dehydrogenase activity (%)									
	7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	2.79	6.27	0.11	5.09	9.18	14.40	36.35	39.09	-1.85	0.08
Oxyfluorfen	4.67	6.59	1.44	5.36	10.54	15.27	42.45	42.90	0.26	0.47
Cyhalofop butyl	6.00	6.64	2.24	6.07	13.22	15.34	42.71	43.35	1.09	1.37
Bispyribac sodium	4.14	6.58	0.55	5.24	10.34	15.23	40.33	41.45	-0.02	0.18
Control	6.21	6.80	10.07	10.36	14.92	15.44	43.33	43.64	2.29	2.40
Absolute control	5.35	9.49	15.88	17.35	18.66	18.97	46.44	47.29	2.39	3.79

Table 5.4 Percentage reduction in dehydrogenase activity with respect to control at different days after herbicide application

Treatment	Reduction in dehydrogenase activity (%)											
	0DAHA		7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	-0.02	0.03	3.21	0.53	9.03	4.81	4.98	0.94	4.86	3.21	4.03	2.30
Oxyfluorfen	0.00	0.01	1.45	0.21	7.84	4.54	3.81	0.16	0.61	0.53	1.99	1.89
Cyhalofop butyl	-0.03	0.02	0.17	0.18	7.09	3.91	1.45	0.12	0.40	0.23	1.14	1.02
Bispyribac sodium	-0.02	0.01	1.94	0.22	8.64	4.65	3.97	0.19	2.07	1.54	2.24	2.18

*S_1: medium O.M. soils S_2: high O.M. soils

5.5.3 Urease activity

Effects of herbicide application on urease activity are presented in Figure 14. The data revealed that urease activity in all the treatments showed an increasing trend up to 60 DAHA and thereafter the activity decreased. The magnitude of increase in activity was lower during initial stages and comparatively higher at 60 DAHA. These results are in agreement with Vandana *et al.* (2012). This might be due to the tolerance of urease enzyme to the applied herbicides. Decrease in enzyme activity at harvest is mainly due to the lack of sufficient soil moisture and also reduced root activity as it reaches late maturity stage. During the harvest of rice, soil was in dry condition, so the metabolic activity of the microorganism might be lower. According to Ojeda *et al.* (2013) soil moisture content is an exceptionally significant factor that changes the biological activity of soil.

The percentage increase in urease activity over the sampling periods was comparatively higher in soils with high organic matter content due to the substrate availability (Table 5.5). Urease activity in medium and high organic matter soils are depicted in Figure 15. Percentage increase in activity with respect to the day of herbicide application (2 h before the application) was higher at 60 DAHA and it varied from 158.09 to 306.90 per cent. This might be due to the utilisation of degraded herbicides by the microorganism as well as release of root exudates during the panicle initiation stage leading to a faster multiplication of the active microflora involved in urease production. Rhizosphere effect was more in this stage as it coincide with panicle initiation stage. This observation is in conformity with the findings of Aparna (2000) who reported that increase of urease activity during panicle initiation stage was mainly due to the excretion and secretion of the roots which augmented the higher level of urease activity.

Percentage increase was considerably lower at harvest due to the effect of soil moisture. Among the herbicide treatments, percentage increase in urease

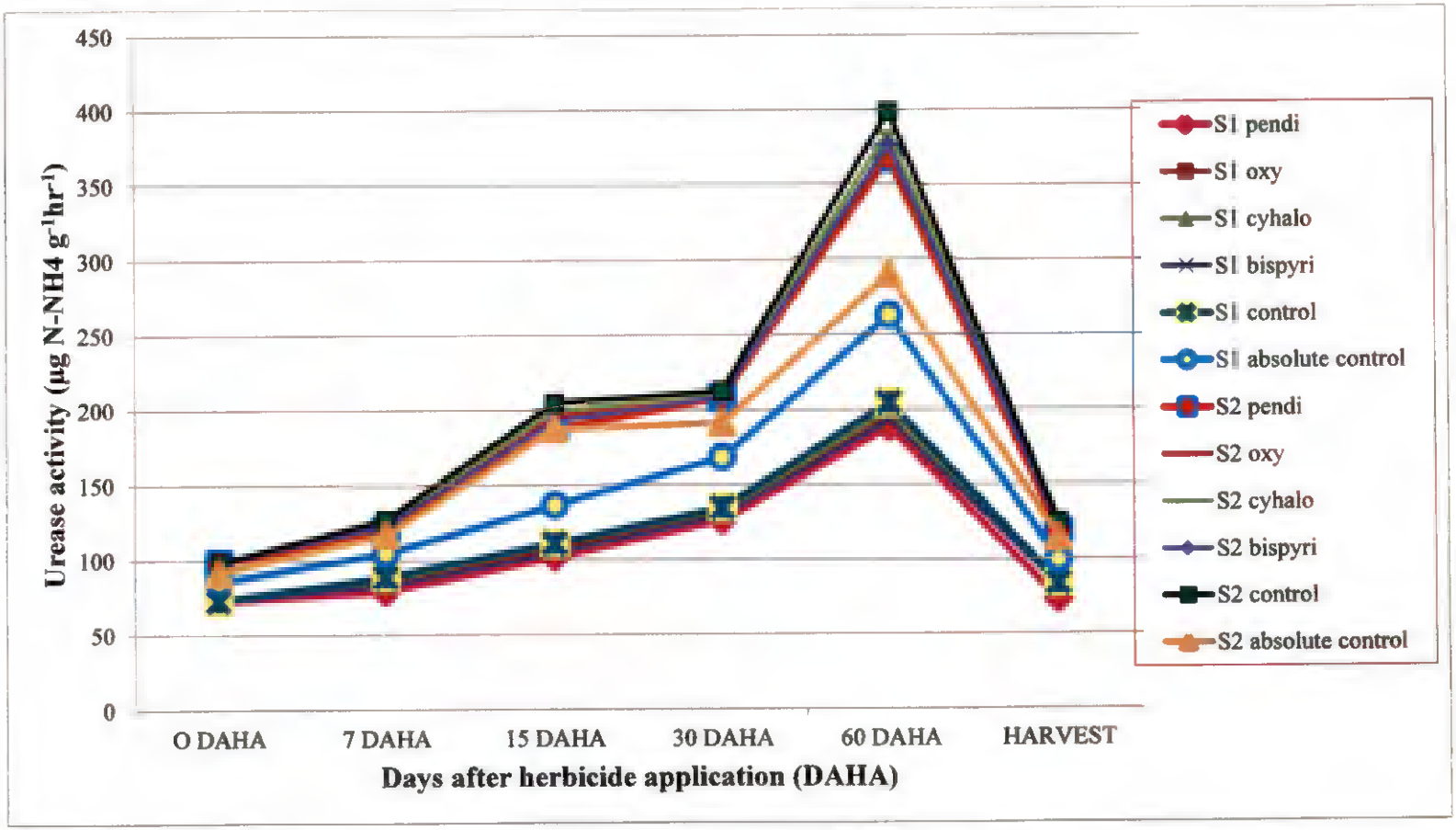

Fig. 14: Effect of herbicide application on urease activity at different days after herbicide application (DAHA)

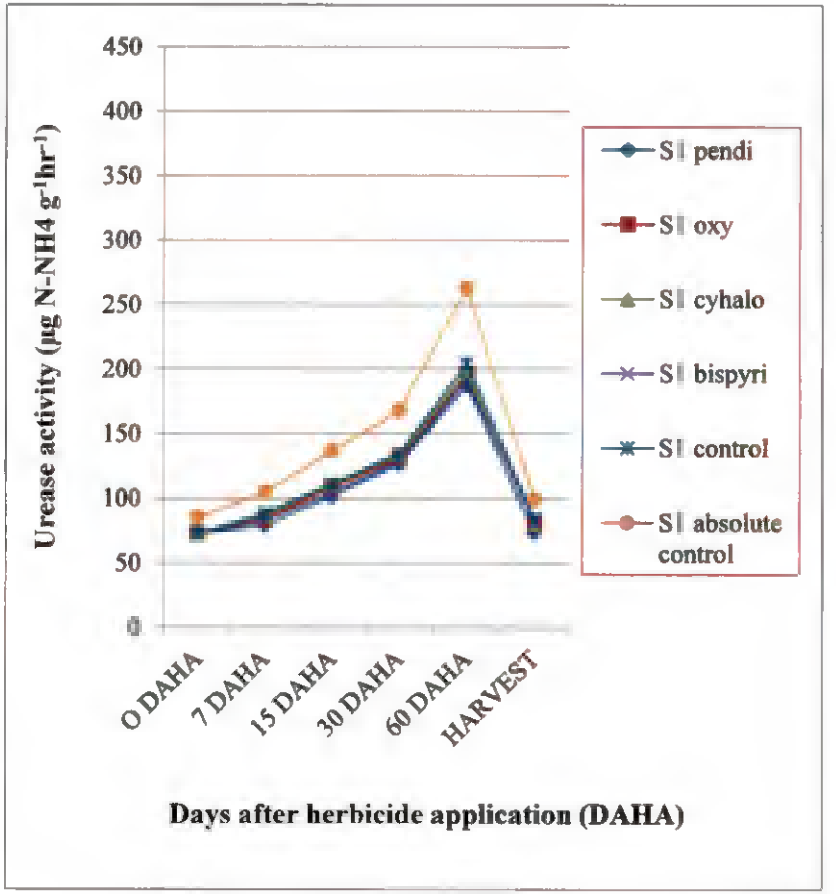

Fig.15 a

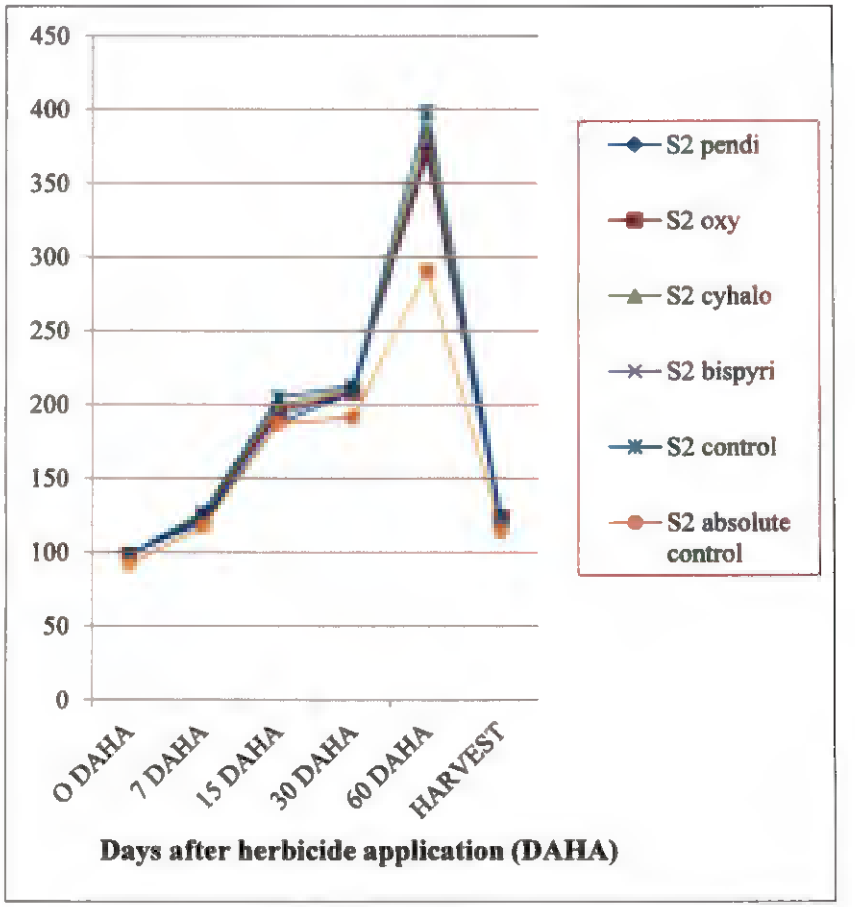

Fig. 15 b

Fig.15: Effect of herbicide application on urease activity in a) medium and b) high organic matter soils

Table 5.5 Percentage increase in urease activity with respect to zero day (2 h before herbicide application)

Treatment	Increase in urease activity (%)									
	7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	8.80	22.44	39.77	90.86	73.78	112.69	158.09	275.00	0.34	20.22
Oxyfluorfen	15.35	26.12	49.16	99.89	88.01	112.77	170.16	277.62	12.58	26.49
Cyhalofop butyl	19.04	28.16	51.54	103.37	82.42	114.63	173.07	292.46	11.28	26.66
Bispyribac sodium	13.77	25.30	43.70	96.27	77.35	112.33	164.51	283.94	12.29	23.00
Control	21.85	29.58	52.95	108.87	85.05	116.74	181.28	306.90	15.02	27.33
Absolute control	22.23	27.52	59.79	103.93	96.67	108.40	207. 31	216.03	15.93	24.29

Table 5.6 Percentage reduction in urease activity with respect to control at different days after herbicide application

Treatment	Reduction in urease activity (%)											
	0DAHA		7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	0.00	-0.03	10.71	5.49	8.62	8.59	6.09	2.12	8.25	7.81	12.76	0.55
Oxyfluorfen	0.00	0.00	5.34	2.68	2.48	4.30	2.73	1.83	3.95	7.20	2.12	0.66
Cyhalofop butyl	0.00	-0.04	2.31	1.05	0.93	2.59	1.43	0.93	2.92	3.51	3.26	0.48
Bispyribac sodium	0.00	-0.03	6.64	3.28	6.04	6.01	4.16	2.01	5.96	5.62	2.37	3.37

*S_1: medium O.M. soils S_2: high O.M. soils

activity over the sampling intervals was higher in cyhalofop-butyl treated soils followed by oxyfluorfen, bispyribac-sodium, and lowest in pendimethalin treated soils throughout the intervals studied. Percentage increase in urease activity was lower in herbicide treatments with respect to control.

The percentage reduction in urease activity with respect to control at different days after herbicide application is presented in Table 5.6. Among the medium and high organic matter soils, percentage reduction in enzyme activity was higher in medium organic matter soil due to reduced buffering activity as compared to soils with high organic matter content. Maximum reduction in enzyme activity was observed at harvest (0.48 to 12.76 %) which was followed by seven days after herbicide application (1.05 to 10.71 %). Reduction in urease activity at harvest might be due to dry condition of soil and reduced root activity as the crop reaches late maturity stage. Reduction in activity after seven days of herbicide application is due to toxic effect of herbicides on urease as it is an extracellular enzyme. Among the four different herbicide treatments, reduction in urease activity with respect to control was lowest in cyhalofop-butyl, followed by oxyfluorfen, bispyribac-sodium and maximum reduction was noticed in pre emergence application of pendimethalin treatment. This might be due to the higher toxicity of pendimethalin and their long term persistence in soil as compared to the other three herbicides. Mammalian toxicity and persistence in terms of half-life of pendimethalin is 4050 mg kg^{-1} and three to four months respectively (RSC, 1987).

5.5.4 Acid phosphatase activity

Acid phosphatase activity followed an increasing trend in all the treatments at all the intervals except at harvest (Fig.16). However, the extent of increase was lower in herbicide treatments. In the case of pendimethalin, there was a decline in the acid phosphatase activity at harvest stage. Decrease in activity at harvest is mainly due to the dry soil condition prevailed during harvest of rice

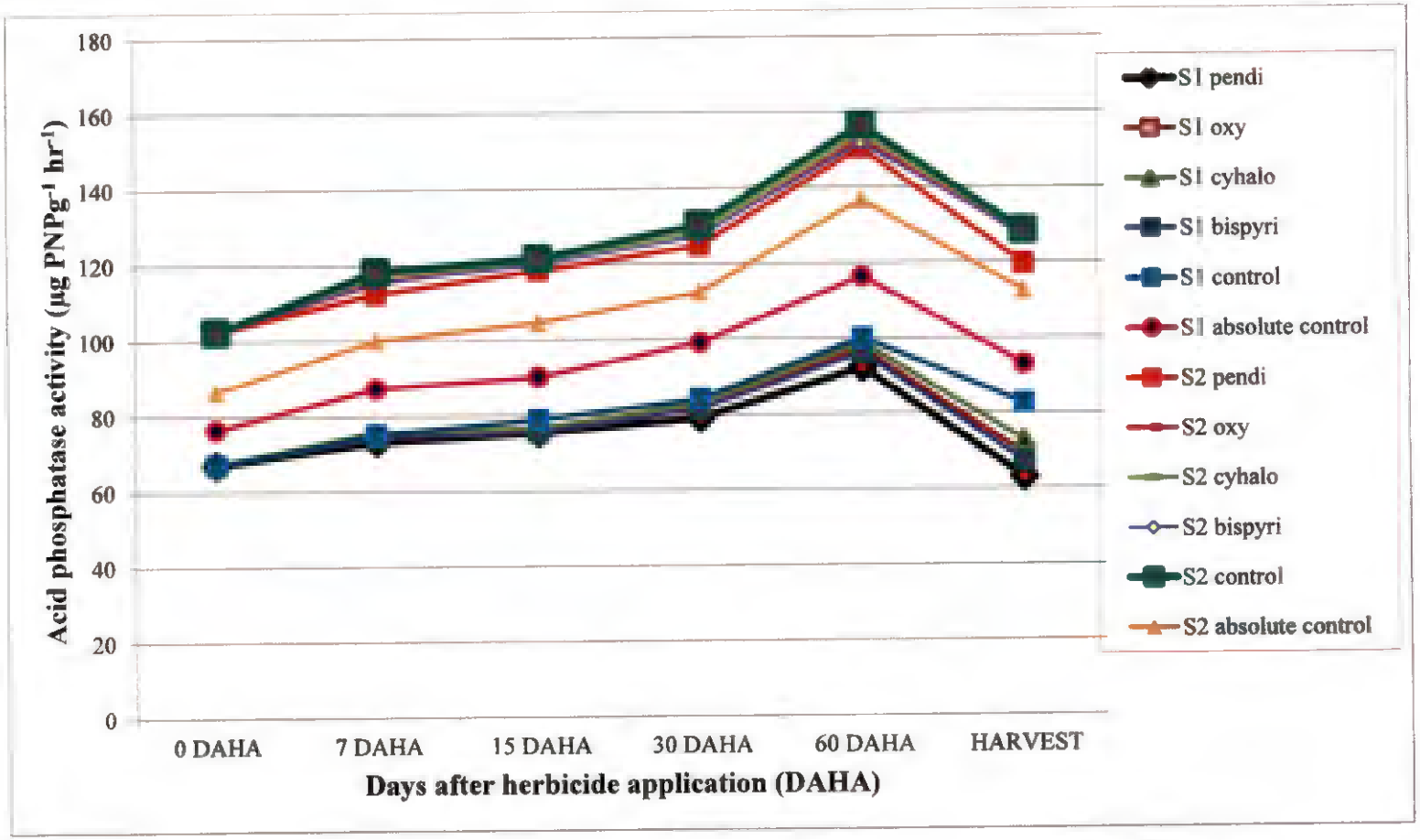

Fig. 16: Effect of herbicide application on acid phosphatase activity at different days after herbicide application (DAHA)

and reduced root activity of rice at its later maturity stage. At 60 DAHA, acid phosphatase activity reached a peak due the effect of root exudates which coincided with the panicle initiation stage. While comparing the soil types, enzyme activity was higher in soil having high organic matter content (Fig.17).

Percentage increase in acid phosphatase activity with respect to the day but two hour before herbicide application was highest in S_2 (high O. M. soil) than S_1 (soil with medium O.M.) throughout the intervals due to the buffering action of organic matter to applied herbicides (Table 5.7). Percentage increase in acid phosphatase activity was 8.93 to 15.61 per cent, 12.38 to 20.76 per cent, 17.86 to 29.81 per cent, 37.38 to 57.86 per cent and -6.03 to 29.84 per cent at 7, 15, 30, and 60 DAHA and at harvest, respectively.

Percentage reduction in acid phosphatase activity with respect to control at different days after herbicide application were analysed and are given in Table 5.8. Percentage reduction in activity was lower in S_2 compared to S_1 at all the intervals studied. Among the treatments, pendimethalin recorded highest percentage of reduction followed by bispyribac-sodium, oxyfluorfen, and cyhalofop- butyl at 7, 15, 30, and 60 DAHA and even during harvest with respect to control and the similar trend was observed in both the soil types.

Among the different intervals studied, percentage reduction in acid phosphatase activity was highest at harvest and it varied from 0.24 to 23.66 per cent. General decrease in acid phosphatase activity observed during harvest could be attributed to the lack of moisture and reduction in release of soluble compounds from roots at its physiological maturity. Aparna (2000) claimed that decrease in enzyme activity was mainly due to the lack of optimum conditions for the availability of moisture, carbon substrates and nutrients at harvest.

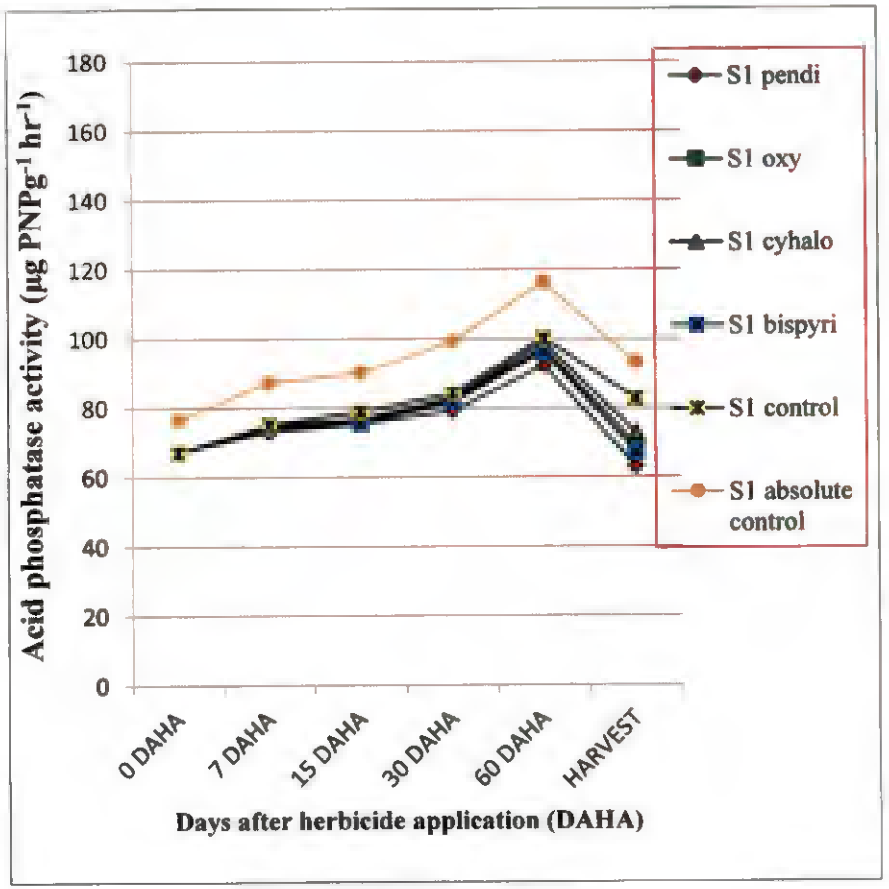

Fig. 17 a

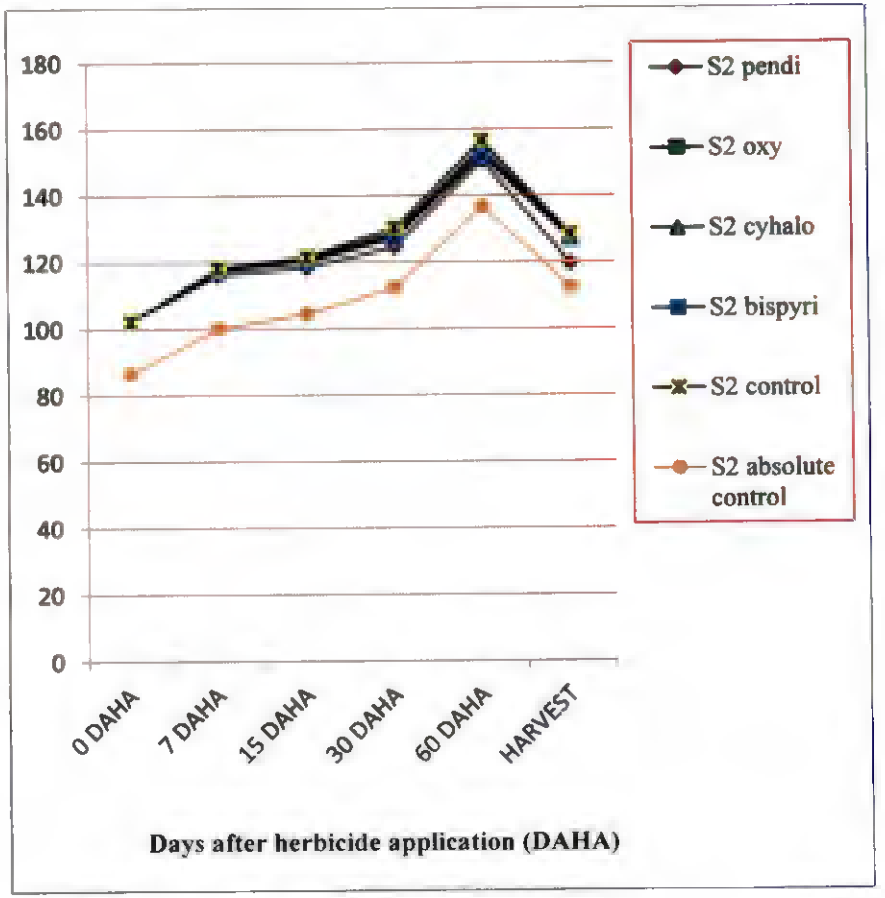

Fig. 17 b

Fig. 17: Effect of herbicide application on acid phosphatase activity in a) medium and b) high organic matter soils

Table 5.7 Percentage increase in acid phosphatase activity with respect to zero day (2 h before herbicide application)

Treatment	Increase in acid phosphatase activity (%)									
	7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	8.93	13.56	12.38	15.71	17.86	21.82	37.38	46.98	-6.03	16.84
Oxyfluorfen	11.21	15.22	12.79	17.93	22.88	25.06	44.71	49.39	3.01	24.08
Cyhalofop butyl	11.76	15.4	14.21	18.24	23.54	25.96	47.13	50.92	8.52	25.13
Bispyribac sodium	10.69	14.61	12.56	17.33	21.56	24.23	43.21	48.35	0.01	23.99
Control	11.92	15.52	17.51	18.85	25.42	27.32	49.11	52.94	23.09	25.43
Absolute control	14.30	15.61	17.99	20.76	29.62	29.81	52.14	57.86	21.61	29.84

Table 5.8 Percentage reduction in acid phosphatase activity with respect to control at different days after herbicide application

Treatment	Reduction in acid phosphatase activity (%)											
	0DAHA		7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	0.00	0.00	2.68	1.70	4.37	2.65	6.03	4.32	7.87	3.90	23.66	6.85
Oxyfluorfen	0.00	0.01	0.65	0.27	4.03	0.79	2.03	1.79	2.96	2.33	16.32	1.09
Cyhalofop butyl	0.00	0.00	0.15	0.10	2.81	0.52	1.50	1.07	1.33	1.32	11.84	0.24
Bispyribac sodium	0.00	0.00	1.11	0.80	4.22	1.29	3.08	2.43	3.96	3.00	18.75	1.15

*S_1: medium O.M. soils S_2: high O.M. soils

Among the applied herbicides, pendimethalin showed maximum reduction in acid phosphatasae activity with respect to control followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl at all the intervals. The magnitude of reduction in acid phosphatase activity shown by the four different herbicides was also in accordance with their LD_{50} values.

5.5.5 Alkaline phosphatase activity

Data on alkaline phosphatase activity are presented in Figure 18. There was an increase in activity upto 60 DAHA and a drastic decrease toward the harvest. The increase in activity upto 60 DAHA might be due to tolerance of alkaline phosphatase activity to the applied herbicides and positive peak at 60 DAHA might be due to utilisation of carbon reserves from the applied herbicides as well as release of root exudates from the rice as it coincide with panicle initiation stage. Decrease in activity at harvest is mainly due to the dry condition during rice harvest. Among the medium and high organic matter soils, alkaline phosphatase activity was higher in high organic matter soils (Fig.19).

The percentage increase in alkaline phosphatase activity with respect to the day of (2 h before) herbicide application is given in Table 5.9. It could be seen that alkaline phosphatase activity increased to an extent of 17.21 per cent to 34.65 per cent in the different treatments at 60 DAHA. However, the magnitude of change was comparatively lower than that of acid phosphatase activity. The effect of herbicides on alkaline phosphatase activity was more pronounced than acid phosphatase activity. Among the medium and high organic matter soils, percentage increase was more in high organic matter soil at all the intervals in case of all treatments. This might be due to the buffering action of organic matter. Percentage increase was lower in herbicide treatments compared to control throughout the period of experiment especially in pendimethalin treatment. This might be due to the persistence of residue and the lethal toxicity as it was included in blue toxicity class.

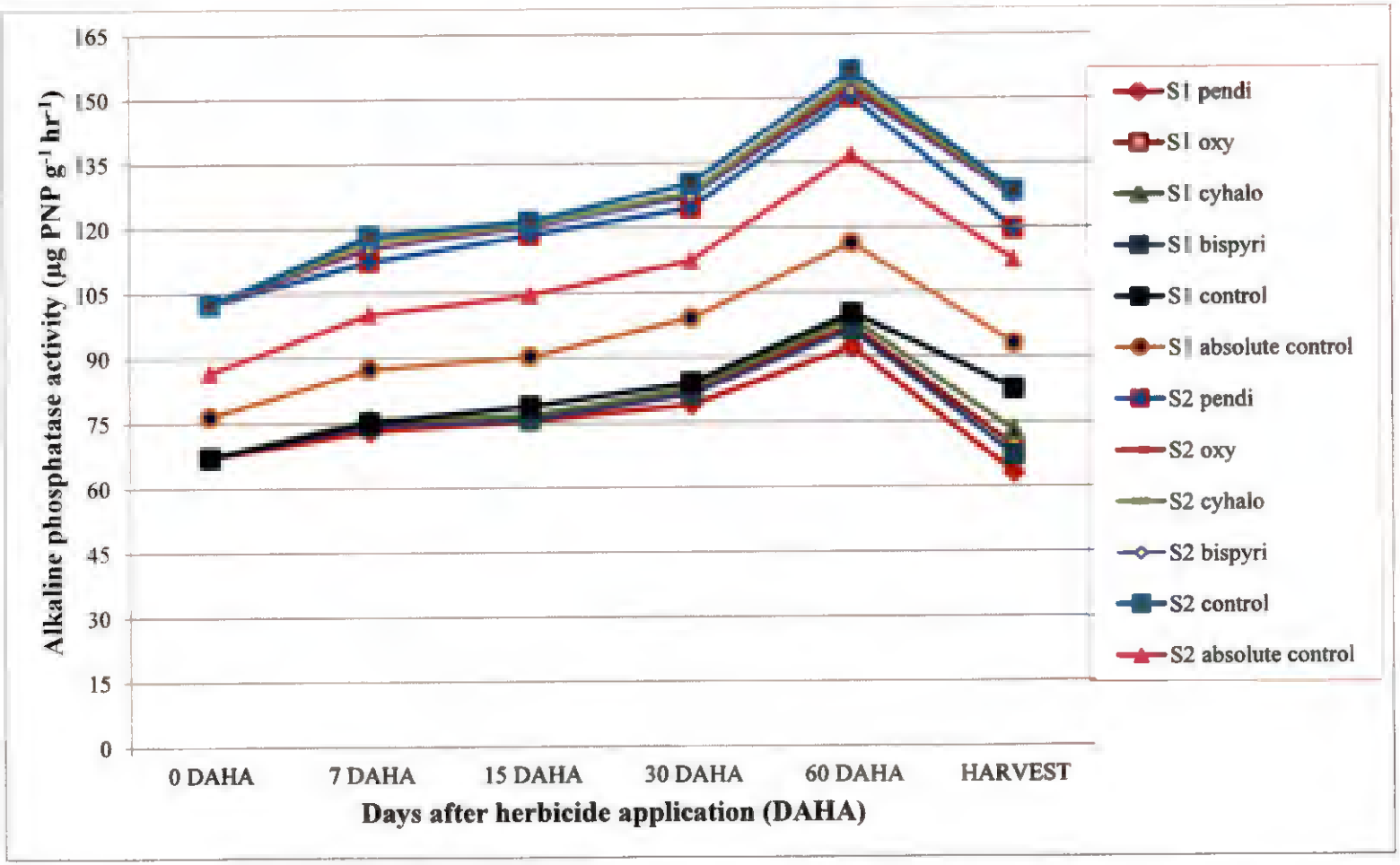

Fig. 18: Effect of herbicide application on alkaline phosphatase activity at different days after herbicide application (DAHA)

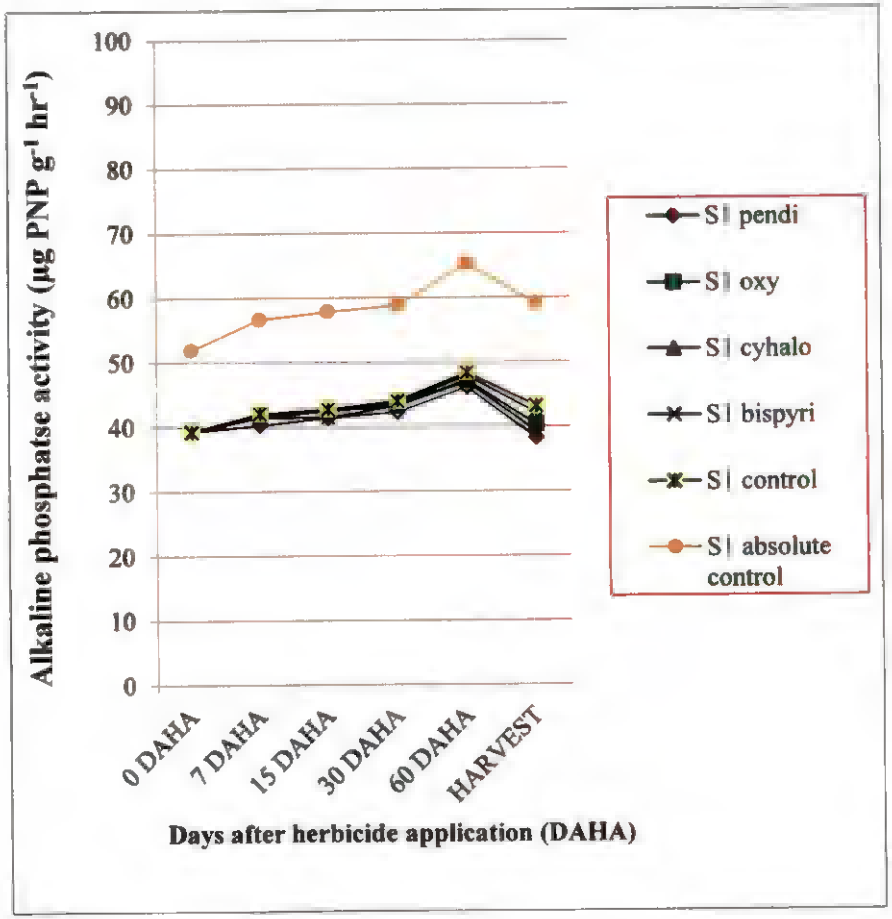

Fig. 19 a

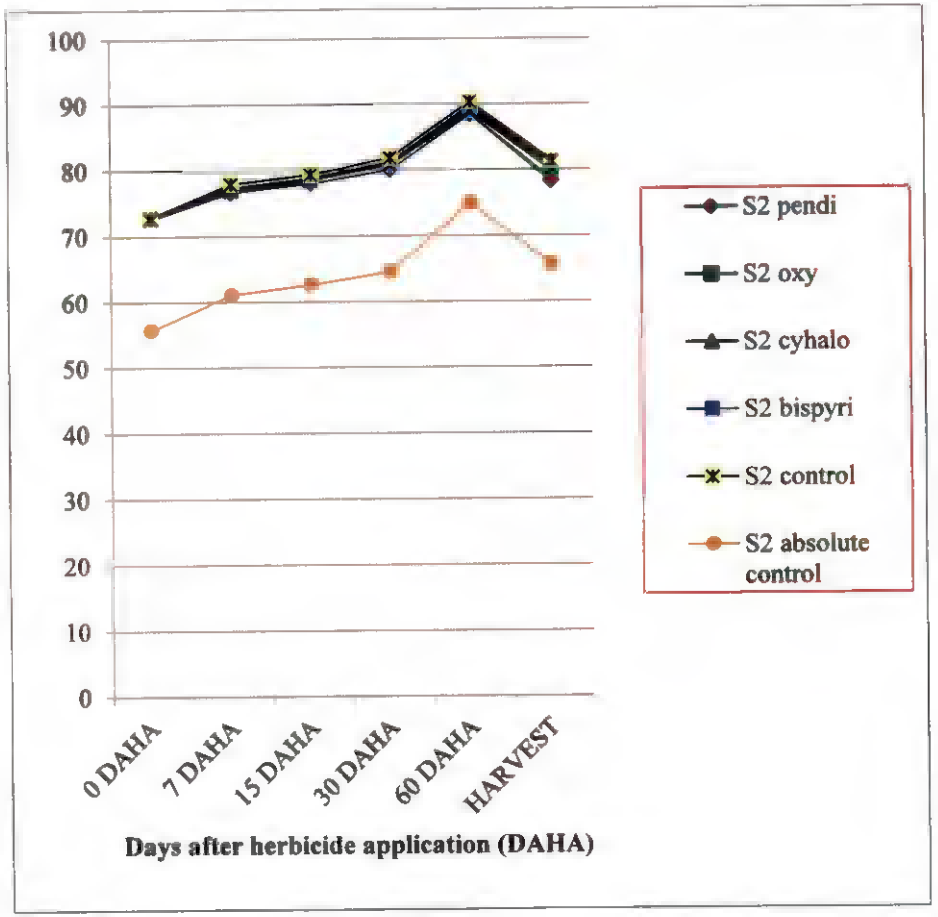

Fig. 19 b

Fig. 19: Effect of herbicide application on alkaline phosphatase activity in a) medium and b) high organic matter soils

Table 5.9 Percentage increase in alkaline phosphatase activity with respect to zero day (2 h before herbicide application)

Treatment	Increase in alkaline phosphatase activity (%)									
	7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	2.47	5.36	5.40	7.20	7.59	10.00	17.21	21.53	-2.60	7.71
Oxyfluorfen	5.80	5.84	7.84	8.22	10.11	11.48	20.11	22.63	2.57	11.03
Cyhalofop butyl	6.21	6.28	7.99	8.36	11.07	11.59	21.84	23.32	7.23	11.52
Bispyribac sodium	5.50	5.82	5.58	7.95	9.88	11.11	19.25	22.16	0.03	10.04
Control	7.13	7.18	8.81	9.10	12.04	12.53	23.09	24.27	9.93	11.66
Absolute control	9.20	9.66	11.57	12.43	13.35	15.90	25.81	34.65	13.91	17.87

Table 5.10 Percentage reduction in alkaline phosphatase activity with respect to control at different days after herbicide application

Treatment	Reduction in alkaline phosphatase activity (%)											
	0DAHA		7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Pendimethalin	0.00	0.00	4.35	1.69	3.14	1.74	3.98	2.25	4.78	2.20	11.39	3.53
Oxyfluorfen	0.00	0.00	1.24	1.24	0.89	0.81	1.73	0.93	2.42	1.32	6.69	0.57
Cyhalofop butyl	0.00	0.00	0.86	0.83	0.75	0.68	0.86	0.83	1.01	0.76	2.45	0.12
Bispyribac sodium	0.00	0.00	1.52	1.26	2.97	1.06	1.93	1.26	3.12	1.69	9.01	1.45

*S_1: medium O.M. soils S_2: high O.M. soils

The mammalian toxicity of pendimethalin in terms of LD_{50} value was 4050 mg kg^{-1} and its half-life in soil was three to four months (RSC, 1987).

The data on percentage reduction in activity with respect to control at different days after herbicide application revealed that pendimethalin had adverse effect on alkaline phosphatase followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl (Table 5.10). Reduction in alkaline phosphatase activity was comparatively of lower magnitude in soil having high organic matter content.

5.6 PLANT CHARACTERS

The observed plant characters showed that organic matter is the major factor determining the plant growth and yield. Adverse effects of herbicides on the plant characters were minimal. The content of major nutrients in rice was higher in high organic matter soils. This might be due to the nutrient availability.

5.7 CORRELATION STUDIES

Soil characteristics *viz.*, pH, organic carbon and biological activity in the soil namely microbial biomass carbon, dehydrogenase activity, urease activity, acid and alkaline phosphatase activity at different sampling intervals were subjected to correlation analysis.

Organic carbon had highly significant and positive correlation with all the biotic components (1 % level). This might be due to the contribution of carbon as an energy source. Soil enzymes are immobilized on soil organic matter and soil organic

matter is indexed by organic carbon content of soil. So significant correlations between enzyme activities with soil organic carbon are expected (Frankenberger and Tabatabai, 1991).

Soil acidity had negative correlation with soil enzyme activity. This result is in conformity with the findings of Hue and Cao (2007). When soil enzymes are exposed to strongly acidic condition, the catalytic activity of enzyme protein decreased probably due its effect on the overall three dimensional structure of the protein. Exposure to high H+ concentration tends to disrupt the ionic and hydrogen bonds needed to maintain the active conformation of the enzyme resulting in loss of biological activity (Dick *et al.*, 1988).

Microbial biomass carbon was significantly and positively correlated with organic carbon. Kunjur and Patel (2012) also reported the positive correlation of microbial biomass carbon and organic carbon content of soil samples.

Dehydrogenase activity was highly correlated with soil organic carbon throughout the intervals. Similar findings were also reported by Madejon *et al.* (2007).

Urease activity was positively and significantly correlated with organic carbon but no significant correlation was noticed with soil pH. This is in conformity with conclusion from the study of Baligar *et al.* (1991) who reported the negative correlation of urease activity with pH (-0.15**) and positive correlation with organic carbon (0.83**).

Soil phosphatase activity had significantly and positively correlated with organic carbon and negatively correlated with soil pH. Siwik-Ziomek and Lemanowicz (2014) concluded that the content of soil organic carbon positively correlated with the activity of alkaline phosphatase (0.72**) and acid phosphatase

(0.82**) at five per cent level of significance. Similar conclusions were also made by Chonkar and Tarafdar (1984).

SUMMARY

6. SUMMARY

The investigation entitled "Effect of herbicides and chemical characteristics of soil on microbial biomass carbon and enzyme activity" was carried out in the Department of Soil Science and Agricultural Chemistry at College of Horticulture, Vellanikkara during 2016-2017. The research programme consisted of pot culture experiment and laboratory analysis of soil samples. The studies were undertaken with the objective to determine the impact of pre and post emergence herbicides on microbial biomass carbon and the soil enzymes namely dehydrogenase, urease, and phosphatase and also to study the influence of chemical characteristics of the soil on microbial biomass carbon and enzyme activity.

Representative soil samples were collected during March-April, 2016 from four sites of Thrissur district *viz*., rice field of Agricultural Research Station, Mannuthy (medium organic matter soil with a history of herbicide application: S_1 Control), non-cropped area of Agricultural Research Station, Mannuthy (medium organic matter soil without a history of herbicide application: S_1 Absolute control), rice field of Kole land, Alappad (high organic matter soil with a history of herbicide application: S_2 Control), and non- cropped area of Kole land, Alappad (high organic matter soil without history of herbicide application: S_2 Absolute control). The samples collected from the four sites were analysed to study the physical, chemical and biological properties.

Pot culture experiment with rice variety Jyothi was conducted in the kharif season of 2016 with twelve treatments and six replications (four herbicides under each soil type + four controls). Pre emergence herbicides (pendimethalin and oxyfluorfen) and post emergence herbicides (bispyribac-sodium and cyhalofop-butyl) were selected for the study. Soil samples were analysed at six intervals *viz*., on the day of herbicide application, but two hour before, then at 7, 15, 30, and 60 days after

herbicide application (DAHA) and at harvest so as to evaluate the changes in the chemical and biological properties. Plant characters like height of the plant and productive tillers were recorded at 60 days after sowing. Yield, yield attributes and major nutrient contents were analysed after the completion of rice.

The salient results of the present study are given below;

- Data on analysis of soil samples before the experiment revealed that all the physical, chemical, and biological characteristics which are more favourable for the growth of microflora are exhibited by rice soils of Kole land, Alappad.
- Based on the organic carbon content, the soils under study can be ranked in the order:
  Rice field, Kole > non- cropped, Kole > non-cropped, ARS > rice field, ARS
- Chemical characteristics of the soil *viz.*, pH, electrical conductivity, and organic carbon followed a decreasing trend towards the harvest in all the treatments.
- Decline in microbial biomass carbon (MBC) was observed upto harvest except on 60 DAHA. Pendimethalin treatment in S_1 soil (medium organic matter) registered the highest per cent reduction in MBC with respect to control at different intervals. Percentage reduction in MBC due to herbicide application was comparatively lower in S_2 (high organic matter) throughout the period of study.
- Out of the five biological parameters analysed, microbial biomass carbon showed the highest variation from control at all the sampling intervals.
- Activity of dehydrogenase, urease, acid phosphatase and alkaline phosphatase was higher in S_2 compared to S_1. In all cases, the enzyme activity increased upto 60 DAHA with slight variations and declined thereafter registering a peak at 60 DAHA. However, the extent of increase was lower in the herbicide treatments compared to control and absolute control.

- Among the four enzymes studied, urease recorded maximum increase at 60 DAHA followed by acid phosphatase, dehydrogenase and alkaline phosphatase.
- Adverse effect of herbicides on biological parameters can be ranked in the order:

  pendimethalin > bispyribac- sodium > oxyfluorfen > cyhalofop- butyl

  This was in accordance with mammalian toxicity (LD_{50}) of the respective herbicides and their persistence in the soil in terms of half- life.

  [LD_{50} and half-life ($t_{1/2}$): 4050 mg kg^{-1} and 3 to 4 months for pendimethalin, 4111 mg $kg^{-1}$and 42 to 115 days for bispyribac- sodium, > 5000 mg $kg^{-1}$and 30 to 56 days for oxyfluorfen, and > 5000 mg $kg^{-1}$and 3 to 11 hrs for cyhalofop-butyl, respectively]

- Among the pre emergence herbicides, pendimethalin had adverse effect on all the biological parameters. In the case of post emergence herbicides, bispyribac-sodium exerted adverse effect.
- Magnitude of change with respect to zero day was the highest in urease activity followed by acid phosphatase, dehydrogenase and alkaline phosphatase
- The impact of herbicides on microbial biomass carbon and enzyme activity was comparatively lower in the soils having high organic matter content.
- All the vegetative and yield parameters of rice were high in S_2 (high organic matter soil) irrespective of the treatment combinations. Adverse effects of herbicides on the plant characters were minimal.
- Whole plant analysis for major nutrients also exhibited appreciably higher values in S_2 compared to S_1.
- All the plant characters were higher in high O.M. soils compared to medium O.M. soils.

- Organic carbon had highly significant and positive correlation with all the biotic components *viz.*, microbial biomass carbon, dehydrogenase, urease, acid and alkaline phosphatase activity.

Future line of work

- Identification of microbial diversity
- Rhizosphere effects on biological activity
- Analysis of biological parameters with respect to crop growth stages (active tillering and panicle initiation stage)
- Field studies with different crops under varying soil conditions
- Effects of new molecules of herbicides on biological activity

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ABSTRACT

EFFECT OF HERBICIDES AND CHEMICAL CHARACTERISTICS OF SOIL ON MICROBIAL BIOMASS CARBON AND ENZYME ACTIVITY

by

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ABSTRACT OF THE THESIS

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ABSTRACT

Weed competition is one of the major factors limiting rice production in the tropics. Due to the scarcity and high cost of labour, weed management with herbicides is widely practiced. The problem associated with herbicides is the persistence of their residues in soil which interact with microorganisms thereby altering the microbial diversity and soil enzyme activity. Hence, the present investigation entitled "Effect of herbicides and chemical characteristics of soil on microbial biomass carbon and enzyme activity" was undertaken at College of Horticulture, Vellanikkara during 2016-2017. The objectives were: (i) to determine the impact of pre and post emergence herbicides on microbial biomass carbon and the soil enzymes namely dehydrogenase, urease, and phosphatase and (ii) to study the influence of chemical characteristics of the soil on microbial biomass carbon and enzyme activity.

Representative soil samples were collected during March-April, 2016 from four sites *viz.*, rice field of Agricultural Research Station, Mannuthy (medium organic matter soil with a history of herbicide application: S_1 Control), non-cropped area of Agricultural Research Station, Mannuthy (medium organic matter soil without a history of herbicide application: S_1 Absolute control), rice field of Kole land, Alappad (high organic matter soil with a history of herbicide application: S_2 Control), and non- cropped area of Kole land, Alappad (high organic matter soil without history of herbicide application: S_2 Absolute control). Physical, chemical, and biological characterization of the soil samples were done before starting the experiment. Pot culture experiment with rice variety Jyothi was conducted in the kharif season of 2016 with twelve treatments and six replications in factorial CRD (four herbicides under each soil type + four controls). The herbicides included pendimethalin, oxyfluorfen, bispyribac-sodium, and cyhalofop-butyl.

Pre emergence herbicides (pendimethalin and oxyfluorfen) were applied at six days after sowing (DAS) and post emergence herbicides (bispyribac-sodium

and cyhalofop-butyl) at 16 DAS. Soil samples were analysed at six intervals *viz.*, on the day of herbicide application, but two hour before, then at 7, 15, 30, and 60 days after herbicide application (DAHA) and at harvest so as to evaluate the changes in the chemical and biological properties.

Data on analysis of soil samples before the period of experimentation revealed that soils from rice field of Kole land recorded physical, chemical, and biological characteristics which are more favourable for the growth of microflora.

The chemical characteristics like pH, electrical conductivity, and organic carbon followed a decreasing trend towards the harvest in all the treatments. Decline in microbial biomass carbon (MBC) was observed upto harvest except on 60 DAHA. Pendimethalin treatment in S_1 soil (medium organic matter) registered the highest per cent reduction in MBC with respect to control at different intervals. Percentage reduction in MBC due to herbicide application was comparatively lower in S_2 (high organic matter) throughout the period of study. Activity of dehydrogenase, urease, acid phosphatase and alkaline phosphatase was higher in S_2 compared to S_1. In all cases, the enzyme activity increased upto 60 DAHA with slight variations and declined thereafter registering a peak at 60 DAHA. Among the four enzymes studied, urease recorded maximum increase at 60 DAHA followed by acid phosphatase, dehydrogenase and alkaline phosphatase.

All the vegetative and yield parameters of rice were high in S_2 (high organic matter soil) irrespective of the treatment combinations. Adverse effects of herbicides on the plant characters were minimal. Whole plant analysis for major nutrients also exhibited appreciably higher values in S_2 compared to S_1.

Out of the five biological parameters analysed, microbial biomass carbon showed the highest variation from control at all the sampling intervals. The adverse effects of herbicides on MBC and enzyme activity followed the order: pendimethalin > bispyribac-sodium > oxyfluorfen > cyhalofop-butyl. Organic carbon had highly significant and positive correlation with all the biotic

components *viz*., MBC, dehydrogenase, urease, acid and alkaline phosphatase activity at different sampling intervals consequent to herbicide application.

Further study should be focused on identification of microbial diversity, effect of rhizosphere on biological activity and analysis of biological parameters with respect to crop growth stages consequent to herbicide application.