IMPACT OF GLYPHOSATE AND CHLORPYRIPHOS ON CHEMICAL AND BIOLOGICAL PROPERTIES OF LATERITIC SOIL

By

SHITHA C. R.

THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"Impact of glyphosate and chlorpyriphos on chemical and biological properties of lateritic soil"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title of any other University or Society.

Shitha C. R.

Vellanikkara
11-06-14

SHITHA C. R.

CERTIFICATE

Certified that this thesis entitled **"Impact of glyphosate and chlorpyriphos on chemical and biological properties of lateritic soil"** is a record of research work done independently by **Miss. SHITHA C. R.** 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.

11/06/2014

Vellanikkara

Dr. K.M. Durga Devi
Chairman, Advisory Committee
Associate Professor (SS & AC),
AICRP on Weed Control
College of Horticulture,
Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Miss. SHITHA C. R.**, a candidate for the degree of **Master of Science in Agriculture** with major field in Agronomy, agree that the thesis entitled **"Impact of glyphosate and chlorpyriphos on chemical and biological properties of lateritic soil"** may be submitted by **Miss. SHITHA C. R.** in partial fulfillment of the requirement for the degree.

11/06/2014

Dr. K. M. Durga Devi

Associate Professor (Soil Science &Agrl. Chemistry),

AICRP on Weed Control, College of Horticulture, Vellanikkara

11/6/14

Dr. P. K. Sushama

Professor and Head (Soil Science & Agricultural Chemistry)

College of Horticulture,

Vellanikkara

11/06/2014

Dr. C. T. Abraham

Professor and Head (Agronomy)

& P.I.AICRP on weed control

College of Horticulture

Vellanikkara

11/06/14

Dr. Sosamma Jacob

Professor and Head (Agrl. Entomology)

College of Horticulture, Vellanikkara

11/6/2014

Dr. D. Girija

Professor and Head (Agrl.Microbiology)

College of Horticulture, Vellanikkara

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SHITHA C. R.

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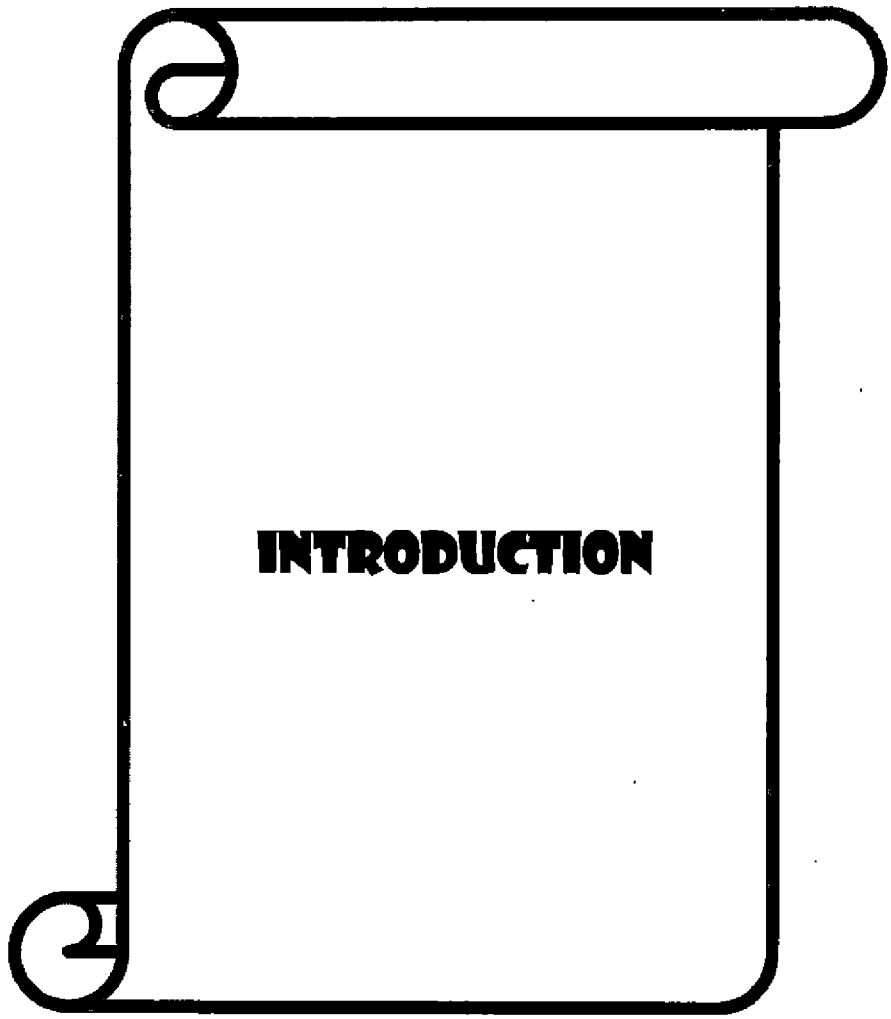
ABBREVIATIONS

a.i.	Active ingredient
%	Percentage
µg g^{-1}	microgram per gram
Ca	Calcium
Cm	Centimeter
CRD	Completely randomized design
Cu	Copper
DAS	Days after spraying
Fe	Iron
Fig.	Figure
G	Gram
K	Potassium
kg ha^{-1}	kilogram per hectare
M	Metre
mg kg^{-1}	Milligram per kilogram
Mg	Magnesium
mL L^{-1}	Millilitre per litre
N	Nitrogen
P	Phosphorous
pH	Hydrogen ion concentration
RBD	Randomized block design
Zn	Zinc


INTRODUCTION

1. INTRODUCTION

Glyphosate and chlorpyriphos are popular pesticides with wide spread applications. In pure chemical terms, glyphosate (N- phosphonomethyl) glycine) is an organophosphate herbicide as it contains carbon and phosphorous. However, it is not a cholinesterase inhibitor and does not affect the nervous system in the same way as organophosphate insecticides like chlorpyriphos.Glyphosate inhibits 5-enolpyruvyl shikimate 3-phosphate synthase, a key enzyme in the synthesis of aromatic amino acids in plants, fungi and bacteria. Chlorpyriphos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is used worldwide as an agricultural insecticide and a termiticide. The general recommendations of glyphosate and chlorpyriphos in Kerala are 0.82-1.64 kg a.i. ha^{-1} and 100-300 g a.i. ha^{-1} respectively.

Organophosphate pesticides are generally considered as safe for use in variety of crops due to their rapid degradation in the environment. However, the effect of pesticides on chemical and biological properties of soil are variable with the nature of pesticides, dose and field conditions. Approximately less than 0.1% of applied pesticide reaches the target pest, leaving the rest to affect the environment (Ardley, 1999). Therefore, there is an increasing concern regarding the effects of the above two pesticide on non target organisms and the soil environment. The data generated in the temperate countries are usually used in the risk assessment of pesticides in the tropical countries which may lead to erroneous results. Information on the effect of glyphosate and chlorpyriphos on the soil micro flora, fauna and chemical properties of lateritic soils of Kerala is too limited.

The soil is a complex ecosystem with a diverse community of organisms. Earthworms representing the greatest part of biomass of terrestrial invertebrates play an important role in soil ecosystem. Earthworms have many chemoreceptors in their body wall (especially in the anterior segments of the body). They show a high sensitivity to chemicals in their environment (Edwards and Bohlen 1996). This sensitivity, coupled with their locomotory abilities, enables them to avoid contaminated areas (Stephenson *et al.*, 1997).They serve as model organisms in toxicity testing and are characterized by high ability to accumulate a lot of pollutants from soil in their tissues. Thus they are used for studying bioaccumulation potential of chemicals.

A study conducted by Springett and Gray (1992) reported that glyphosate reduced the growth rate of the earthworm, *Aporrectodea caliginosa*. A study on Roundup® resistance in

soya field of Argentina showed deleterious effect of these pesticides on earthworm population. Earthworms avoided the soils treated with glyphosate. Exposure to glyphosate also reduced cocoon viability and number of juveniles in earthworms. Both glyphosate and chlorpyriphos caused reduction in the feeding activity under laboratory and field conditions (Casabe *et al.*, 2007). At cellular level DNA damage was also observed in earthworms exposed to chlorpyriphos treated soils. A review of pesticides effects on earthworms showed that there is a negative effect on growth and reproduction by many pesticides (Yasmin and D'Souza, 2010). In addition to the negative impacts on earthworms, there is evidence that pesticides pollution affects numerous other non target species which ultimately affects the food webs and other aspects of ecological community structure.

Soil micro flora plays a key role in maintaining the soil structure, transformation and mineralization of organic matter, availability of plant nutrients, metabolism and degradation of pollutants and pesticides. Microbial activity measurements appear as good indicators of the degree of pollution of contaminated soil. Enzymes are the vital activators in life processes and hence they are known to play a substantial role in maintaining soil health and its environment. The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell-associated or free enzymes. A better understanding of the role of the soil enzyme activity in maintaining the soil health will potentially provide an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management. Although there have been extensive studies on soil enzymes, little has been reported on their roles in maintaining soil health.

Glyphosate is marketed by many agrochemical companies in different solution strength and with various adjuvants under many trade names. The two major formulations used by the farmers in the state as well as in the country are Glycel® and Roundup®. There is controversy on the use of Roundup and there was a move to ban this formulation in the Kerala state. Both Roundup and Glycel formulations contain the surfactant POEA (poly oxy ethylene tallow amine). The surfactant content in Roundup® is 15% and that of Glycel® range from 8-15% depending upon the manufacturer. The surfactant POEA is toxic to fish and to aquatic invertebrates as reported by Cox (1995) and several other scientists. It is about 30 times more toxic to fish than glyphosate (Servizi *et al.*, 1987).

In general, insecticides have a greater direct effect on soil organisms than herbicides. Insecticides like chlorpyriphos, quinalphos, dimethoate, diazinon, and malathion had a range of effects including changes in bacterial and fungal numbers in soil (Pandey and Singh 2004), varied effects on soil enzymes (Menon *et al.,* 2005), as well as reductions in collembolan density (Endlweber *et al.,* 2005). A field study conducted by Fernandez *et al.* (2005) suggested that only long-term and repeated application of glyphosate can affect the soil microbial community.

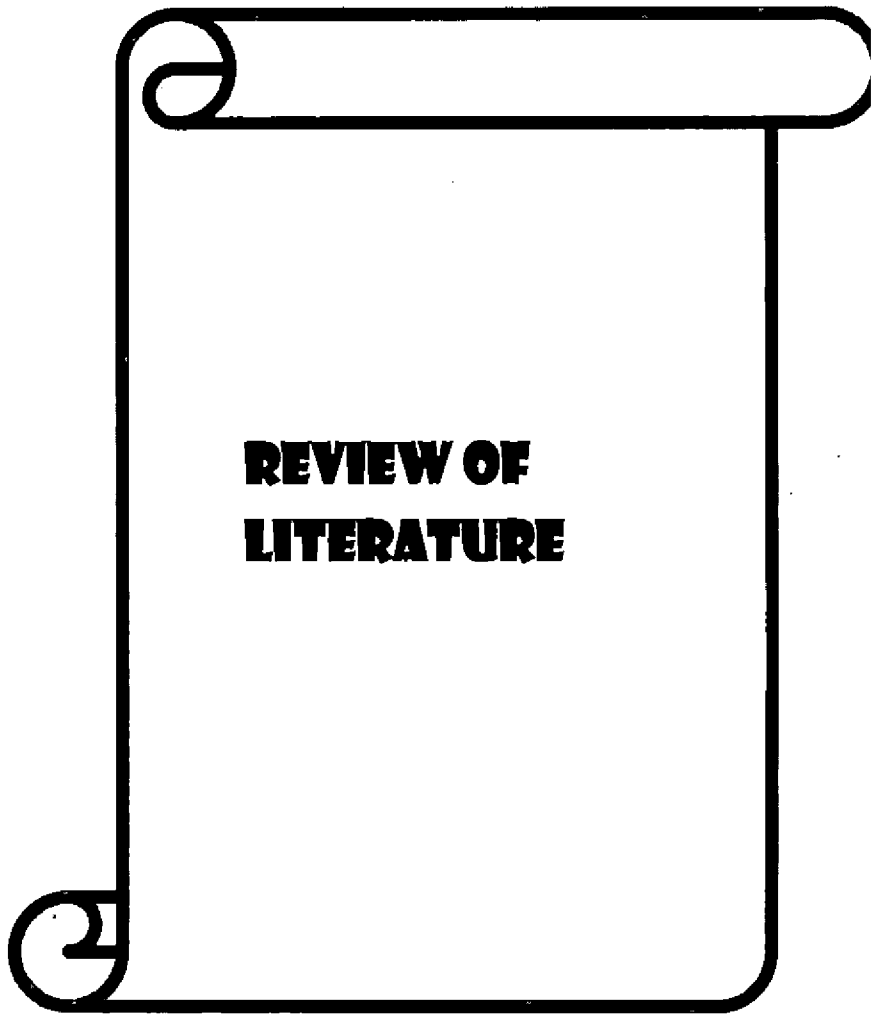
A unique balance of physical, chemical and biological (including microbial especially enzyme activities) components contribute to maintaining soil health. Both laboratory and field studies are important to investigate the impact of pesticides on the soil environment.

In view of the above, the present study entitled "Impact of glyphosate and chlorpyriphos on chemical and biological properties of lateritic soil" was formulated with the following objectives:

1. To monitor the sensitivity of earthworms to varying concentrations of two glyphosate formulations namely Roundup® and Glycel® and one chlorpyriphos formulation *viz.,* Dursban® applied to the soil.

2. To study the effect of application of the above chemicals on soil micro flora and soil enzymes.

3. To evaluate the changes in chemical characteristics *viz.,* pH, organic carbon and available nutrients of the soil due to application of glyphosate and chlorpyriphos


REVIEW OF
LITERATURE

2. REVIEW OF LITERATURE

The literature available on the chemical structure and characteristics of organophosphorous pesticides namely glyphosate and chlorpyriphos and their effects on chemical and biological properties of soil are briefly reviewed below.

2.1. Chemical structure, characteristics and toxicology of the pesticides selected for the study

Organophosphorous herbicides were developed during 1960's. Among this group bensulide and piperophos were introduced in 1964 and 1969 respectively and these two are used as pre-emergent herbicides. However, the post emergence herbicide glyphosate [N-(phosphonomethyl) glycine] is the most widely used chemical for weed control in different crops and cropping systems. It was first sold in 1974 under the trade name Roundup® by Monsanto. It is a broad-spectrum, systemic, post-emergent herbicide. It is a phloem mobile chemical and readily translocated throughout the plant. From the leaf surface, glyphosate molecules are absorbed into the plant cells, where they are translocated to meristematic tissues (Laerke, 1995). Glyphosate's primary action is the inhibition of the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), a chloroplast-localized enzyme in the shikimic acid pathway of plants (Della-Cioppa *et al.*, 1986). This prevents the biosynthesis of essential aromatic amino acids. These acids are used by plants in protein synthesis and to produce many secondary plant products such as growth promoters, growth inhibitors, phenolics, and lignin (Franz *et al.*, 1997).

Glyphosate is generally regarded to be an herbicide with low environmental impact, with low mammalian toxicity. Being water soluble, glyphosate has a low risk of bioaccumulation in food webs. In addition, the phosphate group in glyphosate is readily adsorbed to clay and aluminum and iron oxides in soil, which limits losses from the field and entry into aquatic ecosystems or groundwater. Once adsorbed, the compound is rapidly degraded by soil microorganisms (Franz *et al.*, 1997)

The organophosphate insecticides were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous (they were used in World War II as nerve agents). They are generally highly lipid soluble and may be classified as direct or indirect acetylcholinesterase

(AchE) inhibitors. However; they usually do not persist in the environment. The organophosphates (OPs), because of their widespread use and frequently high acute toxicity, are involved in more pesticide poisonings than any other class of pesticides. (Fukuto, 1990)

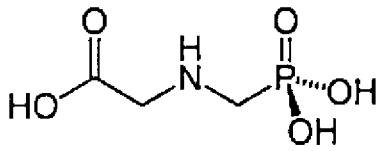
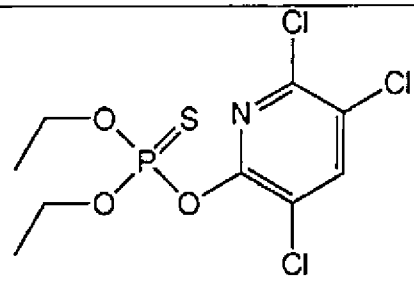
Chlorpyriphos (O, O-diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide. It was introduced in 1965 by Dow Chemical Company and is known by many trade names, including Dursban® and Lorsban®.It is a broad-spectrum contact and stomach poison, with fumigant action. It inhibits the acetyl choline esterase enzyme in the synaptic gap of the insect nervous system acting as a nerve excitatory poison. It has sufficient margins of safety to mammals. It is registered for the control of sucking and chewing insects on a wide range of food crops, oil seeds, pulses, fiber crops, plantation crops and fruits and vegetables (Linan, 1994).

Chlorpyriphos that has been applied to the soil generally stays in the area where it has been applied because it adsorbs to soil particles. There is less chance for washing off chlorpyriphos from the soil and entry into local water systems. Also, since it does not mix well with water even, if it reaches the water bodies it would remain on or near the surface and will evaporate. Volatilization is the major way by which chlorpyriphos disperses after it has been applied. Once it reaches the environment (soil, air, or water), it is broken down by sunlight, bacteria, or other chemical processes. The behaviour of chlorpyriphos in soil was studied intensively due to its long persistence and harmful impacts on organisms (Racke *et al.*, 1998).

Information on the chemical characteristics and toxicity of glyphosate and chlorpyriphos are presented in the table 1.

Table 1. Technical information and toxicity data on glyphosate and chlorpyriphos

Product	**Herbicide**	**Insecticide**
Common name	Glyphosate	Chlorpyriphos
Chemical name	[N-(phosphonomethyl) glycine]	(O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate)

Structure of the chemical		
Trade names	Roundup, Rodeo, Spasor Glifonox, Glycel	Dursban , Eradex, Zidil Lorsban , Detmol
Chemical family	Organophosphorus	Organophosphorus
Molecular formula	$C_2H_8NO_5P$	$C_9H_{11}Cl_3NO_3PS$
Molecular weight	169.08	350.62
CAS Registory no:	1071-83-6	2921-88-2
Physical form	Colourless, crystals	Colourless, crystals
Melting point	200°c	42-43.5°C
Solubility	12g L^{-1} (In water at 25°C)	2 mg L^{-1} (In water at 25°C)
Vapour pressure	2.5m Pa	Negligible
Acute oral LD50 for rats	5600 mg kg^{-1}	135-633 mg kg^{-1}
Acute percutaneous LD50	>5000 mg kg^{-1}	2000 mg kg^{-1}
Mode of action	Non selective systemic herbicide absorbed by the foliage with rapid translocation throughout the plant. Acts on various enzyme systems thus interfereing with the formation of aminoacids. Inactivate on contact with the soil.	Non systemic insectide with contact, stomach and respiratory action. Cholinesterase inhibitor.

Uses	Control of a great variety of annual, biennial, perennial grasses, sedges, broad leaved weeds and woody shrubs.	Control of soil insects and some foliar pest on wide range of crops. Control of insect pest in stored product, household insects etc.
Degradation and metabolism	Environmental: Strongly adsorbed to soil. Microbial degradation is the major cause of loss from the soil with liberation of CO_2, half life in the soil is normally less than 60 days.	Environmental: In soil it is slowly degraded with a half life of 80-100 days to 3,5,6-trichloro-2-pyridinol which is subsequently degraded to organochlorine compounds and CO_2

Source: BCPC, 1979; RSC, 1987

2.1.1. Effect of pesticides on chemical properties of soil

Ali (1990) had shown that the fate of pesticides in soils is greatly affected by the presence of organic matter in the soil by aiding their disappearance. There was significant reduction in percentage organic carbon level after the application from the 14^{th} day of treatment application (Sebiomo et *al.*, 2011).

Singh *et al.* (2003) found that the rate of degradation of chlorpyrifos was low in acidic soils but increased considerably with an increase in soil pH. Significant changes in soil pH and organic matter were obtained in the case of atrazine treated soil 800 g kg^{-1} a.i. as studied by Ayansina and Oso (2006).

Iron deficiency chlorosis is also becoming increasingly prevalent in cropping systems receiving frequent or prolonged applications of glyphosate (Ozturk *et al.*, 2008).The effect of single or repeated application of glyphosate (0, 0.5 X, 1 X, 2 X, and 3 X recommended field rates) was studied by Lane (2012) and found that there was no reduction in the exchangeable K.

The interaction of atrazine, primextra, paraquat and glyphosate with soil minerals was investigated by Sebiomo *et al.* (2012) The treatments were carried out for a period of six weeks for paraquat, glyphosate and primeextra with recommended rate of 3 kg ha^{-1}. The

potassium, magnesium, iron and zinc content increased significantly ($p<0.001$) compared to the control. This study had elucidated the ability of herbicides to chelate with soil minerals thereby reducing their availability for uptake by plants.

The effects of bispyribac sodium 10% SC and butachlor 50% SC on soil physico-chemical properties in transplanted kharif rice were investigated over two seasons (2010 and 2011). No significant changes in soil physico-chemical properties like bulk density, water holding capacity, moisture content, soil pH, organic matter content, electrical conductivity, as well as total nitrogen, available phosphorus and available potassium content (Bera and Ghosh, 2013) were observed.

2.1.2. Earthworm and their importance

Earthworms (Annelida: Oligochaeta) contribute to soil fertility improvement, plant growth and play a key role in converting organic matter and composting garbage. There are about 3,627 species of terrestrial earthworms in the world (Reynolds, 1994).

Earthworms play an important role in the process of soil formation and also in the maintenance of soil fertility. They incorporate organic matter and turn over large amounts of soil by borrowing, feeding and casting. This leads to improved soil structure (Hoogerkamp *et al.*, 1983; Stewart *et al.*, 1988), enhanced nutrient release (Barley and Jennings, 1959) and ultimately a better plant growth (Edwards and Lofty, 1980). They also play a key role in soil biology as versatile natural bioreactors. They effectively harness the beneficial soil microflora, destroy soil pathogens and convert organic wastes into useful products. Earthworms modify soil's physical, chemical and biological properties and it is believed that they enhance nutrient cycling by ingestion of soil and humus and by production of casts. Earthworms are well known for increasing soil fertility. In the process of burrowing, earthworms accelerate litter decomposition, change pore structure, increase aeration and water infiltration, and accelerate C and N mineralization. They also change microbial community composition during gut transit and following excretion in casts and burrow walls (Pedersen and Hendriksen, 1993). The organic materials ingested by earthworms undergo physical, chemical and biological changes and provide macro and micro nutrients, vitamins, enzymes, growth hormones, antibiotics and micro flora thereby playing a vital role in the enrichment of soil fertility (Bhawalkar, 1991) Earthworms increase the soil - air volume from

8% to 30% (Edwards and Lofty, 1977) and make the soil loose and porous thus improving water absorption, drainage and propagation of roots (Noble *et al.*, 1970).

A greater proportion (80%) of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality. This is important for protecting the health of natural environments and is of increasing interest in the context of protecting human health as well as other terrestrial vertebrates which prey upon earthworms. Thus they are important members of the soil fauna and possess a number of characteristics that make them appropriate organisms for use in assessing potential risks of contaminated soils. They are affected by a variety of organic and inorganic compounds, which may cause bioaccumulation. They are also important in the terrestrial tropic system, constituting a food source for a wide variety of organisms, including birds, mammals, reptiles, amphibians, fish, insects and microorganisms.

2.1.2.1. Earthworm Avoidance Test

The Earthworm Avoidance Test was originally developed in USA (Yeardley *et al.*, 1996). It is a practical and sensitive screening method for assessing the effects of pesticides in tropical soils. Little research has been performed on the impact of pesticides on earthworms under tropical conditions. In fact, the data on the effects of agricultural chemicals on tropical soil invertebrates are scarce (Helling *et al.*, 2000). Earthworm avoidance test is quick and easy to perform, and it is known to be sensitive towards a wide range of chemicals. The principle of the test is that the earthworms are simultaneously exposed to the soil sample spiked with the pesticide, and to a control soil. After a test period of two days the location of the animals is determined. The standard protocol for the Earthworm Avoidance Test (ISO, 2006) was modified in terms of test species, substrate and conditions in order to make it suitable for tropical regions (Garcia *et al.*, 2008). Their results indicate that this test gives reproducible and reliable results. Toxicity values (NOEC, EC50) are lower than those determined in 14 day-acute mortality tests and are approximately in the same range such as those found in 56 day-chronic reproduction tests with the same earthworm species, which were performed in parallel. Therefore, the use of the earthworm avoidance tests is recommended as a screening tool for the risk assessment of pesticides. Casabe *et al.* (2007)

reported that reproduction and avoidance tests were sensitive indicators of glyphosate exposure. Their study showed deleterious effects of glyphosate and chlorpyriphos formulations when applied at normal concentration recommended for soya crops.

2.1.2.2. Effect of glyphosate and chlorpyriphos on earthworms

a) Glyphosate

Morowati (2000) found that the glyphosate (0.15 mL/22 mL water/0.2m^2) is detrimental to *P. elongata*, causing severe histochemical changes and histopathological lesions in the intestinal lining and causing at least 50 percent mortality in the earthworm population.

Correia and Moreira (2010) studied the effects of glyphosate (1; 10; 100; 500; 1,000 mg kg^{-1}) on earthworms and found that earthworms were alive in all evaluations and showed gradual and significant reduction in mean weight (50%) in all the test concentrations. No cocoons or juveniles were found in soil treated with the herbicide.

Glyphosate had been reported to reduce the growth of *Aporrectodea caliginosa* when repeatedly applied to laboratory cultures at 2-week intervals, at a rate lower than that commercially recommended (Springett and Gray, 1992), but had no effect on *Aporrectodea caliginosa* in another pot experiment where the chemical was mixed with the soil (Martin, 1982).

Glyphosate showed no toxic effects for both species *Eisenia andrei* and *Pontoscolex corethrurus*, at the concentration tested (47 mg a.i. kg^{-1}), but they displayed avoidance behavior at this concentration at the dose, i.e., 47 mg a.i. kg^{-1} ($p < 0.05$ level) for *Pontoscolex corethrurus* and at 30 mg a.i. kg^{-1} or higher for *Eisenia andrei* (Buch *et al.*, 2013).

A field study in soya field conducted by Casabe *et al.* (2007) showed that glyphosate (1,440 g a.i. ha^{-1}) reduced cocoon viability, decreased the number of juveniles and moreover, earthworms avoided soils treated with glyphosate.

b) Chlorpyriphos

The effect on fecundity of chlorpyrifos was assessed in the earthworm *Aporrectodea caliginosa*. Juveniles were exposed to sub-lethal concentrations (28 and 60 mg kg^{-1}) and laboratory-simulated field rates (4 and 12 mg kg^{-1}) of chlorpyrifos for 4 weeks, and recovery was monitored for 12 weeks in organophosphate-free soil. Growth, maturation, cocoon production and hatching success in resulting adults were monitored. Chlorpyrifos affected growth, maturation rates and fecundity at the highest concentration. (Booth *et al.*, 2000)

In the laboratory-simulated field experiments, Booth and Halloran (2001) found a significant decrease in cocoon production and viability in the earthworm *Aporrectodea caliginosa* exposed to soils containing 28 ppm of chlorpyriphos.

Zhou *et al.* (2007) assessed toxicity of chlorpyriphos (5mg kg^{-1}) with the earthworm *Eisenia foetida* and found that the growth and cocoon production decreased with increase in concentration. But at lower test concentrations, only significant chronic toxic effects could be observed. In the year 2008, Zhou *et al.* found that chlorpyrifos had adverse effect on fecundity in earthworm when they were exposed to this chemical.

c) Effect of other pesticides on earthworms

Earthworms exposed to 50 mg kg^{-1} carbaryl showed no mortality, but exposure resulted in a reduction by more than 50% in cocoon numbers which lead to significant reduction in the population in 2-3 generations (Neuhauser, 1990).

Choo and Baker (1998) also found that cocoon production in *Aporrectodea trapezoides* was inhibited by fenamiphos at normal application rates and methiocarb at 10 times the normal rate.

In a laboratory study conducted by Helling *et al.*, (2000) on the effect of copper oxychloride found that it significantly reduced the growth and cocoon production at concentrations 8.92, 15.92, 39.47, 108.72 and 346.85 mg Cu kg substrate^{-1}.

Mosleh *et al.* (2002) investigated the toxicity of aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, and metalaxyl (LC10, LC25, and LC50) in the earthworm *Aporrectodea caliginosa* and observed a reduction in growth rate in all the pesticide-treated worms.

The toxic effect of benomyl on the ultrastructure of the male reproductive system and spermatozoa of the earthworm *Eisenia fetida* was studied at three different concentrations of benomyl (8.3, 56, 112 mg kg^{-1} dry soil) for one week. These applications caused abnormalities in ultrastructure of the cytophore, the spermatogonia, spermatids, and spermatozoa. The alterations viz., incomplete forms of acrosomes, nuclear distortion and disruption of microtubules were observed. These micro morphological changes should be included in a model for predicting environmental hazards. (Sorour and Larink, 2001)

Bustos-Obregon and Goicochea (2002) explored the effect of exposure to commercial parathion on *Eisenia fetida* at concentrations of 444, 739 and 1478 mg kg^{-1} of soil at three time intervals of 5, 15 and 30 days. It was found that body weight and survival rates were observed to decrease with concentration.

Mosleh *et al.* (2003) found that the levels of transaminase and phosphatase activities in the earthworm *Aporrectodea caliginosa* increased when organisms were exposed to pesticides at LC_{25} values of aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine and metalaxyl.

Gupta and Saxena (2003) studied the effects of carbaryl (0.5-2ppm) on the reproductive profiles of the earthworm, *Metaphire posthuma* and found that locomotion and geotaxis were significantly affected, even after a 20-minute exposure to 0.125ppm carbaryl. The hatching of cocoons was altered at 0.5ppm, whereas cocoon production was retarded even at 0.125ppm carbaryl. No cocoon production was observed at 2.0 ppm carbaryl. Sperm head abnormalities were reported even at the lowest test concentration of 0.125ppm. Wavy head abnormalities were observed at 0.125ppm carbaryl, whereas at 0.25ppm and 0.5ppm, the sperm heads became amorphous and the head nucleus was turned into granules deposited within the wavy head.

Espinoza-Navarro and Bustos-Obregon (2004) found that malathion 300 mg kg^{-1} soil had a direct cytotoxic effect, coiling of the tail, altering sperm count etc in the treated earthworms. Xiao *et al.* (2006) suggested that acetochlor had no long term effects on the reproduction of *Eisenia fetida* at field dose (5-10 mg kg^{-1}). In 2007, Yasmin and D'Souza investigated the impact of carbendazim (3.2 mg kg^{-1} soil), glyphosate (8 mg kg^{-1} soil) and dimethoate (1.6 mg kg^{-1} soil) on *Eisenia fetida* and found a significant reduction in the earthworm growth in a dose-dependent manner. Reinecke and Reinecke (2007) reported that

no significant differences in the density of earthworms were observed in orchards and adjacent areas after application of chlorpyriphos but predicted adverse chronic effects.

De Silva in 2009 reported avoidance of chlorpyriphos and carbofuran by both *E. andrei and P. excavates.* The concentrations tested were 1, 3, 10, 30, 100, 300 and 900 mg a.i. kg^{-1} dry soil for chlorpyrifos, 0.5, 1, 2, 4, 8, 16 and 32 mg a.i. kg^{-1} dry soil for carbofuran and for mancozeb 1, 3, 10, 30, 100, 300, 900 and 1200 mg a.i. kg^{-1} dry soil. The toxicity decreased in the order of carbofuran > chlorpyrifos > mancozeb. It was also found that these chemicals were more toxic to *P. excavatus* than to the standard test species *E. andrei* at temperature representative of tropical conditions

An experiment was conducted by Correia and Moreira in 2010 to compare the effects of various concentrations of glyphosate and 2, 4-D on earthworms (*Eisenia fetida*). It was found that glyphosate did not kill the test organisms, but decreased the mean weight, which indicated the chronic effect of this herbicide. In soils treated with 2, 4-D, they observed death of specimens after a few hours of exposure and loss of weight indicating consistent acute toxicity symptoms. Morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate and 2,4 D after 30 days of exposure.

Study conducted by Caceres *et al.,* in 2011 revealed that fenamiphos did not affect the survival of *E. fetida* up to a concentration of 50 mg kg^{-1} in soil. However 100% mortality was observed at 200 mg kg^{-1} soil. The earthworms exposed to the highest pesticide concentration (200 mg kg^{-1} soil) exhibited avoidance behaviour during the first week of incubation.

De Sousa and De Andrea (2011) conducted an experiment in earthworms *Eisenia andrei* and found that they avoided the soils treated with cypermethrin and there was no dose-related response within the range of concentration tested, independently of being formulated as wettable powder or technical grade. Farrukh and Ali (2011) studied effects of dichlorovos at concentration 19, 38, and 76 mg kg^{-1} dry weight of soil and found that increasing concentration significantly affected the growth, reproduction, and avoidance behavior of earthworm *Eisenia fetida.*

2.1.3. Soil micro flora

Soil microbes are the driving force behind many soil processes including transformation of organic matter, nutrient release and degradation of xenobiotic (Zabaloy *et al.*, 2006). Number and mass of microorganisms are basic properties of ecological studies, and which can be related to parameters describing microbial activity and soil health (Bolter *et al.*, 2006).

Early studies on direct effects of pesticides on microorganisms demonstrated that various pesticides affected the growth of bacteria (Worth, 1948) fungi (Eipper, 1959) and algae (Martin 1966). Microorganisms were found to be one of the major factors in the breakdown of many pesticides in nature (Audus, 1949).From such studies it is apparent that different species vary in their sensitivity to individual pesticides (Ukeles and Wilkinson, 1962; Cowley *et al.*, 1970). It has been demonstrated that members of the microbial world vary widely in response to pesticide and several factors may influence the toxicity of pesticide (Durham, 1971). There are some agrochemicals which are not utilizable by soil micro flora and might be degraded in soil by microorganisms through co-metabolism (Bollag and Liu 1990).The microbial tolerance may be affected by growth and physiological condition of cells and various factors. In general, the impact of pesticides on soil micro flora was variable and the effects were not only from the reaction of microorganisms to an active substance and formulation additives but also from the development of specific group of microorganisms (Nowak *et al.*, 1999). Some microbial groups were able to use an applied pesticide as a source of energy and nutrients to multiply (Johansen *et al.*, 2001).

2.1.3.1. Effect of glyphosate and chlorpyriphos on soil micro flora

a) Glyphosate

The herbicide is inactivated and biodegraded by soil microbes under both aerobic and anaerobic conditions. Rates of decomposition were dependent on soil and microfloral population types (Eriksson, 1975). Glyphosate's primary route of decomposition in the environment is through microbial degradation in soil (Franz *et al.*, 1997). The effect of glyphosate on soil microorganisms has been widely studied, with conflicting results. As soil microbial communities are diverse, responses to glyphosate use are varied.

Wardle and Parkinson (1990) found that microbial respiration was enhanced by glyphosate at an application rate of 200 μg g^{-1} soil. Haney *et al.* (2000) found that the glyphosate application at rates of 47, 94, 140, and 234 mg a.i. g^{-1} stimulated soil microbial activity but did not affect soil microbial biomass. Studies by Busse *et al.* (2001) showed no effect of glyphosate when applied at 0, 5, 50 and 500 mg a.i. kg^{-1}. Hanley *et al.* (2002) indicated that glyphosate (234 mg a.i. kg^{-1}) application increased the soil microbial biomass, respiration and carbon and nitrogen mineralization

Detrimental effects of glyphosate on soil microflora had also been reported by many scientists. A study conducted by Busse *et al.*, in 2001 found that glyphosate was lethal to bacteria and fungi when added to soil-free media. At the recommended spray concentration of 50 mM, glyphosate reduced bacterial viability 1000-fold on solid media, and completely eliminated fungal growth. Increasing glyphosate to 500 mM stopped all bacterial growth. Bacterial growth rate in liquid media also declined following additions of glyphosate. A number of studies revealed that glyphosate amendment had no significant effect on microbial community activity and composition (Busse *et al.*, 2001; Liphadzi *et al.*, 2005; Ratcliff *et al.*, 2006; Weaver *et al.*, 2007). Glyphosate has been shown to reduce populations of Mn-reducing microorganisms active in soil nutrient cycling (Huber, 2007). Partoazar *et al.* (2011) found increased heterotrophic bacterial population in a soil with a long history in the use of glyphosate.

Araujo *et al.* (2003) found that glyphosate (2.16 mg kg^{-1} soil) amendment did not affect culturable bacterial populations, while fungi and actinomycetes populations increased. This effect was larger in soils that had greater previous exposure to glyphosate. A review of the effect of genetically resistant crops on soil microbial communities concluded that microbial diversity can be altered by the use of genetically resistant plants in conjunction with glyphosate, although the observed changes were variable and transient (Dunfield and Germida, 2004).

b) Chlorpyriphos

A study was conducted by Pozo *et al.*, (1995) on the effects of the insecticide chlorpyrifos on soil microbial activity. They analyzed the parameters including the total bacterial populations, fungi, aerobic N_2-fixing bacteria, denitrifying bacteria, nitrifying bacteria etc and found that the presence of 2.0, 3.5, 5.0, and 10.0 kg ha^{-1} of chlorpyrifos

significantly ($p < 0.05$) decreased aerobic nitrogen-fixing bacteria where as the total number of bacteria increased significantly at concentrations of 2.0 to 10.0 kg ha^{-1}. Fungal populations, nitrifying bacteria, and denitrifying bacteria were not affected as a consequence of the addition of the insecticide.

The effects of chlorpyriphos on soil microbial characteristics (including microbial biomass carbon and nitrogen, microbial populations, microbial respiration, enzymatic activities, and nitrogen cycling) have also been frequently studied and found that there is a temporary or short-term inhibitory effect on soil microbial functional diversity. (Singh *et al.*, 2006; Menon *et al.*, 2004; Adesodun *et al.*, 2005; Menon *et al.*, 2005; Shan *et al.*, 2006).

A laboratory and field study was conducted to determine the effects of chlorpyrifos 40EC, at four concentration 125, 250, 500, and 1000 ppm. Results obtained from both studies revealed that chlorpyrifos caused significant reduction in number of soil bacteria, but in case of field experiment, effect disappeared at 21 day after application (Ahmed and Ahmed 2006). Shan *et al.*, (2006) also indicated that soil bacterial, fungal, and actinomycete populations in soil were inhibited by chlorpyriphos at a concentration of 10 mg kg^{-1}.

It had been reported that soil microbial biomass was reduced by 25% and 50% after chlorpyriphos treatment at concentrations of 10 and 50 mg kg^{-1}, respectively (Vischetti *et al.*, 2007).

c) Effect of other pesticides on soil micro flora

The study conducted by Mewatankarn and Sivasithamparam 1987 showed that diquat and paraquat (1ppm) increased fungal populations. An increase of the number of soil bacteria was observed during the biodegradation of chlorinated hydrocarbon, organophosphate and synthetic pyrethroid insecticides (Das *et al.*, 1995, Rache and Coats, 1988). Das and Mukherjee (2000) found that the growth of microorganisms was stimulated by addition of hexachlorocyclohexane (HCH), phorate and fenvalerate to soil. Similar results were also obtained by Araujo *et al.* (2003) and Das *et al.* (2003) after application of glyphosate and phorate respectively. The negative impact of other fungicides such as captan, benomyl and paclobutrazol on fungal population size was also observed in the studies conducted by Martinez-Toledo *et al.* (1998), Smith (2000) and Silva *et al.* (2003) respectively.

Three post-emergence herbicides (2, 4-D, picloram and glyphosate) were applied to samples of an Alberta agricultural soil at concentrations of 0, 2, 20, and 200 μg g^{-1}. The effects of these chemicals on certain microbial variables were monitored over 27 days. All the herbicides caused enhancement of basal respiration but only for 9 days following application, and only for concentrations of 200 μg g^{-1}. Substrate-induced respiration was temporarily depressed by 200 μg g^{-1} picloram and 2, 4-D, and briefly enhanced by 200 μg g^{-1} glyphosate. It was concluded that because changes in microbial variables only occurred at herbicide concentrations of much higher than that which occurs following field application, the side-effects of these chemicals is probably of little ecological significance (Wardle and Parkinson 1990).

Kucharski and Wyszkowska (2008), found that even the recommended dose of Apyros 75 WG (8.9, 8 9.0, 890 μm kg^{-1} soil) caused microbiological changes in the treated soil with least affect on fungi.

2.1.4. Soil enzymes

Soil enzymes are a group of enzymes whose usual inhabitants are the soil and are continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility, and soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al.*, 1991). They are important in catalyzing several vital reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling, hence playing an important role in agriculture (Dick *et al.*, 1994; Dick 1997). All soils contain a group of enzymes that determine soil metabolic processes (McLaren, 1975) which, in turn, depend on its physical, chemical, microbiological, and biochemical properties. The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content, composition, and activity of its living organisms and intensity of biological processes. In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for microorganisms (Kiss *et al.*, 1978). These enzymes may include amylase, arylsulphatases, beta-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease, and urease

released from plants animals organic compounds, and microorganisms and soils (Gupta *et al.*, 1993; Ganeshamurthy *et al.*, 1995).

2.1.4.1. Dehydrogenase enzyme

The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978). This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are the part of respiration pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions (Kandeler *et al.*, 1996). Since these processes are the part of respiration pathways of soil microorganisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility as well as soil health. A study by Brzezinska *et al.*, (1998) suggested that soil water content and temperature influence dehydrogenase activity indirectly by affecting the soil redox status. After flooding the soil, the oxygen present is rapidly exhausted so that a shift of the activity from aerobic to anaerobic microorganisms takes place. Such redox transformations are closely connected with respiration activity of soil microorganisms. They may serve as indicators of the microbiological redox systems in soils and can be considered a possible measure of microbial oxidative activity (Tabatabai, 1982). For instance, lack of oxygen may trigger facultative anaerobes to initiate metabolic processes involving dehydrogenase activities and the use of Fe (III) forms as terminal electron acceptors, a process that may affect iron availability to plants in the ecosystem (Benckiser *et al.*, 1984). Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil (Reddy and Faza, 1989; Wilke 1991), as well as a direct measure of soil microbial activity (Garcia and Hernandez, 1997).

a) Effect of glyphosate and other herbicides on soil enzymes

Fenamiphos at 18.6 kg ha^{-1} reduced the activity of urease under field conditions but after 5months, activity was the same as in the control while no effect was observed under laboratory conditions (Ross and Speir, 1985). Glyphosate was found to inhibit dehydrogenase, phosphatase and urease activities in a sandy loam soil (Dzantor and Felsot,

1991). Reduced enzymatic activities were also found by Perucci and Scarponi (1990) and by Dzantor and Felsot (1991) in studies on the interference of atrazine with the phosphatase, dehydrogenase and esterase activity of soil.

Min *et al.* (2002) reported that after the application of butachlor with concentrations of 5.5 μg g^{-1} dried soil, 11.0 μg g^{-1} dried soil and 22.0 μg g^{-1} dried soil, the activity of dehydrogenase was found to be increasing at increasing concentrations. Soil dehydrogenase showed the highest activity on the 16th day after application of butachlor at rate of 22.0 μg g^{-1} dried soil.

Lu *et al.* (2004) observed that enzymes differed markedly in their response to quinclorac. Quinclorac inhibited proteinase, hydrogen peroxidase, phosphorylase, and urease activities. The higher the concentration of quinclorac applied, the more significant the inhibition to these observed activities with a longer time required to recover to the level of the control. However, the highest dehydrogenase activity was detected in soils with 2 μg quinclorac g^{-1} soil on the twenty-fifth day after treatment. The study revealed that quinclorac was relatively safe to the soil ecosystem when applied at a normal concentration (0.67 μg g^{-1} dried soil) but would have some effects on soil enzymes at higher concentrations.

b) Effect of chlorpyriphos and other insecticides on soil enzymes

Madhuri and Rangaswamy (2002) observed that soil samples receiving 2.5 kg ha^{-1} of the insecticides dichlorvos, phorate and methomyl and also in soil samples receiving 5.0 kg ha^{-1} of the insecticides *viz.*, chloropyrifos and methyl parathion, the activity of phosphatase was significantly more at 20 days period of incubation and decreased progressively with increasing period of incubation.

Kalam *et al.* (2004) observed maximum inhibition of soil phosphatase activity (46.6%) in presence of propiconazole (100 mg kg^{-1}) after 120 days. Profenofos affected the soil dehydrogenase activity by 47% at 1000 mg kg^{-1} concentration after 80 days and thereafter, the extent of toxicity decreased little. Soil urease activity was significantly affected in the presence of profenofos and was 62% at 1000 mg kg^{-1} level after 80 days.

Kennedy and Arathan (2004) reported that application of carbofuran at 1.0 and 1.5 kg a.i. ha^{-1} significantly reduced the activity of soil enzymes, *viz.*, alpha -amylase, beta -glucosidase, cellulase, urease and phosphatase up to 30 days after carbofuran application.

However, application of carbofuran at the recommended level (0.5 kg a.i. ha^{-1}) had no significant effect on the activity of soil enzymes which are biologically significant as they play an important role not only in the soil chemical and biological properties but also affect the nutrient availability to plants.

Shiyin *et al.* (2004) reported that catalase activities are inhibited when fenvalerate are added to soil in 15 days, and then the activities began to be stimulated. As to chlorpyrifos, catalase activity was stimulated at lower level of chlorpyrifos (1 ppm), and inhibited at higher concentrations (10-80 ppm) after 1-day incubation. The hydrolysates of fenvalerate had stronger inhibitory effect on catalase activity at the initial stage of exposure (approximately 1-5 days). Maximum inhibition of hydrolysates appeared at 1st day at 80 ppm.

Shan *et al.* (2006) observed that chlorpyriphos at 2.0 mg kg^{-1} showed a slight effect on acid phosphatase; it enhanced the activity at 7 and 14 days. Alkaline phosphatase was inhibited significantly at 7 days due to the addition of chlorpyriphos. After incubation for 14 days, the activity was recovered to the level of the control. Urease activity in the soil treated with chlorpyriphos at 2.0 mg kg^{-1} was decreased at days 1 and 7, whereas it recovered to the level of the control after incubation for 14 days. A significant reduction in urease activity was observed in the soil treated with chlorpyriphos at 4.0 and 10.0 mg kg^{-1}. Catalase activity was negatively affected by the addition of chlorpyriphos at 4.0 and 10.0 mg kg^{-1}. This negative effect on catalase activity disappeared at 21 days

The influence of four pesticides *viz.*, profenofos, deltamethrin, thiram and difeneconazole and two insecticide combinations *viz.*, profenofos+cypermethrin, deltamethrin+endosulfan at 0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} were assessed by Madakka *et al.*, in 2010. The effect of selected pesticides on dehydrogenase activity was dose dependent. This enzyme activity increased with increasing concentrations of the pesticides up to 5.0 kg ha^{-1}. Higher rates of (7.5, 10.0 kg ha^{-1}) of these pesticides were either toxic or innocuous to the dehydrogenase activity. Significant stimulation in the activity of dehydrogenase was noticed with 2.5 kg ha^{-1} of pesticides in black soil, where as in red soil it was 2.5 kg ha^{-1} of profenofos, deltamethrin, thiram and 5.0 kg ha^{-1} difeneconozole. In case of insecticide combinations the significant stimulation of dehydrogenase activity was observed with 2.5 kg ha^{-1} of profenofos+cypermethrin, 1.0 kg ha^{-1} of deltamethrin+endosulfan in black soil, where as in red soil it was observed at 1.0 kg ha^{-1} of profenofos+cypermethrin, deltamethrin+endosulfan. On further incubation, the activity of dehydrogenase was

significantly more at 21 day and enzyme activity decreased progressively with increasing period of incubation.

Sushma and Singh (2006) applied chlorpyrifos 20 EC and quinalphos 25 EC, in groundnut (*Arachis hypogaea* L.) field as seed treatment at 25 mL kg^{-1} and soil treatment at 4.0 L ha^{-1} in 1998 and 1999. The residues of these insecticides were monitored during the entire crop season and their effect on the soil enzymes dehydrogenase, phosphomonoesterase and arginine deaminase were studied. It was observed that in most of the cases insecticides had temporary inhibitory effect on soil enzymes. However, inhibition was smaller in seed treated soil than in direct soil treatment.

Yang *et al.* (2006) reported that addition of repeated dose of chlorimuron-ethyl and furadan to the soil resulted in a change in the invertase activity of -12-7% and -6%-7%, respectively, indicating that the toxicity of the two pesticides was relatively small. Organic substances showed obvious buffering action on the toxicity produced by joint pollution.

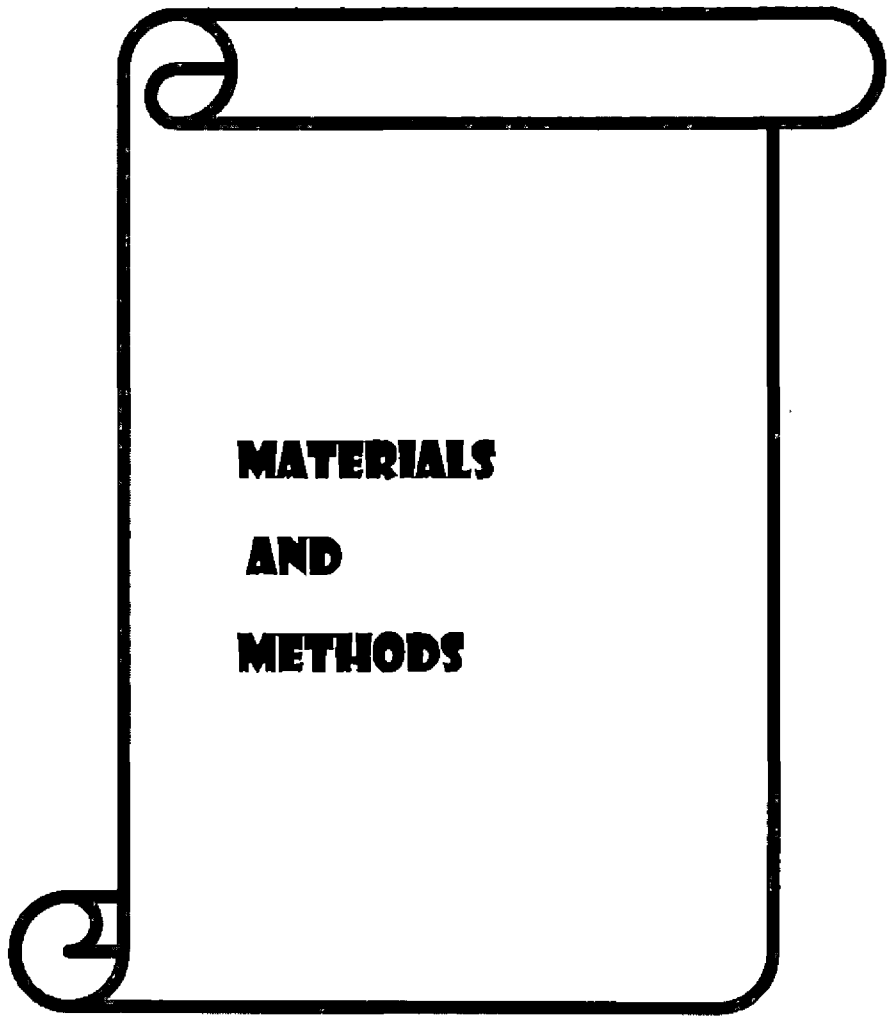
Yao *et al.* (2006) reported that acetamiprid had a strong negative influence on soil respiration and phosphatase activity, The activity of dehydrogenase was enhanced after acetamiprid application for 2 weeks and the enzyme activities in samples treated with 0.5, 5 and 50 mg kg^{-1} dry soil was about 2.5, 1.5 and 2 fold to that of the control on sample day 28. Overall, acetamiprid at normal field dose would not pose a toxicological threat to soil enzymes, but a certain potential threat to soil respiration.

The effects of two different categories of pesticides *viz.*, chlorpyrifos (an organophosphate) and endosulfan (an organochlorine cyclodiene) soil dehydrogenase activity were evaluated by Kumar (2011). The medium and higher dose of endosulfan resulted in the significant decrease in dehydrogenase activity till third and fourth week of treatment, respectively. In case of chlorpyrifos, decrease in dehydrogenase activity was observed till second week at medium dose and till fourth week at higher dose, compared to untreated control. In comparison to control, the activity of dehydrogenase decreased by 16% and increased by 25% in presence of 10 ppm each of endosulfan and chlorpyrifos, respectively, after 14 days. The combined effect of chlorpyrifos and endosulfan showed inhibitory effect at both medium and higher doses, for the treatment duration of four weeks. The maximum inhibition (62%) in soil dehydrogenase activity was noticed in presence of 50 ppm each of chlorpyrifos and endosulfan after first day of treatment.

c) Effect of fungicides on soil enzymes

Kodka *et al.* (2004) conducted a pot experiment on black soil samples of light dusty clay to investigate the effect of 2 fungicides, i.e. copper oxychloride(without adjuvant) and Miedzian 350 Extra SC (with adjuvant), on peroxidase, dehydrogenase activity and ATP content in the soil and peroxidase activity and ATP content in radish. Three concentrations of each preparation was used, i.e. field rate, 10 times the field rate and 100 times the field rate. Great differences between activities of the enzymes and ATP contents caused by the fungicides were observed, especially when the rates used were higher than the field rate.

Cycon *et al.* (2005) investigated the effects of insecticide (diazinon), herbicide (linuron) and fungicide (mancozeb+dimethomorph) on the enzymatic activities in sandy soil for 28 days under laboratory conditions. The results showed that the influence of tested pesticides on dehydrogenase, acid and alkaline phosphatase and urease was diversified. Furthermore, these studies confirmed that enzyme activities are a useful tool for the detection of pesticide side effects in soil and they can be used as indicators of soil pollution. He *et al.* (2003) reported that dimehypo significantly inhibited soil urease activity and the characteristic parameters of soil urease, such as its activity, maximum reaction velocity V and velocity constant k excluding K, were decreased with increasing dimehypo concentrations.


MATERIALS
AND
METHODS

3. MATERIALS AND METHODS

The research programme entitled "Impact of glyphosate and chlorpyriphos on chemical and biological properties of lateritic soil" consisted of laboratory, pot culture and field experiments. The experiments were carried in the laboratory and fields of the College of Horticulture, Vellanikkara during 2013. The details of the experiments and methodology are given in this section.

3.1. Laboratory experiments

3.1.1. Studies on avoidance behaviour of earthworm

3.1.1.1. Test soil

Laboratory experiments were conducted with soil samples collected from the existing field banana (ratoon crop) of Vermicomposting unit under Department of Soil Science and Agricultural Chemistry. Soil samples were taken using a spade at 0-15cm depth from five different sites in the field. The samples were pooled, dried in shade, sieved and stored for conducting laboratory and pot culture experiments.

3.1.1.2. Physico-chemical properties of soil

Major physical and chemical properties of the soil viz., texture, pH, organic carbon and the content of available N, P, K, Ca, Mg and micronutrients before the experiment were determined. The methods adopted for the analysis are given in the Table 2.

3.1.1.3. Test organism

Native earthworms *Perionyx excavatus* (Family: Megascolecidae) were used for this experiment. Earthworms were collected from the College campus at Thrissur on the day of the experiment and brought to the laboratory. The collected earthworms were cleaned with distilled water thoroughly and then kept on a moist filter paper in a tray for two hours in order to clear their guts (Plate 1)

Table 2. Methods for analysis of major physico chemical properties of soil

Sl. No.	Properties	Method	References
1.	Soil texture	International pipette method	Gupta and Dakshinamoorthi (1980)
2.	Bulk density	Keen-Raczkowski brass cup	Piper (1942)
3.	Particle density	Keen-Raczkowski brass cup	Piper (1942)
4.	Water holding capacity	Keen-Raczkowski brass cup	Piper (1942)
5.	pH	1:2.5 soil water suspension	Jackson (1958)
6.	Organic carbon	Walkley-Black method	Jackson (1958)
7.	Available N	Alkaline permanganate method	Subbiah and Asija (1956)
8.	Available P_2O_5	Ascorbic acid reduced molybdophosphoric blue colour method	Watanabe and Olsen (1965)
9.	Available K_2O	Neutral normal ammonium acetate extract using flame photometer	Jackson (1958)
10.	Available Ca & Mg	Neutral normal ammonium acetate extract using Atomic Absorption Spectrophotometer	Sims and Johnson (1991)
11.	Available micronutrients	HCl- Atomic Absorption Spectrophotometer	Sims and Johnson (1991)

3.1.1.4. Test procedure for avoidance behaviour of earthworm

The procedure described in ISO N 281 (2004) was followed. The experiments were conducted in rectangular plastic container of size 20 x 10 x 10 cm. The containers were divided into two compartments with a plastic split. Holes were provided on the lids of containers using a poker to ensure sufficient aeration. Accurately weighed soil samples of 520 g each were placed in the two compartments and water was added to adjust the moisture content to 80% of water holding capacity. The pesticides were applied to one compartment and the other was kept as control. After application of treatments the split was removed and ten adult earthworms were introduced in the middle of the box. It was left undisturbed for three days. On the fourth day, the split was reintroduced and the number of earthworms in each compartment was counted.

3.1.1.5. Standardization of the moisture content

In order to find out the optimum moisture conditions for the survival of earthworm, standardization of moisture content was carried out with four levels of moisture viz., 50% of water holding capacity (WHC), 60% of WHC, 70% of WHC and 80% of WHC. For this, soil sample (520 g) was passed through a 2mm sieve and then was mixed with specified amount of distilled water i.e. 80 mL, 98 mL, 117 mL and 136 mL respectively so as to attain the above mentioned moisture levels. These soil samples were kept in both compartments of the containers and ten native earthworms of uniform size and age were placed on the centre line after removing the plastic split. The containers were closed and kept undisturbed for three days. On the fourth day, the split was again introduced and earthworm count was taken. The number of dead and live worms was counted separately and percentage mortality calculated.

3.1.1.6. Method of application of pesticides

The experiment were conducted in two ways *viz.*, 1) spraying on the soil surface and 2) mixing the pesticides with the soil

In the first experiment, the pesticides were sprayed on the surface of one compartment using two mL disposable syringe. Spray volume was 0.3mL for each treatment. This volume was calculated based on the surface area of the container taking 500 L ha^{-1} spray volume as the standard.

Table 3. Details of treatments

Sl.No.	Treatments	Formulation concentration used for the study
1	Absolute control	No pesticides
2	Glyphosate 41%SL: 1.2 kg a.i. ha^{-1}	Roundup: 6 mL L^{-1} (0.6%)
3	Glyphosate 41%SL : 2.4 kg a.i. ha^{-1}	Roundup: 12 mL L^{-1} (1.2%)
4	Glyphosate 41%SL : 1.2 kg a.i. ha^{-1}	Glycel: 6 mL L^{-1} (0.6%)
5	Glyphosate 41%SL : 2.4 kg a.i. ha^{-1}	Glycel: 12 mL L^{-1} (1.2%)
6	Chlorpyriphos 20%EC: 400 g a.i. ha^{-1}	Dursban: 4 mL L^{-1} (0.4%)
7	Chlorpyriphos 20%EC: 800 g a.i. ha^{-1}	Dursban: 8 mL L^{-1} (0.8%)

Replication: 3 Design: CRD

Plate 1: Studies on earthworm avoidance behaviour

1. Cleaning earthworm in distilled water

4. Application of the treatments

2. Earthworms placed on a moist filter paper

5. Placing the earthworms on the center line of each box

3. Soil were placed on the two sides of containers

6. The box kept undisturbed for 3 days

The second experiment was conducted by mixing the chemicals with the soil. To ensure uniform mixing, the soil samples (520g each) were taken in polyethene cover; chemicals were added as per treatment (0.3mL each) mixed thoroughly and transferred fully to the one side of the containers. The other side was kept as control, for which 0.3 mL distilled water was mixed with the soil and filled in compartment.

3.1.1.7. Observations

Earthworm count was recorded as detailed in 3.1.1.4. Adult and juvenile earthworms were counted separately. Adult earthworms have well defined clitella.

3.1.2. Pot culture experiment

3.1.2.1. Method of planting the lawn

Lawn grasses (Korean carpet grass: *Zoysia matrella*) were purchased from the nursery and from these circular pieces of 0.9 square foot were taken and planted in each pot. Full coverage of the lawn grass over the soil surface was obtained within a week. (Plate 2)

Moisture condition of 80% WHC was taken as the optimum for the pot experiments also. Irrigating with 500 mL water two times a day was sufficient to maintain the moisture level in the pot.

3.1.2.2. Procedure

About 150 kg of 2mm sieved soil was kept ready for pot culture studies. From this, 7.5 kg soil was added to pots of size 30cm diameter and 25cm depth and mixed with 70 g of dried cow dung. Moisture content was adjusted to 80% of WHC of the soil by adding water as standardized in the laboratory experiment. Fifty native adult earthworms collected from different areas of College and home premises were allowed to acclimatize in each pot. After one week, number of earthworms survived in each pot was counted and their total number was made to fifty by introducing additional worms as required. Then the lawn grass was planted and irrigated twice a day with one litre of water. When lawn grass planted was fully established, treatments were introduced (Table 3). Disposable syringe of two mL capacity was used to spray the chemical. The spray volume for the pot was calculated as five mL based on the surface area, taking 500L ha^{-1} as standard.

Plate 2. Pot culture study

a. Methodology

1. 7 kg of soil taken for each pot

3. Fixing the lawn after one week

2. Cow dung was mixed and introduced 50 earthworms and allowed to acclimatize

4. Filling each pot completely

b. Sample collection

1. Soil sample collection

2. Counting the earthworm

3.1.2.3. Observations

Earthworm population

Live earthworm count was taken from each pot at 30 and 60 days after application of treatments and the data were recorded.

Microbiological studies

During the pot culture studies, enumeration of soil microflora was done at 1 week and one month after imposing of the treatments. About 100g soil samples were taken from three sites in each pot at a depth of 15cm and the pooled sample was used for microbiological studies.

Quantitative estimation of microflora

The quantitative estimation of microflora was carried out by serial dilution plate count method (Johnson and Curl, 1972). The soil sample (10.0g) was added to 90 mL sterile distilled water in 250mL conical flask and shaken for 30min in an orbital shaker. Ten mL of this soil dilution was then transferred to another flask containing 90mL sterile distilled water to get 10^{-2} dilution. Later, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were prepared from this by serial dilution. The composition of media used for estimating microbial population in the soil microorganisms is presented in Appendix 2.

Estimation of fungal population

One mL each from 10^{-2} and 10^{-3} soil dilutions were pipetted into sterile petridishes to which 20mL melted and cooled Martin's rose Bengal streptomycin agar medium was poured. Three petridishes were kept as replications for each sample. The petridishes with the medium were swirled thoroughly to get uniform distribution. After solidification, the petridishes were incubated at room temperature for three days. The fungal colonies developed were counted and expressed as number of colony per gram of dry soil.

Estimation of bacterial population

Bacterial population was estimated using 10^{-5} and 10^{-6} dilutions in Nutrient agar medium. The dishes were incubated for 48 hours at room temperature. The bacterial colonies developed were counted and expressed as number of colony per gram of dry soil.

3.2. Field experiments

3.2.1. Effect of glyphosat application in a cropped area

Layout

The field experiment was laid out in plots of 5 m x 4 m in the field where banana was cultivated earlier. The field was fully covered with weeds at the time of experimentation (Plate 3.)

Treatments

The treatments as detailed in the Table 4 were applied with a hand sprayer. The spray volume was calculated based on the area of the plot and it was one litre, based on 500 L ha^{-1} spray volume as standard.

3.2.2. Effect of application of chlorpyriphos in lawns

Layout

The experiment was laid out in the established lawn of College of Horticulture, Vellanikara. The plot size was 2 m x 2 m and the number of replication was seven (Plate 3).

Treatments

The treatments were applied in the lawn as detailed in the Table 5. The spray volume was 200 mL with 500 L ha^{-1} spray volume as standard.

Collection of soil sample:

From the field where glyphosate was applied, approximately 200 g soil sample was taken at 30 and 60 days after spraying the pesticides. The samples were taken from three randomly located sites of each plot at 0-15 cm depth using a spade. In the case of chlorpyriphos applied lawn fields, soil samples were taken at 0-10 cm depth due to the presence of gravels and stones. The three samples from each plot were pooled, air dried and processed for chemical and biological analysis

Table 4. Details of treatments applied in banana ratoon field

Sl. No.	Treatments
1.	Absolute control (no pesticide)
2.	Glyphosate 41%SL (Roundup): 6 mL L^{-1}
3.	Glyphosate 41%SL (-do-) :12 mL L^{-1}
4.	Glyphosate 41%SL (Glycel) : 6 mL L^{-1}
5.	Glyphosate 41%SL (Glycel) : 12 mL L^{-1}

Design: RBD Replication: 4 Plot size: 5m x4m

3.2.3. Observations

Chemical properties of soil

Major chemical properties of the air dried soil viz., pH, organic carbon and the content of available N, P, K, Ca, Mg and micronutrients at 30 and 60 days after application of treatments were determined. Methods adopted for the analysis are the same as given in the table 1.

Earthworm population

Earthworm population was recorded at 30 and 60 days after application of the treatments.

Soil microflora

Total count of bacteria and fungi in the soil at 30 and 60 days after application of the treatments was estimated by using serial dilution plate count method

Dehydrogenase enzyme activity

Enzyme activity at 30 and 60 days after application of the pesticides was determined by using Triphenyl formazon extraction method (Tabatabai, 1977). One gram air dried soil sample was taken in tight screw capped test tube and 0.2 mL of 3% 2, 3, 5-triphenyl tetrazolium chloride (TTC) and 0.5 mL of 1% glucose solution were added. The test tube was incubated at 28 °C for 24 hours. After incubation 10 mL of methanol was added, shaken vigorously and allowed to stand for 6 hours. The contents were filtered through Whatman no. 42 filter paper and the absorbance of supernatant liquid was read in spectrophotometer at wavelength of 485 nm and expressed the enzyme activity as μg TPF formed per hour per gram soil.

Statistical analysis

From the data obtained in the Earthworm Avoidance Test, net response was calculated for each replicate by using the formula NR=C-T/10*100, where NR is the net response; C is the total number of earthworms in control; T is the total number of earthworms in treated compartment and 10 is the total number of earthworms introduced into the plastic container.

Table 5. Details of treatments applied in lawn

Sl. No:	Treatments
1.	Absolute control (no pesticide)
2.	Chlorpyriphos 20%EC (Dursban) : 4 mL L^{-1}
3.	Chlorpyriphos 20%EC (-do-) : 8 mL L^{-1}

Design: RBD Replication: 7 Plot size: 2m x2 m

Plate 3. Field experiment (General view)

a. Application of glyphosate in a cropped area (banana ratoon field)

1. Field before sparying

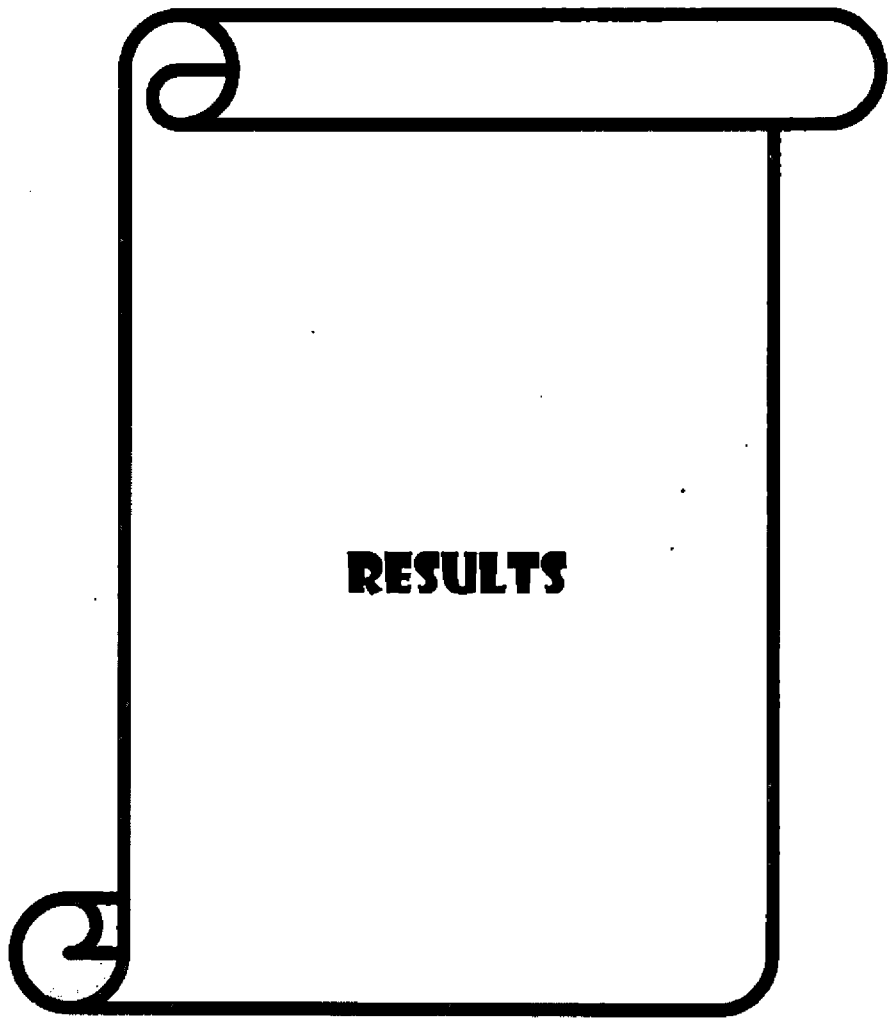
2. Field after one month

b. Application of chlorpyriphos in lawn

3. Field at Olericulture department

4. Field at bioinformatics center

The experimental data under the pot culture and field experiments were statistically analyzed by applying the analysis of Co- variance. Square root transformation was performed wherever necessary.


RESULTS

4. RESULTS

The impact of glyphosate and chlorpyriphos on the non-target organisms in the soil namely, earthworm, bacteria, fungi and major chemical properties of the soil were studied during the year 2013. It was evaluated by conducting a series of experiments in the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Vellanikkara. The results are furnished below.

4.1. Laboratory studies

Laboratory studies were conducted in plastic containers and in pots filled with laterite soil collected from the college premises. These studies were conducted to assess the sensitivity of earthworms and micro flora to two formulations of glyphosate 41% SL (@ 6 and 12 mL L^{-1}) and single formulation of chlorpyriphos 20% EC (@ 4 and 8 mL L^{-1}).

4.1.1. Physico-chemical characteristics of the soil

The major physico-chemical characteristics of the soil used for the laboratory studies are presented in the Table 6.

The soil was sandy clay loam in texture with 48.1 % sand, 23.5 % silt, and 28.40 % clay. The average pH and organic carbon content of the soil samples were 5.2 and 1.39. The soil contained 246.40 kg ha^{-1} available nitrogen; 3.84 kg ha^{-1} available phosphorous; 179.58 kg ha^{-1} available potassium; 375.00 mg kg^{-1} available calcium; 35.50 mg kg^{-1} available magnesium; 36.00 mg kg^{-1} available iron; 1.55 mg kg^{-1} available zinc; 2.60 mg kg^{-1} available copper and 34.05 mg kg^{-1} available manganese.

4.1.2. Studies on avoidance behavior of earthworm

4.1.2.1. Standardization of moisture content

Moisture content is the major factor influencing the activity of earthworm in the soil. In order to standardize the optimum moisture content for the activity of earthworms four levels of moisture i.e. 50% WHC, 60% WHC, 70% WHC and 80% WHC were tried.

Table 6. Physico chemical characteristics of the soil in the experimental field

Soil characteristics	Average content
Sand %	48.10
Silt %	23.50
Clay %	30.40
pH	5.20
Organic carbon %	1.39
Available Nitrogen kg ha^{-1}	246.40
Available Phosphorus kg ha^{-1}	23.84
Available Potassium kg ha^{-1}	336.58
Available Ca mg kg^{-1}	375.00
Available Mg mg kg^{-1}	35.50
Available Fe mg kg^{-1}	36.00
Available Zn mg kg^{-1}	1.55
Available Cu mg kg^{-1}	2.60
Available Mn mg kg^{-1}	34.05

Table 7. Effect of moisture on the earthworm count in the two compartments of the plastic box

Treatments	Number of earthworms			Mortality (%)
	Compartment 1	Compartment 2	Total	
50% WHC	3.7*	5.0*	8.7	13.0
60% WHC	3.0	6.0	9.0	10.0
70% WHC	4.7	4.7	9.4	6.0
80% WHC	5.3	4.7	10.0	0.0

No: of earthworms released box^{-1}: 10

*Mean of three replicates

Results showed that 80% WHC was the optimum for the survival of the earthworm (Table 7). It could be seen that mortality (13%) was maximum at 50% WHC followed by 60% WHC (10%) and 70% WHC (6%).

4.1.2.2. Effect of glyphosate and chlorpyriphos on avoidance behavior of earthworm

Spraying the pesticides on the soil surface

In this experiment the above pesticides were sprayed over the soil surface and the net response was worked out from the number of earthworms in control and treated compartments (Table 8).

In no pesticide treatment (control), both the compartments were having equal number of earthworms and therefore the net response was zero. In case of Roundup 6 mL L^{-1} and Glycel 6 mL L^{-1} treatments, the average earthworm count was 3.7 in treated side and 6.3 in control side indicating a net avoidance response of 26.7%. Maximum avoidance response was noticed in Roundup 12 mL L^{-1} treatment (46.7%) which was followed by Glycel 12 mL L^{-1} and Dursban 8 mL L^{-1}, indicating an avoidance response of 40.0% each. In Dursban 4 mL L^{-1} treatment, the average earthworm count in treated side was 4.0 and in control side 6.0 with avoidance response of 20.0%.

Mixing the pesticides with the soil

Effect of pesticides was studied by mixing the chemical with the soil and the results are given in table 9.

In the control treatment, both the sides were having an equal number of earthworms; therefore the net response was zero. In case of Roundup 6 mL L^{-1}, the average earthworm count was 3.0 in treated side and 7.0 in control side indicating avoidance response of 40%. As in the case of spraying the pesticides maximum avoidance response was obtained in Roundup 12 mL L^{-1} (73.3 %) followed by Glycel 12 mL L^{-1} (70.0%). Both Glycel 6 mL L^{-1} and Dursban 4 mL L^{-1} treatments showed same avoidance response (33.3%). In Dursban 8 mL L^{-1} treatment, the average earthworm count was 1.7 in treated side and 7.7 in control side, with avoidance response of 60.0%.

Table 8. Effect of spraying pesticides on earthworm avoidance behavior

Sl. No.	Treatments	Number of earthworms		Average net response (%)
		Treated compartment	Untreated compartment	
1	Absolute control (Distilled water)	5.0*	5.0*	0.0*
2	Glyphosate 41% SL (Roundup): 6 mL L^{-1}	3.7	6.3	26.7
3	Glyphosate 41% SL (-do-) :12mL L^{-1}	2.7	7.3	46.7
4	Glyphosate 41% SL (Glycel) : 6mL L^{-1}	3.7	6.3	26.7
5	Glyphosate 41% SL (-do-) : 12mL L^{-1}	3.0	7.0	40.0
6	Chlorpyriphos 20% EC (Dursban) : 4mL L^{-1}	4.0	6.0	20.0
7	Chlorpyriphos 20% EC (-do-) : 8mL L^{-1}	2.7	7.7	40.0

No. of earthworms released/plot: 10 *Mean of three replicates

NR=C-T/10 x100, where NR is the Net Response; C =total number of earthworms in control; T = total number of earthworms in treated compartment and 10 = total number of earthworms introduced into the plastic container

Table 9. Effect of pesticides mixing with soil on earthworm avoidance behaviour

Sl. No	Treatments	Number of earthworms		Average net response (%)
		Treated compartment	Untreated compartment	
1	Absolute control (Distilled water)	5.0*	5.0*	0.0*
2	Glyphosate 41%SL (Roundup): 6 mL L^{-1}	3.0	7.0	40.0
3	Glyphosate 41% SL (-do-) :12mL L^{-1}	1.3	8.7	73.3
4	Glyphosate 41% SL (Glycel) : 6mL L^{-1}	3.3	6.7	33.3
5	Glyphosate 41% SL (-do-) : 12mL L^{-1}	1.3	8.3	70.0
6	Chlorpyriphos 20% EC (Dursban) : 4mL L^{-1}	3.0	6.3	33.3
7	Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	1.7	7.7	60.0

No. of earthworms released/plot: 10 *Mean of three replicates

NR=C-T/10 x100, where NR is the Net Response; C =total number of earthworms in control; T = total number of earthworms in treated compartment and 10 = total number of earthworms introduced into the plastic container.

The results showed that all the chemicals were avoided by earthworm and the maximum avoidance was obtained with the application of Roundup 12 mL L^{-1} followed by Glycel 12 mL L^{-1} and Dursban 8 mL L^{-1}. Even though similar trends were obtained for mixing or surface spraying the pesticides, mortality percentage was higher for mixing the pesticides. In case of spraying the chemicals, the avoidance response was in the range of 20.0 to 46.7% whereas for mixing the pesticides with soil, the response was in the range of 33.3 to 73.3%.

4.2. Pot culture study on the survival of earthworm

Pot culture experiment was carried out for detecting the extent of survival of the earthworms exposed to pesticides. In this study, the impact of pesticide application on the earthworm count at 30 and 60 days after application of the pesticides was evaluated. Total microbial population (bacteria as well as fungi) in the soil was also assessed at one week and 30 DAS after application of the pesticides and presented in the Table 10.

4.2.1. Effect of treatments on earthworm count

At 30 days after application of treatments, the earthworm counts in the control (24.0/pot), Roundup 6 mL L^{-1} (24.3/pot) were on par. Glycel 6 mL L^{-1} (29.7/pot) and Dursban 4mL L^{-1} (28.7/pot) were also on par. Glycel 12 mL L^{-1} recorded significantly higher count of earthworms (38.7/pot) than the control. Higher concentrations of Roundup (15.3/pot) and Dursban (10.0/pot) gave comparable counts. It could be noticed that the recommended rates of application of all the three chemicals registered count of earthworms on par with that of control. Roundup and Glycel 12mL L^{-1} also did not cause any harmful effect on earthworms (Table 10).

At 60 DAS, no significant difference was observed between the treatments. Here also Glycel 12 mL L^{-1} recorded the highest count (41.7/ pot) followed by Glycel 6 mL L^{-1}. The lowest value was recorded in the treatment sprayed with Roundup 12 mL L^{-1} (Table 10).

Table 10. Effect of glyphosate and chlorpyriphos on immature and adult stages of earthworms

Treatments	Adult earthworm count		No. of cocoons		No. of young ones	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Absolute control (Distilled water)	24.0* (6.96)	15.7 (5.63)	18.3 (5.90)	(5.52) 15.3	4.7 (3.03)	(2.21) 3.0
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	24.3 (7.00)	18.0 (5.98)	14.7 (5.13)	(6.12) 19.3	6.7 (3.67)	(2.47) 3.0
Glyphosate 41%SL (-do-) :12mL L^{-1}	15.3 (5.49)	13.3 (5.09)	17.0 (5.68)	(6.88) 24.7	10.0 (4.38)	(4.66) 11.3
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	29.7 (7.72)	26.7 (7.33)	38.7 (8.63)	(8.06) 32.3	7.7 (3.97)	(3.41) 5.7
Glyphosate 41%SL (-do-) : 12mL L^{-1}	38.7 (8.81)	41.7 (9.15)	4.0 (2.56)	(4.01) 10.7	6.7 (3.63)	(4.54) 10.3
Chlorpyriphos 20%E (Dursban) : 4mL L^{-1}	28.7 (7.39)	26.0 (6.08)	5.3 (2.94)	(2.12) 4.0	3.3 (1.98)	(2.83) 5.0
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	10.0 (4.15)	14.0 (5.01)	7.3 (3.87)	(3.13) 4.7	19.0 (5.77)	(6.65) 28.3
CD (0.05)	1.73	NS	3.49	3.25	NS	NS
CV (%)	19.25	33.74	37.46	35.04	41.55	53.75

*Original values

Values in parentheses indicate transformed values [Percentage square root transformation]

4.2.2. Effect of treatments on count of earthworm cocoons

At 30 DAS, though significant difference was observed between treatments, they were on par with control. Glycel 6 mL L^{-1} (38.7/pot) gave significantly higher count than that of Glycel 12 mL L^{-1} (4.0/pot) and Dursban 4mL L^{-1} (5.3/pot) and 8 mL L^{-1} (7.3/pot) (Table 10).

Similar trends were observed at 60 daays after spraying. However Dursban 4 mL L^{-1} gave significantly lower counts (4.0/pot) (Table 10).

4.2.3. Effect of treatments on count of juvenile earthworm

No significant differences between the treatments were obtained in case of young ones at 30 and 60 days after application of treatments (Table 10).

The study revealed that neither glyphosate nor chlorpyriphos had any deleterious effects on the multiplication of earthworm.

4.2.2. Effect of treatments on soil microflora

4.2.2.1. Effect on soil fungi

At one week after application of pesticides, the total count of soil fungi was highest in the control treatment (64.00 x 10^2 cfu g^{-1} soil) followed by Glycel 6 mL L^{-1} and Roundup 6 mL L^{-1} (57.53 and 54.10 x 10^2 cfu g^{-1} soil respectively). These three treatments did not differ significantly. Roundup 6 mL L^{-1}, Glycel 6 mL L^{-1} and Dursban 4 mL L^{-1} (49.27 x 10^2 cfu g^{-1} soil) treatments were on par. Likewise, the treatments Glycel 12 mL L^{-1} (40.80 x 10^2 cfu g^{-1} soil) and Dursban 8 mL L^{-1} (36.13 x 10^2 cfu g^{-1}soil) were also on par. Roundup 12 mL L^{-1} recorded the lowest count of 18.87 x 10^2 cfu g^{-1} soil (Table 11).

At 30 days after application of treatments, the total count of soil fungi was highest in control (74.20 x 10^2 cfu g^{-1}soil) and was on par with Roundup and Glycel 6 mL L^{-1} (61.77 and 69.87 x 10^2 cfu g^{-1} soil respectively). Lowest count was registered by Roundup 12mL L^{-1} (27.53 x 10^2 cfu g^{-1}soil). Roundup 6 mL L^{-1} and Dursban 4 mL L^{-1} (61.77 and 57.97 x 10^2 cfu g^{-1} soil respectively) treatments gave similar counts.

Table 11. Effect of glyphosate and chlorpyriphos on soil microflora in pot culture studies

Treatments	Fungi ($x10^2$cfu g^{-1} soil)		Bacteria ($x10^5$cfu g^{-1} soil)	
	7 DAS	30 DAS	7 DAS	30 DAS
Absolute control (Distilled water)	64.00	74.20	61.43	75.37
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	54.10	61.77	38.90	33.00
Glyphosate 41%SL (-do-) : 12mL L^{-1}	18.87	27.53	17.43	17.90
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	57.53	69.87	47.20	42.57
Glyphosate 41%SL (-do-) : 12mL L^{-1}	40.80	52.93	34.10	32.10
Chlorpyriphos 20%EC (Dursban) : 4mL L^{-1}	49.27	57.97	52.67	56.57
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	36.13	48.33	43.33	49.57
CD (0.05)	9.79	14.28	13.22	13.35
CV (%)	6.79	6.70	8.58	8.28

The treatments viz., Glycel 12 mL L^{-1} (52.93 x 10^2 cfu g^{-1} soil) and Dursban 8 mL L^{-1} (48.33 x 10^2 cfu g^{-1}soil) recorded significantly lower count than control (Table 11).The results showed that higher concentration of glyphosate formulation and both the concentration of chlorpyriphos significantly affected the fungal population

4.2.2.2. Effect on soil bacteria

At one week after application of treatments, the highest bacterial population was recorded in control (no pesticide) with a total count of 61.43 x 10^5 cfu g^{-1} of soil and lowest in Roundup 12 mL L^{-1} treatment (17.43 x 10^5 cfu g^{-1} of soil). Glycel 6mL L-1 (47.20 x 10^5 cfu g^{-1} of soil) and Dursban 4 mL L^{-1} (52.67 x 10^5 cfu g^{-1} of soil) treatments were on par. Dursban 8mL L^{-1} (43.33 x 10^5 cfu g^{-1} of soil), Roundup 6 mL L^{-1} (38.89 x 10^5 cfu g^{-1} of soil) and Glycel 6 mL L^{-1} and 12 mL L^{-1} (47.2 and 34.10 x 10^5 cfu g^{-1} of soil respectively) did not differ significantly.

At 30 DAS, the total count of bacteria was found to be the highest at control (75.37 x 10^5 g^{-1} of soil) and the lowest in Roundup 12 mL L^{-1} (17.90 x $10^5 g^{-1}$ of soil). The two levels of Roundup were significantly different from each other whereas the two concentration of Glycel were on par. In the case of Dursban also, the two doses were on par with regard to the total bacterial population. All the chemicals inhibited the bacterial population considerably at one week as well as 30 days after spraying at both the levels tried.

In this study it was found that both soil fungi and bacteria were affected by application of glyphosate and chlorpyriphos. Roundup @ 12 mL L^{-1} had more deleterious effect on soil fungi as well as bacteria followed by Dursban @ 8 mL L^{-1} in case of soil fungi and Glycel @ 12 mL L^{-1} in case of soil bacteria.

4.3. Field studies

Field studies were conducted in the banana ratoon plot and established lawns for estimating the effect of glyphosate and chlorpyriphos application on the earthworm, soil micro flora and chemical properties of the soil.

4.3.1. Effect of treatments on biological properties of the soil

Biological parameters namely population of earthworm, soil fungi and bacteria and dehydrogenase activity, were assessed in the soil samples collected from the two field experiments.

4.3.1.1. Earthworm population

Observations on earthworm counts in the control and treated plots were taken at 30 and 60 days after spraying. No earthworms were observed in both the experiments at a depth of 0-20 cm.

4.3.1.2. Effect of glyphosate on soil microflora

The effect of glyphosate on total soil micro flora was studied and presented in the table 12.

4.3.1.2.1. Effect on soil fungi

At 30 days after application of pesticides, highest population was observed in case of control plot (77.88 x 10^3 cfu g^{-1} soil) and was on par with Glycel 6 mL L^{-1} (67.00 x 10^3 cfu g^{-1} soil). Significant reduction was found in treatments sprayed with Roundup 6 mL L^{-1} (46.88 x 10^3 cfu g^{-1} soil) and Roundup 12 mL L^{-1} (46.75 x 10^3 cfu g^{-1} soil) as well as Glycel 12 mL L^{-1} (37.25 x 10^3 cfu g^{-1} soil) and these three treatments were on par (Table 12).

No significant differences were observed at 60 days after application of the treatments. The population of soil fungi in soil ranged from 52.00 to 78.38 x 10^3 cfu g^{-1} (Table 12).

4.3.1.2.2. Effect on soil bacteria

No significant reduction was found in case of total bacterial population at 30 and 60 days after application of the treatments. The total count of soil bacteria at 30 and 60 DAS month ranged from 14.75 to 19.88 x 10^6 cfu g^{-1} soil and 27.88 to 29.25 x 10^6 cfu g^{-1} soil respectively (Table 12).

Table 12. Effect of glyphosate on soil microflora

Treatments	Fungi ($x10^3$cfu g^{-1} soil)		Bacteria ($x10^6$cfu g^{-1} soil)	
	30 DAS	60 DAS	30 DAS	60 DAS
Absolute control (Distilled water)	77.88	78.38	17.50	29.25
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	46.88	66.38	17.63	27.88
Glyphosate 41%SL (-do-) :12mL L^{-1}	46.75	52.00	14.75	27.88
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	67.00	79.25	18.38	28.38
Glyphosate 41%SL (-do-) : 12mL L^{-1}	37.25	62.63	19.88	28.63
CD (0.05)	18.18	NS	NS	NS
CV (%)	21.43	14.89	23.32	13.13

4.3.1.3. Effect on dehydrogenase enzyme activity

Dehydrogenase activity in the soil was not significantly affected by the application of pesticides. The enzyme activity ranged from 0.08 to 0.22 µg TPF g^{-1} hr^{-1} at 30 DAS and 0.12 to 0.26 µg TPF g^{-1} hr^{-1} at 60 days after application of pesticides (Table 13).

The biological studies showed that application of glyphosate caused short term inhibitory effect on soil fungus up to 30 days after spraying only. Soil bacteria count and dehydrogenase enzyme activity was unaffected by glyphosate application.

4.3.1.3. Effect of chlorpyriphos on soil microflora

Population of microflora at 30 and 60 days after application of chlorpyriphos at different concentration was estimated and given in table 14.

4.3.1.3.1. Effect on soil fungi

No significant effects on soil fungi were observed in case of fungi at 30 and 60 days after application of treatments. The fungal count ranged from 23.93 to 29.86 x 10^2 cfu g^{-1} soil and 24.57 to 31.71 x 10^2 cfu g^{-1} soil at 30 and 60 days after application of pesticides (Table 14).

4.3.1.3.2. Effect on soil bacteria

Significant reduction in the soil bacteria was observed in the treatments at 30 days after application of the chemical. The highest count of 27.00 x 10^5 cfu gm^{-1} soil was found in control followed by Dursban 4 mL L^{-1} (24.64 x 10^5 cfu g^{-1} soil). The lowest count of 20.86 x 10^5 cfu g^{-1} soil was registered by Dursban 8 mL L^{-1} which was significantly different from control (Table 14).

No significant differences were observed between the treatments at 60 days after application of treatments. The total bacterial count ranged from 21.71 to 28.41 x 10^5 cfu g^{-1}.

Table 13. Effect of glyphosate on soil dehydrogenase activity

Treatments	Dehydrogensae activity (µg TPF g^{-1} soil hr^{-1})	
	30 DAS	**60 DAS**
Absolute control (Distilled water)	0.130* (3.22)	0.247 (5.01)
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	0.107 (3.26)	0.260 (5.04)
Glyphosate 41%SL (-do-) :12mL L^{-1}	0.082 (2.79)	0.120 (3.52)
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	0.170 (4.13)	0.138 (3.75)
Glyphosate 41%SL (-do-) : 12mL L^{-1}	0.215 (4.62)	0.148 (3.90)
CD (0.05)	NS	NS
CV (%)	37.16	16.69

*Original value

Values in parentheses indicate transformed values [R sine transformation]

Table 14. Effect of chlorpyriphos on soil micro flora

Treatments	Fungi ($x10^2$cfu g^{-1}soil)		Bacteria ($x10^5$cfu g^{-1} soil)	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control (Distilled water)	29.86	31.71	27.00	28.41
Chlorpyriphos 20% EC (Dursban): 4mL L^{-1}	23.93	30.14	24.64	25.5
Chlorpyriphos 20% EC (-do-): 8mL L^{-1}	24.93	24.57	20.86	21.71
CD (0.05)	NS	NS	4.58	NS
CV (%)	16.14	27.19	16.26	19.68

Table 15. Effect of chlorpyriphos on soil dehydrogenase activity

Treatments	Dehydrogensae activity (µg TPF g^{-1} soil hr^{-1})	
	30 DAS	**60 DAS**
Absolute control (Distilled water)	0.274 *(4.82)	0.116 (3.44)
Chlorpyriphos 20% EC (Dursban) : 4mL L^{-1}	0.197 (3.92)	0.209 (4.22)
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	0.313 (4.55)	0.611 (6.16)
CD (0.05)	NS	NS
CV (%)	55.20	75.72

*Original value

Values in parentheses indicate transformed values [R sine transformation]

4.3.1.4. Effect on dehydrogenase enzyme activity

No significant effects were found in case of dehydrogenase activity at 30 and 60 days after application of treatments. Dehydrogenase activity in the treatments ranged from 0.20 to 0.31 and 0.12 to 0.61 µg TPF g^{-1} hr^{-1} at 30 and 60 days after spraying respectively.

It could be concluded that application of chlorpyriphos caused only a short term inhibitory effect on soil bacteria (at 30 DAS). No effects were observed on soil fungi and dehydrogenase activity.

4.3.2. Effect of treatments on chemical characteristics of the soil

4.3.2.1. Effect of glyphosate on soil chemical properties

Effect of glyphosate on the chemical characteristics of the soil was studied in the banana field by analyzing soil sample for pH, organic carbon and available nutrients at 30 and 60 days after spraying.

4.3.2.1.1. pH and organic carbon

No significant differences were observed in pH and organic carbon content at 30 and 60 days after application of treatments (Table 16). The pH ranged from 4.7 to 4.9 and 4.6 to 4.7 at 30 and 60 days after spraying respectively. Organic carbon content ranged from 0.90 to 0.99 % and 0.79 to 0.92 % at 30 and 60 DAS.

4.3.2.1.2. Available N, P and K

In case of available nitrogen and phosphorus, no significant differences were observed between the treatments. The available nitrogen content ranged from 347.20 to 397.60 kg ha^{-1} (30 DAS) and 354.38 to 388.88 kg ha^{-1} (60 DAS), whereas available phosphorus ranged from 16.24 to 23.80 kg ha^{-1} at 30 DAS and 16.80 to 19.88 kg ha^{-1} at 60 DAS (Table 17).

The available potassium content of the plot ranged from 307.08 to 329.83 kg ha^{-1} at 30 DAS and 302.80 to 327.32 kg ha^{-1} at 60 DAS. No significant differences were found in available K between the treatments at 30 as well as 60 days after application of glyphosate.

Table 16. Effect of glyphosate on pH and organic carbon of the soil

Treatments	pH		Organic carbon (%)	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control (Distilled water)	4.70	4.68	0.91	0.79
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	4.78	4.70	0.97	0.84
Glyphosate 41%SL (-do-) :12mL L^{-1}	4.65	4.70	1.02	0.86
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	4.88	4.65	0.91	0.85
Glyphosate 41%SL (-do-) : 12mL L^{-1}	4.70	4.63	1.02	0.92
CD (0.05)	NS	NS	NS	NS
CV (%)	3.99	2.30	5.38	8.94

Table 17. Effect of glyphosate on available N, P and K content of the soil

Treatments	Nitrogen (kg ha^{-1})		Phosphorus (kg ha^{-1})		Potassium (kg ha^{-1})	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control (Distilled water)	347.20	354.38	16.24	18.76	329.83	327.32
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	375.20	373.20	18.76	19.60	320.00	313.68
Glyphosate 41%SL (-do-) :12 mL L^{-1}	369.60	388.88	23.80	19.88	320.70	302.80
Glyphosate 41%SL (Glycel): 6 mL L^{-1}	397.60	354.38	23.24	16.80	307.08	308.53
Glyphosate 41%SL (-do-): 12 mL L^{-1}	386.40	357.50	21.84	16.80	314.10	319.26
CD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	8.94	15.54	36.03	42.96	15.09	18.75

4.3.2.1.3. Available calcium and magnesium of the soil

Significant differences were not observed in the calcium content in the banana field at 30 and 60 days after application of treatments. The calcium content ranged from 257.00 to 267.5 mg kg^{-1} at 30 DAS and 242.50 to 262.50 at 60 DAS (Table 18).

No significant differences were observed in case of available magnesium content at 30 and 60 DAS. Available magnesium content of the soil ranged from 124.13 to 140.63 mg kg^{-1} and 109.38 to 131.88 mg kg^{-1} at 30 and 60 days after spraying (Table 18).

4.3.2.1.4. Available micronutrients of the soil

Available Fe, Mn, Zn and Cu contents were estimated in the soil samples taken at 30 and 60 days after application of Roundup and Glycel. No significant differences were observed between the treatments. The available iron content ranged from 5.43 to 13.60 mg kg^{-1} at 30 DAS and 6.92 to 10.96 mg kg^{-1} at 60 DAS, available zinc content from 0.59 to 0.71 mg kg^{-1} at 30 DAS and 0.61 to 0.81 mg kg^{-1} at 60 DAS, available copper (2.68 to 3.50 mg kg^{-1}) at 30 DAS and (2.81 to 3.69 mg kg^{-1}) at 60 DAS and available manganese(15.46 to 26.32 mg kg^{-1}) at 30 DAS and (12.18 to 12.95 mg kg^{-1}) at 60 DAS (Table 19) .

4.3.2.2. Effect of chlorpyriphos on chemical properties of soil

Effect of chlorpyriphos on the soil chemical properties was studied in the lawns. Changes in the chemical properties *viz.*, pH, organic carbon and available nutrients were studied.

4.3.2.2.1. pH and Organic carbon

No significant differences were observed between the treatments in the case of pH and organic carbon content of the soil sample taken at one and two month after application of chlorpyriphos. The pH ranged from 6.5 to 6.6 (over a period of 60 DAS) and organic carbon from 1.19 to 1.27 % at 30 DAS and 0.86 to 1.09 % at 60 days after application of treatments (Table 20).

Table 18. Effect of glyphosate on available calcium and magnesium content of the soil

Treatments	Calcium (mg kg^{-1})		Magnesium (mg kg^{-1})	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control (Distilled water)	267.50	262.50	124.13	109.38
Glyphosate 41%SL (Roundup) : 6 mL L^{-1}	261.00	242.50	137.50	118.75
Glyphosate 41%SL (-do-) : 12mL L^{-1}	257.00	245.00	124.38	118.75
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	263.80	260.00	140.63	131.88
Glyphosate 41%SL (-do-) : 12mL L^{-1}	258.00	253.80	133.13	116.88
CD (0.05)	NS	NS	NS	NS
CV (%)	19.35	6.71	8.89	12.11

Table 19. Effect of glyphosate on available micronutrients

Treatments	Iron (mg kg^{-1})		Zinc (mg kg^{-1})		Copper (mg kg^{-1})		Manganese(mg kg^{-1})	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Absolute control (Distilled water)	6.82	9.47	0.71	0.77	2.68	3.36	21.86	12.53
Glyphosate 41% SL (Roundup) : 6 mL L^{-1}	5.88	10.96	0.68	0.77	3.09	2.84	22.02	12.95
Glyphosate 41% SL (-do-) : 12mL L^{-1}	5.43	9.12	0.76	0.81	3.18	3.69	26.32	12.20
Glyphosate 41% SL (Glycel) : 6mL L^{-1}	13.60	10.80	0.59	0.68	2.79	3.14	15.46	12.18
Glyphosate 41% SL (-do-) : 12mL L^{-1}	7.79	6.92	0.68	0.61	3.50	2.81	23.60	12.30
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	81.63	28.47	16.83	27.16	17.18	23.76	38.95	15.44

Table 20. Effect of chlorpyriphos on pH and organic carbon of the soil

Treatments	pH		Organic carbon (%)	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control	6.47	6.46	1.27	0.87
Chlorpyriphos 20% EC (Dursban) : 4mL L^{-1}	6.57	6.49	1.26	0.86
Chlorpyriphos 20% EC (-do-) : 8mL L^{-1}	6.53	6.50	1.19	1.09
CD (0.05)	NS	NS	NS	NS
CV (%)	1.76	1.92	11.98	16.20

4.3.2.2.2. Available N, P and K

In case of available nitrogen, phosphorus and potassium, no significant differences were observed between the treatments at 30 and 60 days after application of treatments. Available nitrogen content ranged from 141.57 to 166.66 kg ha^{-1} at 30 DAS and 145.96 to 152.69 kg ha^{-1} at 60 DAS. Available phosphorous content of the soil significantly did not differ between the treatments at 30 and 60 DAS after spraying and it ranged from 9.11 to 11.21 kg ha^{-1} and 8.36 to 9.57 kg ha^{-1} at 30 and 60 DAS. In case of available potassium content, it ranged from 469.14 to 479.06 kg ha^{-1} at 30 DAS and 396.96 to 444.97 kg ha^{-1} at 60 DAS (Table 21).

4.3.2.2.3. Available calcium and magnesium

No significant differences were observed in the calcium content of the soil samples in the various treatments at 30 and 60 days after application of treatments. The calcium content ranged from 169.97 to 183.79 mg kg^{-1}at 30 DAS and 186.20 - 215.51 mg kg^{-1} at 60 DAS (Table 22).

No significant differences were observed in case of available magnesium content of the soil sample taken at one and two months after application of treatments and their values ranged from 35.06 to 35.60 mg kg^{-1} (30 DAS) and 32.96 to 33.94 mg kg^{-1}(60 DAS) respectively.

4.3.2.2.4. Available micronutrients

In case of available Fe, Mn, Zn and Cu, significantly difference was not observed at 30 as well as 60 DAS. The available iron content ranged from 19.63 to 22.23 mg kg^{-1} (30 DAS) and 24.81 to 29.91 mg kg^{-1} (60 DAS), available zinc content 1.33 to 1.66 mg kg^{-1} (30DAS) and 1.14 to 1.50 (60 DAS), available copper 2.54 to 3.56 mg kg^{-1} (30 DAS) and 2.13 to 3.04 mg kg^{-1} (60 DAS) and available manganese 33.36 to 37.29 mg kg^{-1} (30 DAS) and 27.20 to 41.76 mg kg^{-1} (60 DAS) after application of chlorpyriphos (Table 23).

Table 21. Effect of chlorpyriphos on available N, P and K of the soil

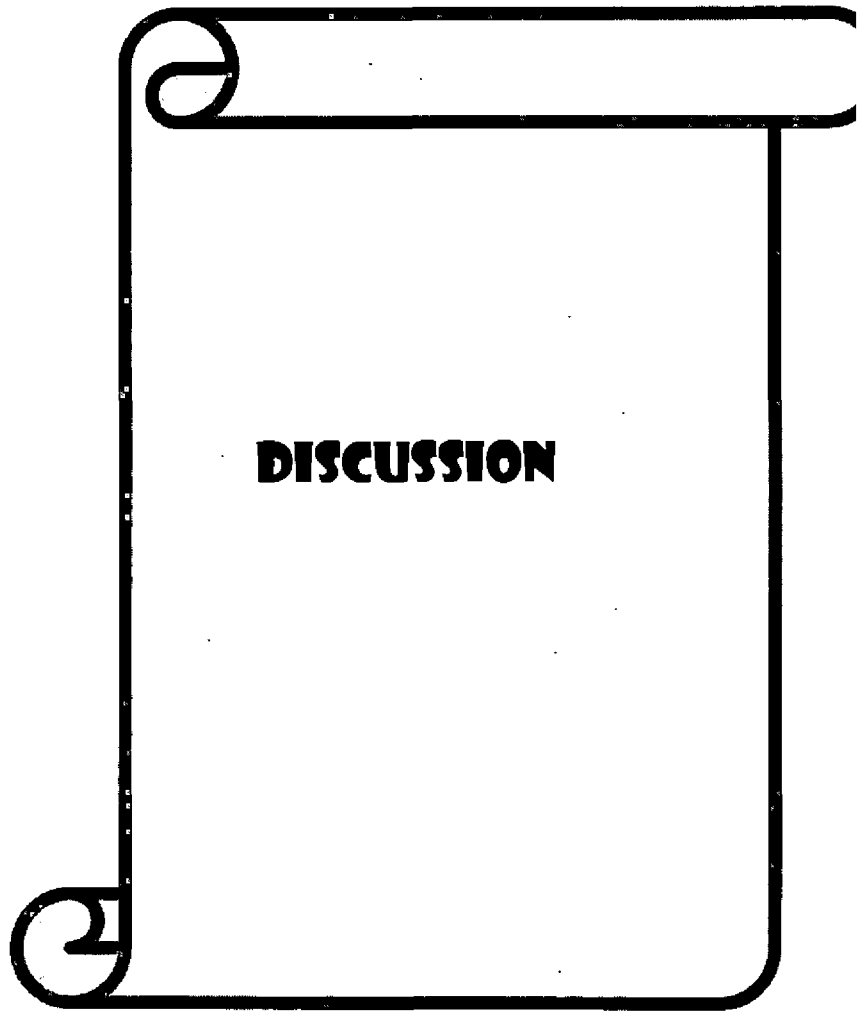
Treatments	Nitrogen (kg ha^{-1})		Phosphorus (kg ha^{-1})		Potassium (kg ha^{-1})	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control (Distilled water)	166.66	145.96	9.129	8.46	479.06	396.96
Chlorpyriphos 20%EC(Dursban) : 4mL L^{-1}	141.57	148.46	9.114	8.36	469.14	444.97
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	154.11	152.69	11.214	9.57	469.76	431.84
CD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	14.48	6.86	29.10	12.48	8.43	12.57

Table 22. Effect of chlorpyriphos on available calcium and magnesium of the soil

Treatments	Calcium (mg kg^{-1})		Magnesium (mg kg^{-1})	
	30 DAS	**60 DAS**	**30 DAS**	**60 DAS**
Absolute control (Distilled water)	183.79	186.20	35.60	33.94
Chlorpyriphos 20% EC (Dursban) : 4mL L^{-1}	169.97	215.51	35.06	33.91
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	174.24	189.06	35.20	32.96
CD (0.05)	NS	26.499	NS	NS
CV (%)	8.84	11.55	2.43	3.50

Table 23. Effect of chlorpyriphos on available micronutrients

Treatments	Iron (mg kg^{-1})		Zinc (mg kg^{-1})		Copper (mg kg^{-1})		Manganese (mg kg^{-1})	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Absolute control (Distilled water)	20.71	24.81	1.33	1.50	2.54	3.00	33.36	41.76
Chlorpyriphos 20%EC (Dursban) : 4mL L^{-1}	22.23	25.31	1.66	1.20	3.56	3.04	34.61	30.63
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	19.63	29.91	1.47	1.14	2.89	2.13	37.29	27.20
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	24.81	18.92	41.58	17.91	64.85	33.28	37.92	24.00


DISCUSSION

5. DISCUSSION

The results of the studies conducted on "Impact of glyphosate and chlorpyriphos on the biological and chemical properties of lateritic soil" conducted at the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Vellanikkara are discussed in this section.

5.1 Laboratory studies

The laboratory studies were conducted to assess the sensitivity of earthworm and microflora to the applied chemicals namely glyphosate and chlorpyriphos.

The soils used for the laboratory experiments were coarse textured, acidic in reaction (pH 5.0-5.4) with medium fertility status. Therefore the soil conditions were feasible for survival of earthworms. Garcia *et al.* (2008) reported that heavy textured soil and pH less than 4.0 were not suitable for conducting avoidance test. Guild (1948) also reported that soils with coarse texture would have a better survival condition than those with sandy and clayey texture. Satachell (1967) reported that soil pH had vital effect on the earthworm life. Earthworms were mostly present and active between the pH ranges of 5.0-7.4 but their growth was limited at pH range of 3.5-4.5 and completely ceased below pH 3.5.

5.1.1. Standardization of moisture content

Earthworms prefer moist and well aerated soil. Around 85 % of the fresh weight of earthworms is water; a considerable part is being in the coelomic fluid and blood; so they must be able to prevent excessive water losses in order to survive (Ismail and Murthy, 1985). If soils are dry, earthworms may move to deeper soil layers, die, or revert to a hibernation condition called diapause. Earthworms in diapause are tied up in a knot in a little hole that is lined with a slimy substance to avoid moisture loss. Earthworms can live under submerged conditions if the oxygen content of the water is high enough. In most cases, however, earthworms will die when exposed to excessive water logging. They move to the surface when the soil is saturated, to avoid suffocation. So maintenance of optimum water level is important.

Not all species have the same moisture requirements and within a species, the moisture requirements for earthworm population from different regions of the world can be quite different. The earthworm *Aporrectodea caliginosa* goes into diapause at a soil moisture content below 25.30% and did not survive well below 20% soil moisture (Baltzer, 1956; Zicsi, 1958), whereas in *Aporrectodea caliginosa* and *Aporrectodea rosea* are active in soils with a moisture content as low as 15% (Ljungstrom and Emiliani, 1971; Ljungstrom *et al.*, 1973). Madge (1969) placed earthworms of the species *Hyperiodrilus africanus* in moisture gradients, and reported that they prefer soil between 12.5 and 17.2% moisture content. Soil with a moisture content of about 23.3% appears to be optimal for them to produce casts. The earthworms that live in compost or dung heaps like *Perionyx excauatus* prefer a moisture content of about 80% in cattle manure (Hallet *et al.*, 1992) and 80-85% in other organic materials (Edwards, 1988). In the present study, the lower moisture levels (<80%) resulted in mortality of the earthworms.

5.1.2. Effect of treatments on avoidance behaviour of earthworm

The result showed that the earthworm avoidance test is sufficiently sensitive to assess the risk associated with use of pesticides in the soil. It has been reported by Casabe *et al.* (2007) that avoidance test was sensitive indicator of glyphosate exposure but not so for chlorpyriphos. However Garcia *et al.* (2008) found that the use of earthworm avoidance test could be recommended as a screening tool for the risk assessment of the pesticides. In the present study also, greater response was obtained for glyphosate, especially for Roundup formulation (Fig 1).

Different earthworm species often have different sensitivities to the same pesticide (De Silva *et al.*, 2009).The earthworms avoided the soil treated with all the pesticides used in the study. Similar results were obtained by Buch *et al.* (2013). In their study, that earthworms *Pontoscolex corethrurus* and *Eisenia andrei* exhibited avoidance behavior against glyphosate at the doses 47 mg a.i. kg^{-1} and 30 mg a.i. kg^{-1}. Zhou *et al.* (2007) also found that earthworms avoided the soil treated with chlorpyriphos at 40 mg kg^{-1}. From these, it is clear that both glyphosate and chlorpyriphos were avoided by the earthworms. They have chemoreceptors and sensory tubules which are capable of detecting the chemicals. (Edwards and Bohlen, 1996)

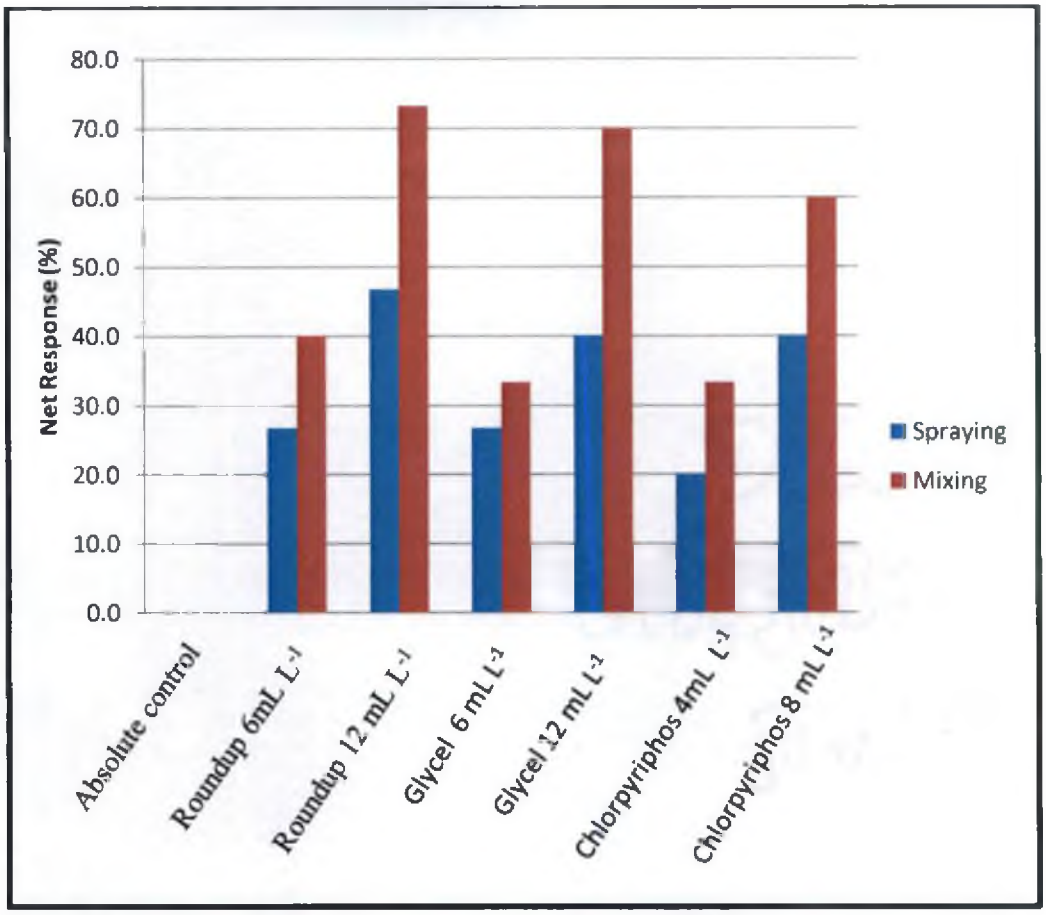


Fig1. Avoidance behaviour of earthworm in the laboratory study

Earthworm avoidance test done by two methods of application (mixing as well as spraying) showed similar trends. However, higher net response was obtained by mixing the pesticides in soil. Spraying on the surface imitated the field situation, in which the avoidance response ranged from 20.0 to 46.7 %, while in the case of mixing the pesticides with the soil, the avoidance response ranged from 33.3 to 73.3 %. When the soil was mixed with the chemicals, the earthworms had direct contact with the pesticides throughout the treated soil section and thus they had shown greater tendency to avoid the area. More dead or missing earthworms were also recorded in case of mixing the chemicals with soil than in spraying on the soil surface.

5.2. Pot culture studies

5.2.1. Study on survival of earthworms

In the pot culture study, the chemicals showed no significant effect on the earthworm density and reproduction at two months after spraying (Fig. 2 and 3). Though significant differences were observed between treatments, cocoon production in all the treatments was on par with control. The result suggested that glyphosate and chlorpyriphos did not influence the growth and reproduction of the earthworm, when they were applied at the rate of 1-2 kg and 400-800g respectively. Herbicides such as glyphosate as well as insecticides of organophosphate group including chlorpyriphos, isofenphos and trichlorpfon were considered non toxic to earthworms when applied at normal dose rates (Wang *et al.*, 2012). However, detrimental effects of chlorpyriphos had been reported by various researchers. Booth *et al.* (2000) observed a reduction in growth rate of earthworms by application of chlorpyriphos. Zhou *et al.* (2007) found that chlorpyriphos was toxic to earthworms at 5 mg kg^{-1} exposed for eight weeks.

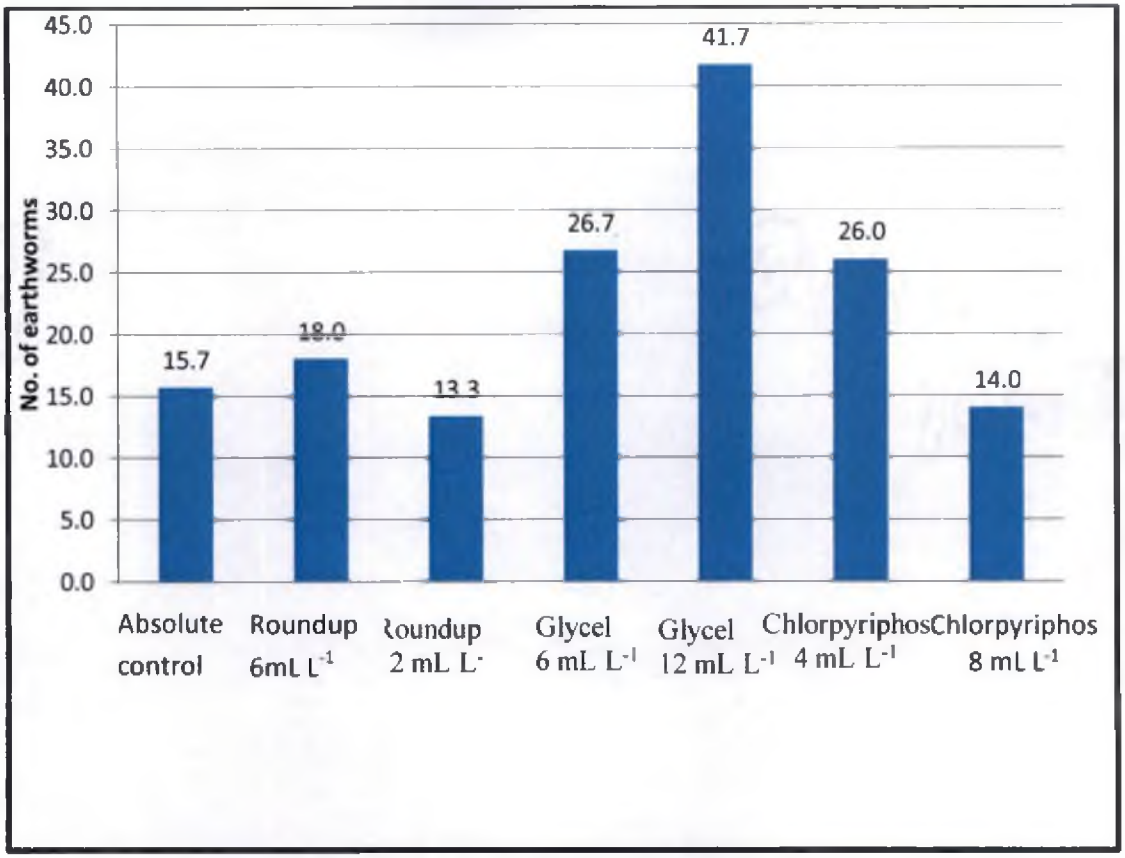


Fig 3. Effect of pesticides on adult earthworm count at 60 DAS

Chlorpyriphos application to soil reduced earthworm and termite abundance and organic matter breakdown even at the recommended dose of 0.6 kg a.i. ha^{-1} (De Silva, 2009).

Water is the most limiting factor for earthworm survival (Lee, 1985). Earthworms need appropriate water levels and have no anatomical adaptations to cope with water loss. Most of the water they acquire is consumed with their food although they do drink water stored in soil pores. They also respire through their skin, which must be kept moist, and acquire oxygen from the air or from oxygenated water in the soil. Suitable range of soil moisture is species-specific, but in general, they will survive in soils near field capacity (Lee, 1985).

According to Paoletti (1999) and Curry *et al.* (2002), earthworm populations in cultivated land are generally lower than those found in undisturbed habitats. Agricultural activities such as ploughing, several tillage operations, fertilizing and application of chemical pesticides have detrimental effect on invertebrate animals. Any management practice applied to soils is likely to have some (positive or negative) effects on earthworm abundance and diversity; these effects are primarily the result of changes in soil temperature, soil moisture and organic matter quantity or quality (Hendrix and Edwards, 2004).

Climate in tropical temperature regimes is mainly warm and more uniform (yearly average). It has been well identified that temperature is a key element that affects toxicity of chemicals and knowledge on temperature-effect relationships is essential for a proper risk assessment of pesticides used in tropical regions. Therefore, comparative studies of temperature effects on pesticide toxicity are to be done. In tropical environments, abiotic factors like higher temperature and moisture could play a more important role in decomposition rates than in temperate climates. (De Silva *et al.*, 2010)

Soil type may also affect toxicity of the pesticides, which could be linked with sorption. Sorption is a key process that governs bioavailability of chemicals and largely depends on soil organic matter content (Spark and Swift, 2002; Coquet, 2003).

To cope up with adverse conditions in their environment, earthworms exhibit several behaviours that increase their survival rates. When the soil surface becomes too dry or cold, they migrate to deeper layers. If avoiding the situation is not possible, they may become inactive until conditions improve. In response to low soil temperatures and periods of

drought, earthworms will enter a state of hibernation referred to as aestivation (Edwards and Bohlen, 1996; Lee, 1985). Finally, because egg cocoons can tolerate desiccation and extreme temperatures better than the worms, cocoons are deposited at the onset of adverse conditions, remaining in the soil to hatch when conditions improve, thereby increasing reproductive capabilities (Edwards and Bohlen, 1996).

Earthworms are sensitive to certain chemicals and pesticides commonly used on lawns and turf as well as high amounts of ammonium nitrate and salts, most likely originating from winter road salting. Populations can be reduced using the insecticides carbaryl (1-naphthyl methylcarbamate) and diazinon (O,O-diethyl-O-(2-isopropyl-6-methyl-pyrimidine-4-yl)phosphorothioate), and to a lesser extent, the herbicide 2,4-D (2,4-Dichlorophenoxyacetic acid) (Card *et al.*, 2002) and fungicides, such as the carbendazim-based benomyl, can be toxic to earthworms (Edwards and Lofty, 1980). Various studies like species selection in earthworm toxicity tests mainly depends on life cycle and the potential for laboratory culturing.

It may be concluded that the effects of pesticides on earthworms mainly depend on the type of pesticide, method and rate of their application; earthworm species, species specific sensitivity and age.

5.2.2. Effect on soil fungi

The results of the pot culture study indicated that fungal and bacterial populations were inhibited by the application of glyphosate (@ 6 and 12 mL L^{-1}) and chlorpyriphos(@ 4 and 8 mL L^{-1}). Percent decline in the fungal population was 10.1 to 70.5 at one week and 5.8 to 62.9 at one month after application. Higher concentration of Roundup (12 mL L^{-1}) showed the maximum decline at both sampling intervals whereas Glycel at lower concentration showed the minimal decline at both sampling intervals (Table 23).The adverse effect of chemicals followed the order Roundup 12 mL L^{-1} > Dursban 8 mL L^{-1} > Glycel 12 mL L^{-1} > Dursban 4 mL L^{-1} > Roundup 6 mL L^{-1} >Glycel 6 mL L^{-1}. Therefore, it was seen that glyphosate formulation namely Glycel at the recommended rates of application did not cause any adverse effect on the fungal population in soil. Roundup 6 mL L^{-1} was also comparatively safe. Though it produced a negative effect on fungi at one week, their population was comparable with control at one month. However the higher concentration

significantly reduced the fungal count in soil. Chlorpyriphos formulation caused significant decline in the fungal count at both the sampling intervals. Therefore it could be concluded that toxicity to soil fungi is more with the application of chlorpyriphos. Even if chlorpyriphos is an organophosphorus pesticide, toxicity to fungi is manifested to a greater magnitude than that of glyphosate. It could be concluded that the results of the present study are in agreement with previous studies conducted indicated that the effects of glyphosate on soil microflora at recommended rates of application were insignificant.(Preston and Trofymow, 1989; Busse *et al.*, 2001).

5.2.3. Effect on soil bacteria:

As in the case of fungi, greater reduction in their population was noticed with the application of higher concentration of Roundup (12 mL L^{-1}). At one week, percentage decline was ranging from 14.3 to 71.6. At first month, the percentage decline ranged from 24.9 to 76.3 indicating that the adverse effect of chemical persisted for one month after application of the pesticides. At one week the percentage decline followed the order Roundup 12 mL L^{-1} > Glycel 12 mL L^{-1} > Roundup 6 mL L^{-1} > Dursban 8 mL L^{-1} > Glycel 6 mL L^{-1} > Dursban 4 mL L^{-1} (Table 24).

At one month also Roundup 12 mL L^{-1} caused maximum reduction in the bacterial population followed by Glycel 12 mL L^{-1} and this was followed by Roundup 6 mL L^{-1}, then Glycel 6 mL L^{-1}, Dursban 8 mL L^{-1} and Dursban 4 mL L^{-1}. Therefore, the effect of Dursban on bacteria was comparatively lower than that of glyphosate formulation at both the concentrations.

Table 24: Extent of reduction in the population of soil microflora by application glyphosate and chlorpyriphos in the pot culture study

Treatments	Percentage decline over control (fungal population)		Percentage decline over control (bacterial population)	
	7 DAS	30 DAS	7 DAS	30 DAS
Absolute control (Distilled water)	0.0	0.0	0.0	0.0
Glyphosate 41%SL(Roundup): 6 mL L^{-1}	15.47	16.75	36.68	56.22
Glyphosate 41%SL (-do-) :12mL L^{-1}	70.52	62.90	71.62	76.25
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	10.11	5.84	23.16	43.52
Glyphosate 41%SL (-do-) : 12mL L^{-1}	36.25	28.67	44.49	57.41
Chlorpyrifos 20%EC (Dursban) : 4mL L^{-1}	23.02	21.87	14.26	24.94
Chlorpyrifos 20%EC (-do-) : 8mL L^{-1}	43.55	34.87	29.46	34.23

5.3. Field studies

5.3.1. Effect of glyphosate and chlorpyriphos on soil microflora

Effect of glyphosate on soil microflora revealed that both the formulations significantly reduced the fungal population in the soil when they were applied at higher levels (Table 25). Roundup 12 mL L^{-1} significantly reduced the fungal population to an extent of 39.9%. The lower concentration of Roundup also inhibited fungal population at one month after spraying. Percentage decline in the treatments ranged from 13.9 to 52.2 % with the lowest being recorded by glycel 6 mL L^{-1}. However at two months after spraying fungal population was found increasing and the differences between the treatments become insignificant. Thus, it was evident that glyphosate formulations did not adversely affect the soil fungi.

In studying the effect of glyphosate on the number of microorganisms in the soil, Stratton and Stewart (1992) observed no negative or positive effects in respect to the number of microorganisms. These findings are consistent with the results of the present study. The lack of negative effect on the micro biota can be explained by the fact that glyphosate is probably not directly toxic, leading to the acute death of sensitive types. Rather, it might exert a gradual effect caused by relative changes in growth efficiency (slowly decreasing the abundance of sensitive types which waste energy due to the stress response and increasing the abundance of those adapted for rapid use of the free resources resulting from metabolism) (Zabaloy *et al.*, 2008). In the case of Saharan soil, a significant increase in bacteria, fungi and actinomycetes populations was observed for treated sample. Thus, the results are in agreement with the results of Gimsing *et al.* (2004) and Ratcliff *et al.* (2006) who reported an increase in viable soil microorganisms counts after glyphosate addition.

Studies on the effect of chlorpyriphos on soil microflora showed that the insecticide did not alter the population of soil microflora. Decline in the bacterial population was observed only at one month after spraying and the percentage decline ranged from 8.7 to 22.7 in the samples taken at two months after spraying did not show any significant change from control (Table 26).

Table 25. Extent of reduction in the population of soil microflora by application glyphosate in the banana ratoon field

Treatments	Percentage decline over control (fungal population @ 30 DAS)
Absolute control (no pesticide)	0.0
Glyphosate 41%SL (Roundup): 6 mL L^{-1}	39.80
Glyphosate 41%SL (-do-) :12mL L^{-1}	39.97
Glyphosate 41%SL (Glycel) : 6mL L^{-1}	13.97
Glyphosate 41%SL (Glycel) : 12mL L^{-1}	52.17

Table 26: Extent of reduction in the population of soil microflora by application chlorpyriphos in the lawn

Treatments	Percentage decline over control (bacterial population @ 30 DAS)
Absolute control (no pesticide)	0.0
Chlorpyriphos 20%EC (Dursban) : 4mL L^{-1}	8.7
Chlorpyriphos 20%EC (-do-) : 8mL L^{-1}	22.74

The half-life of chlorpyiphos was 34-46 days and hence it had negligible effect on soil microflora (Singh *et al.*, 2002). Studies conducted by Fang *et al.*(2009) observed that residues of chlorpyiphos in the soil had only a temporary inhibitory effect on microbial communities.

5.3.2. Effect of glyphosate and chlorpyriphos on dehydrogenase activity

Dehydrogenase activity was not significantly affected by the application of glyphosate and chlorpyriphos formulations. Among the glyphosate formulations, Glycel treatment registered higher values of enzyme activity at one month than control. Roundup formulation at the recommended rate of application 6 mL L^{-1} also registered increased dehydrogenase enzyme activity over the period from first to second month of treatments. Varying effects of glyphosate on dehydrogenase activity in soil were observed by different scientists. Under laboratory conditions, a normal dose of glyphosate stimulated dehydrogenase activity during the period of six weeks after application (Schuster and Schroder, 1990). Glyphosate caused activation of soil enzymes (Sannio and Gianfreda, 2001). Andrea *et al.* (2003) also showed that dehydrogenase activity was higher than the control at one month after glyphosate application. Increased adaptation of microbial community to the stress caused by increase in concentration of herbicides had been reported by Sebiomo *et al.* (2011). Glyphosate was found to inhibit dehydrogenase activity in sandy loam soil (Dzantor and Felsot, 1991). No effects on soil dehydrogenase activity were detected by Lethbridge *et al.*, (1981) and Nakamura *et al.* (1990).

Chlorpyriphos formulation namely Dursban 20% EC did not cause any significant alteration in the enzyme activity of the soil studied. Over a period from one month to two months after spraying, dehydrogenase activity showed a decreasing trend in the control treatment however both the concentrations of the Dursban showed increasing values over the period. Contrary results were obtained in the case of application of chlorpyriphos. Pozo *et al.* (1995) found that dehydrogenase activity was significantly decreased initially at chlorpyriphos concentrations of 2.0 to 10.0 kg ha^{-1}, but recovered after 14 days to levels similar to those in control soil without chlorpyriphos, whereas a work conducted by Rani *et al.* (2008) found that dehydrogenase activity was sensitive with increasing concentrations of chlorpyriphos.

5.3.3. Effect of glyphosate and chlorpyriphos on chemical properties of the soil

Changes in chemical properties of the soil namely pH, organic carbon, N, P, K, secondary nutrients (Ca & Mg) and micronutrients such as Fe, Mn, Zn and Cu due to application of glyphosate and chlorpyriphos were analyzed by conducting field studies. The results revealed that the application of glyphosate in the banana ratoon field and chlorpyriphos in lawns did not produce any significant effects on any of chemical characteristics of the soil (Fig 4-13). Studies conducted by Sharma *et al.* (2010) showed that physico-chemical properties of the soil were not affected by pesticide application. Devi *et al.* (2013) also reported that application of herbicides *viz.*, butachlor, pertilachlor and 2,4-D did not affect the chemical properties of lateritic soil, when they were applied at recommended levels.

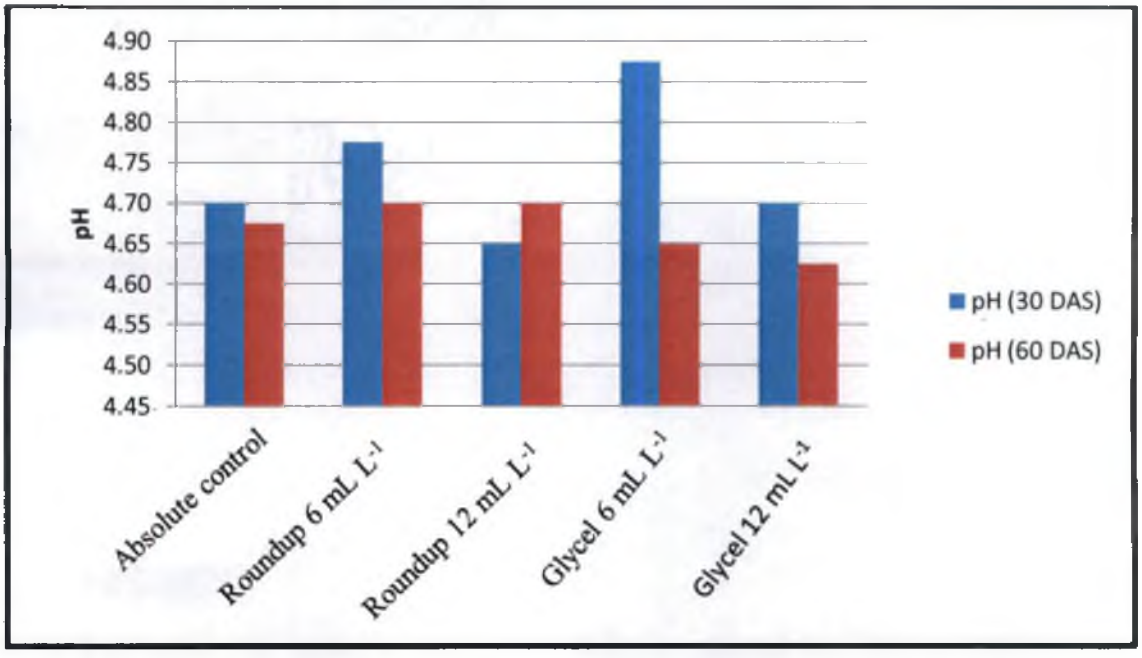


Fig 4. Effect of glyphosate on soil pH

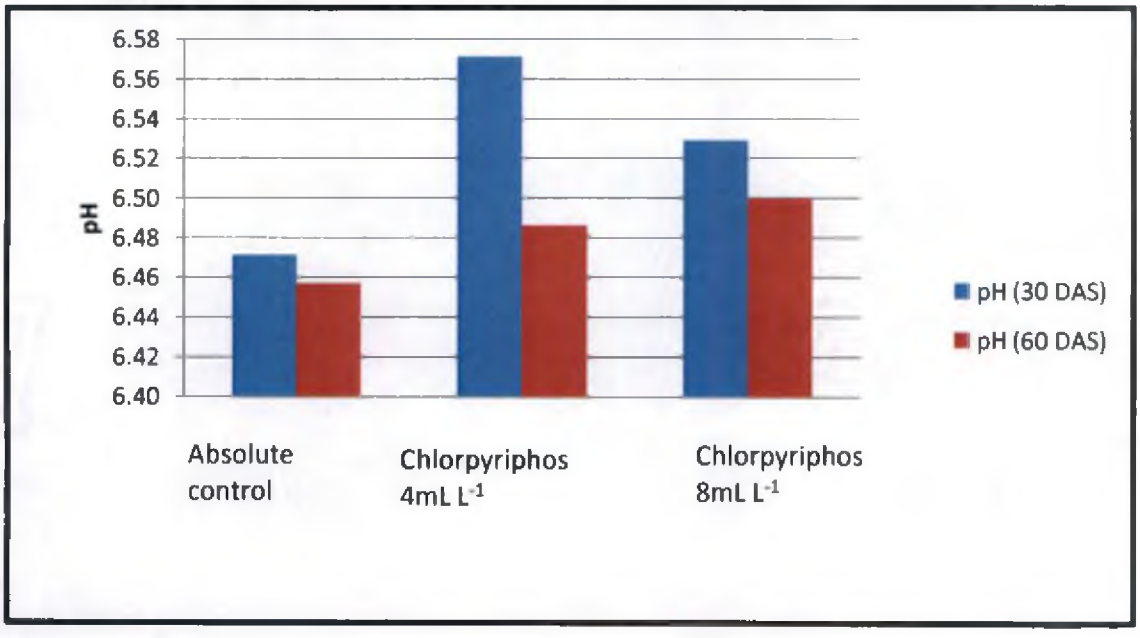


Fig 5. Effect of chlorpyriphos on soil pH

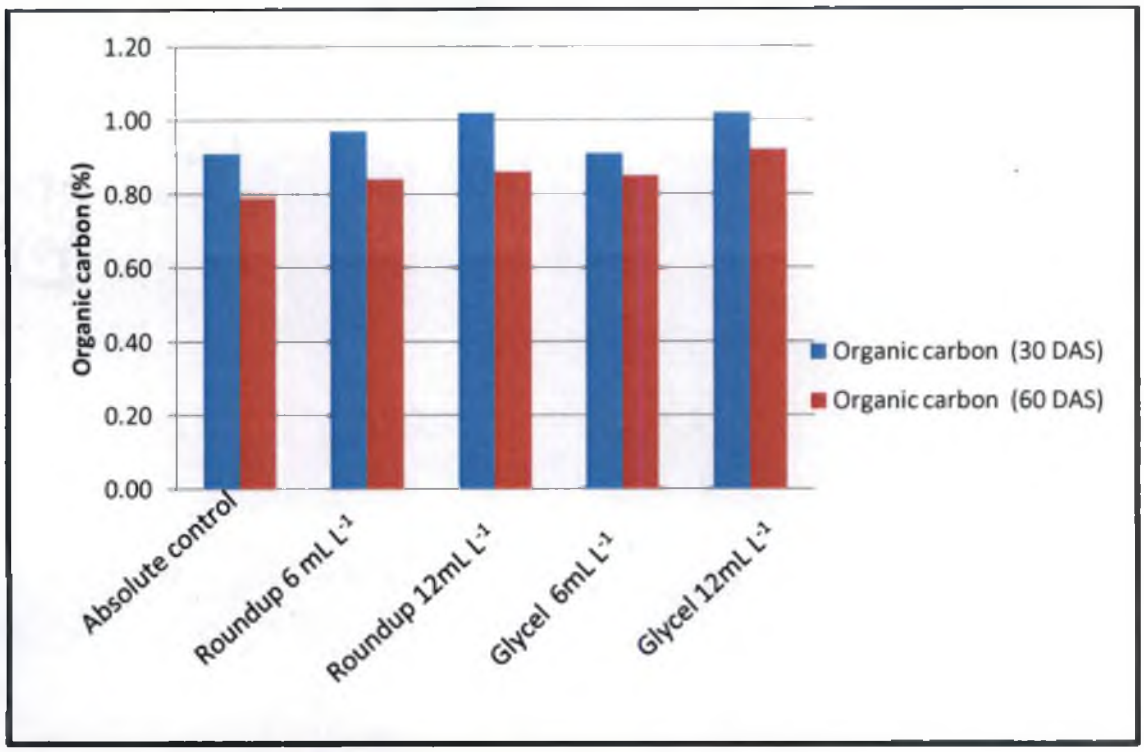


Fig 6. Effect of glyphosate on soil organic carbon content

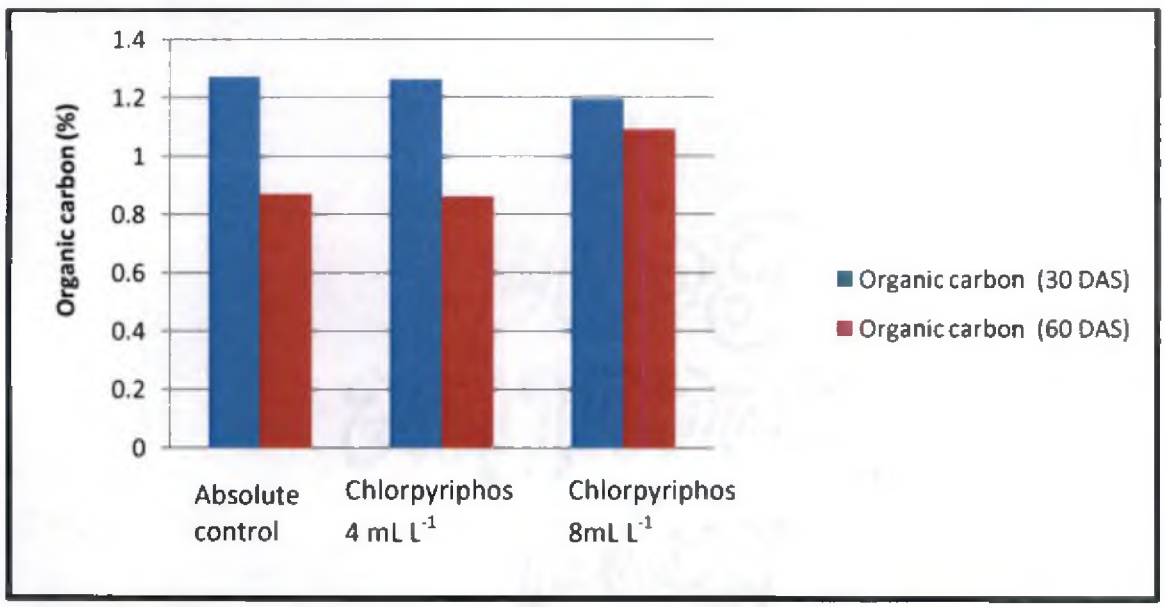


Fig 7. Effect of chlorpyriphos on soil organic carbon content

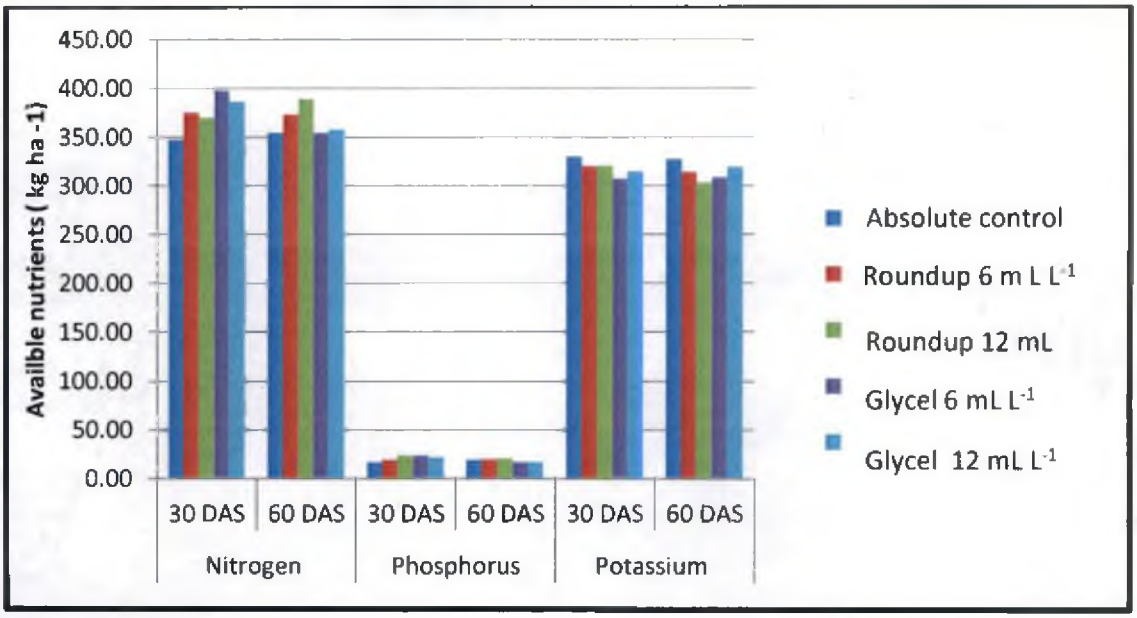


Fig 8. Effect of glyphosate on available N, P and K in the soil

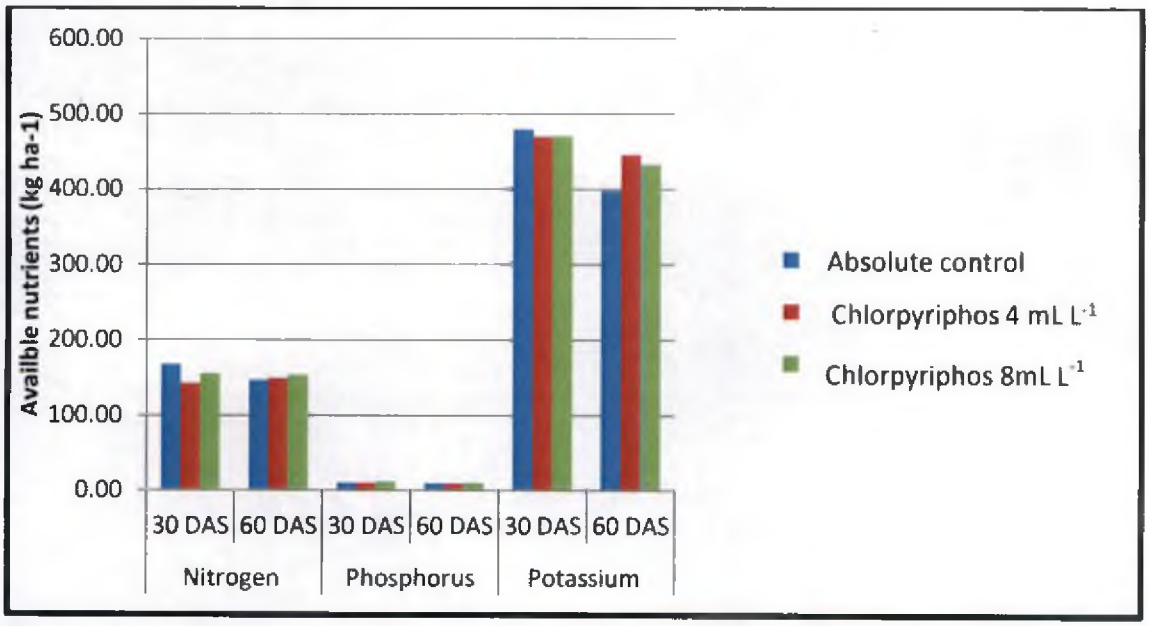


Fig 9. Effect of chlorpyriphos on available N, P and K in the soil

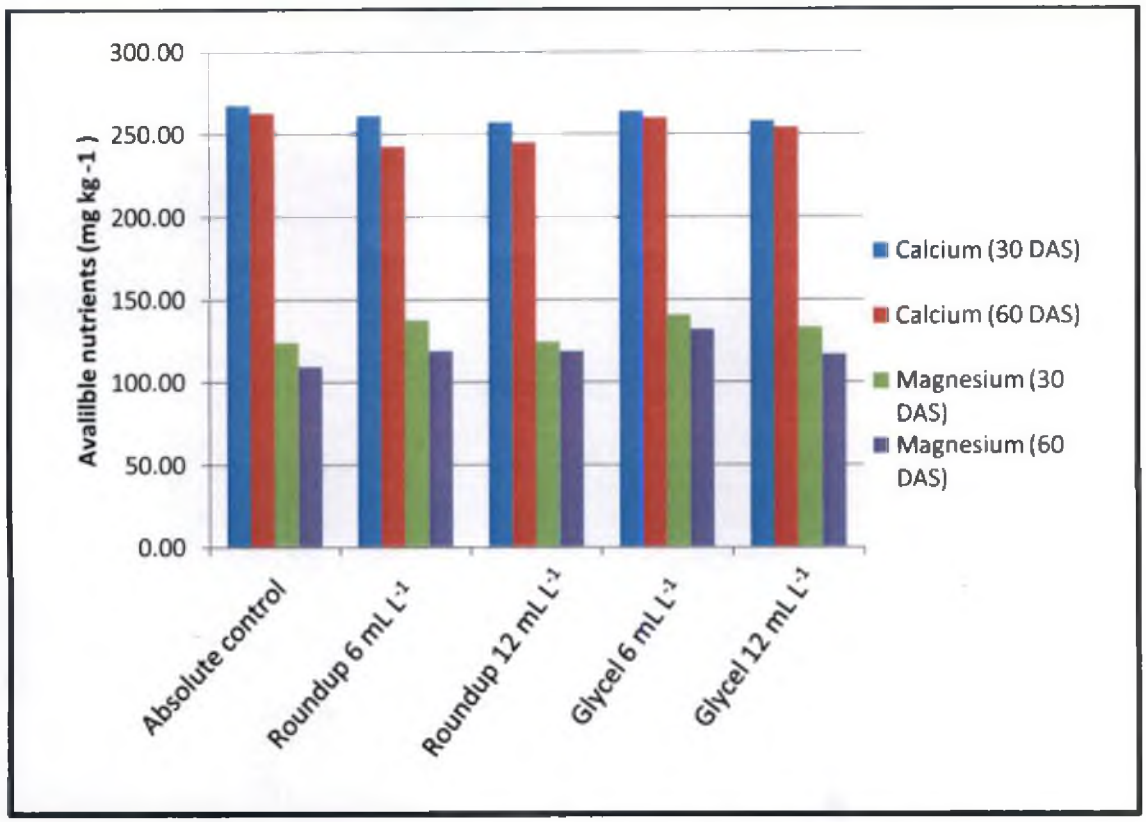


Fig 10. Effect of glyphosate on available Ca and Mg in the soil

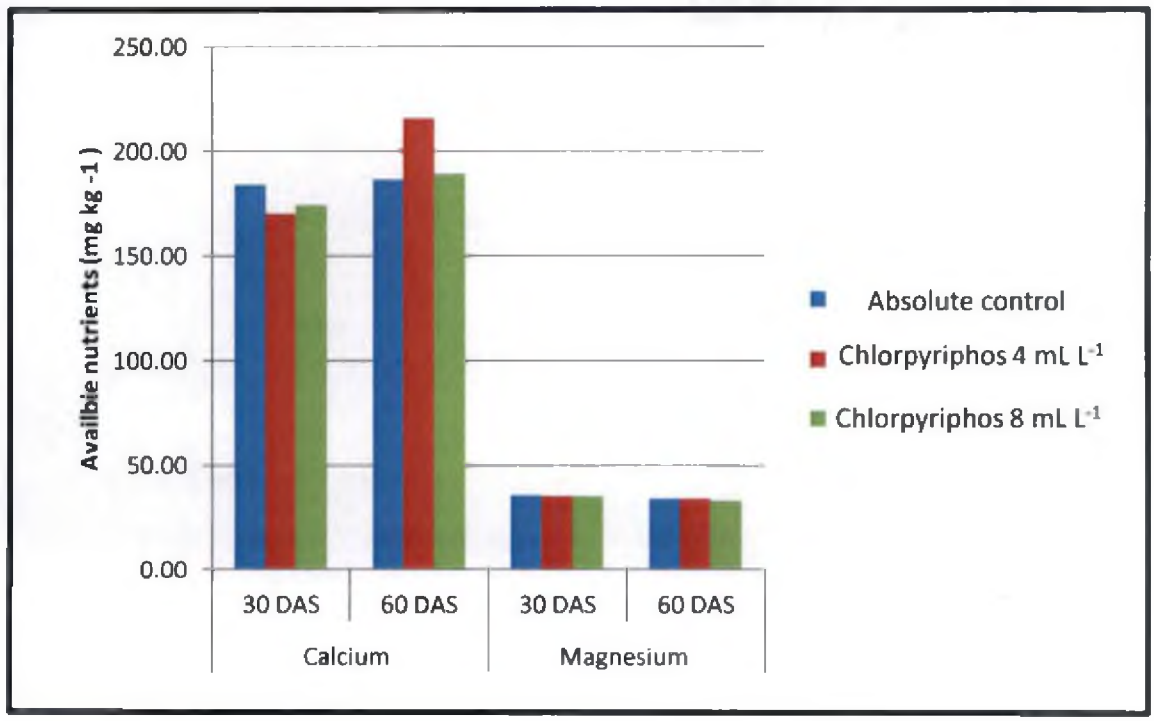


Fig 11. Effect of chlorpyriphos on available Ca and Mg in the soil

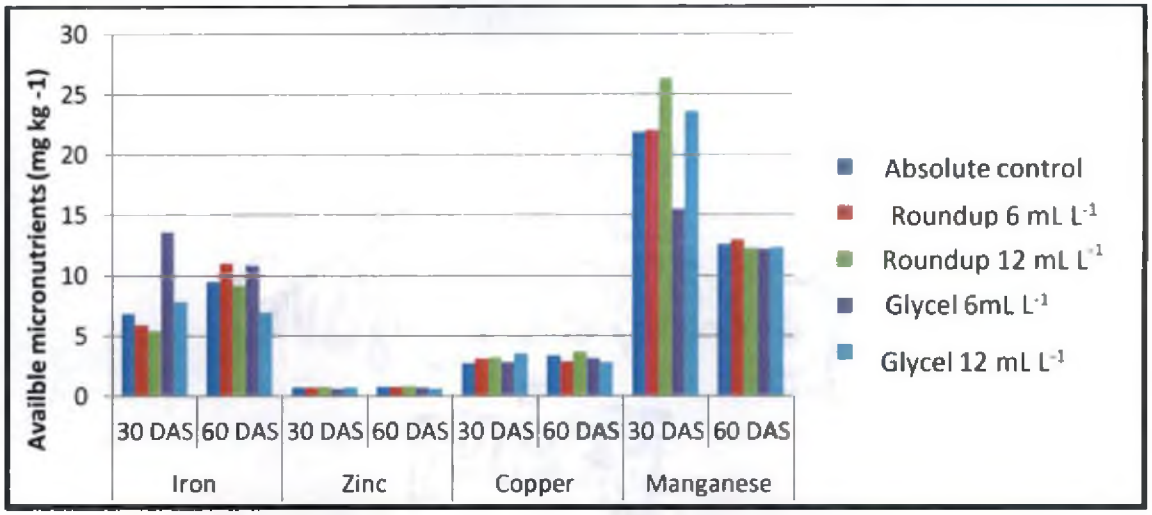


Fig 12. Effect of glyphosate on available micronutrients in the soil

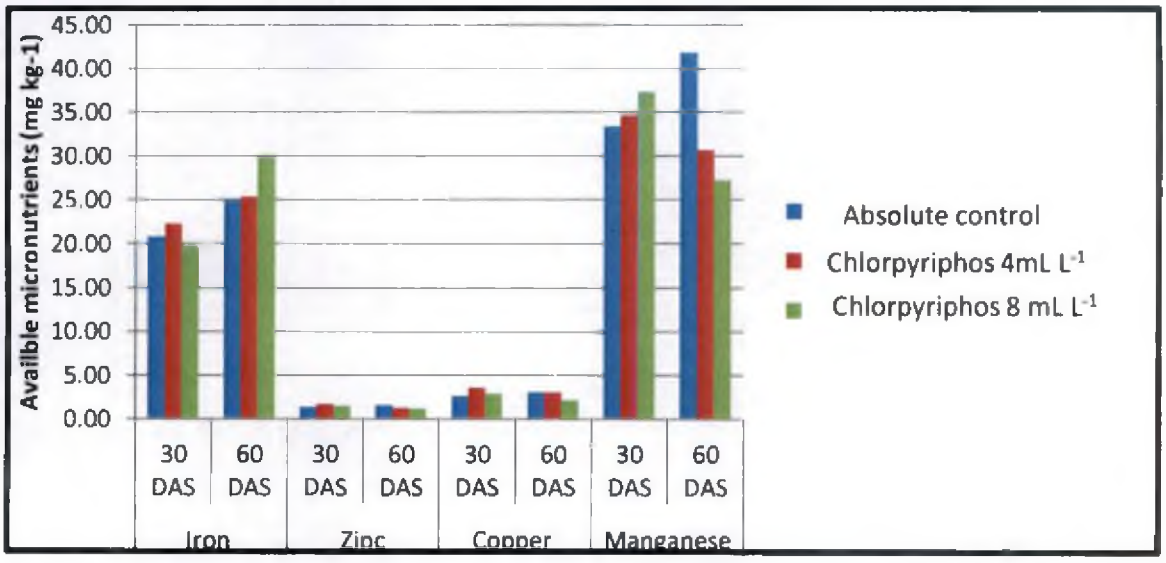
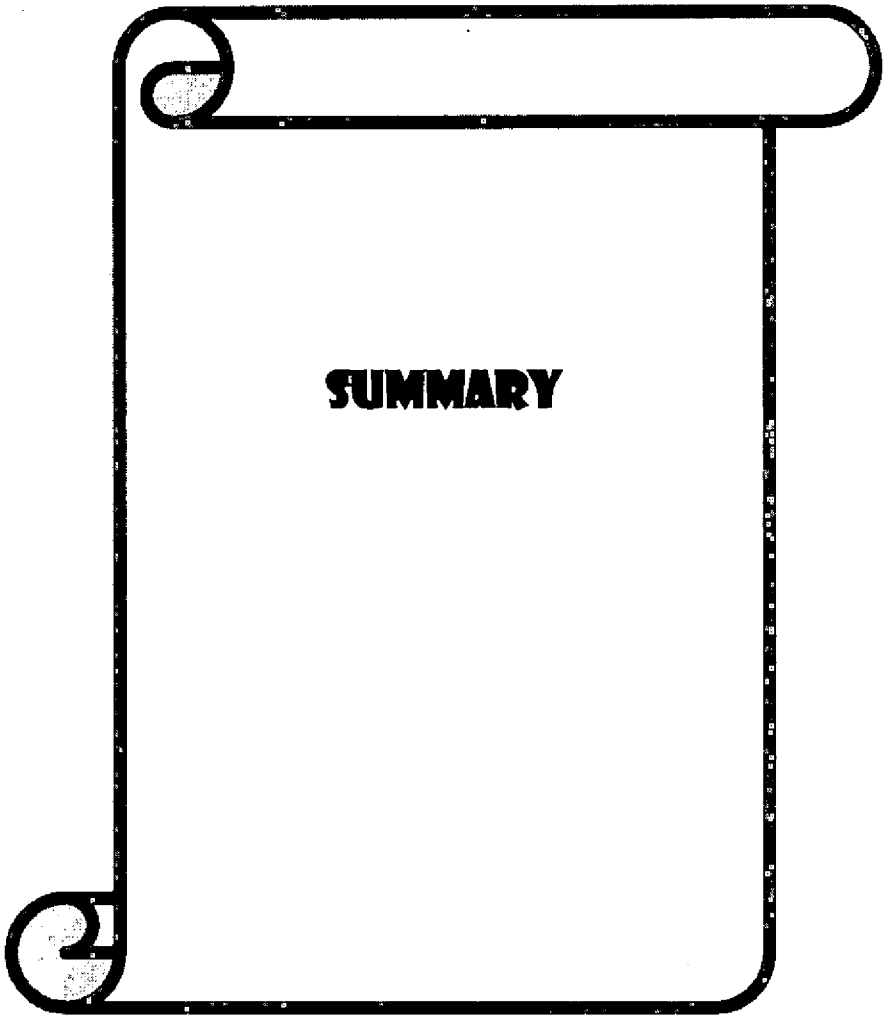


Fig 13. Effect of chlorpyriphos on available micronutrients of the soil


SUMMARY

6. SUMMARY

The research programmme entitled "Impact of glyphosate and chlorpyriphos on chemical and biological properties of the lateritic soil" was carried out in the year 2013 in the Department of soil science and Agricultural Chemistry, College of Horticulture, Vellanikara. The programme consisted of two laboratory experiments *viz.*, (1) avoidance behaviour and 2) survival rates of earthworms and two field experiments *viz.*, application of (1) glyphosate and (2) chlorpyriphos in cropped area and lawn respectively. Organophosphate pesticides *viz.*, two formulations of glyphosate (Roundup and Glycel at two concentrations 6 and 12 mL L^{-1}) and single chlorpyriphos formulation (Dursban) at two concentrations 4 and 8 mL L^{-1} were applied as per treatments. The salient results obtained in the present study are summarized below:

1. The earthworm avoidance study using earthworms showed that all the chemicals were avoided by earthworms.

2. The maximum avoidance was obtained with the application of Round up 12 mL L^{-1} followed by Glycel 12 mL L^{-1} and Dursban 8 mL L^{-1}.

3. The pot culture study revealed that neither glyphosate nor chlorpyriphos at recommended doses had deleterious effects on the multiplication of earthworm.

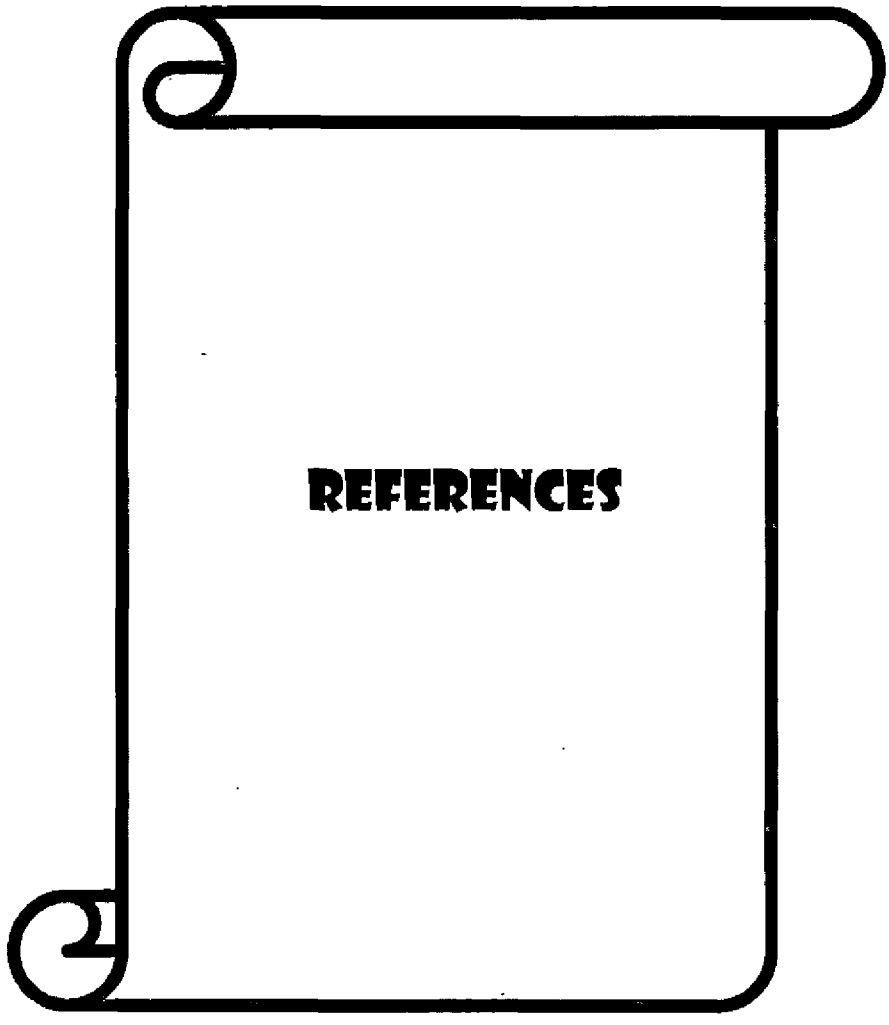
4. This study also showed that both fungal and bacterial populations in the soil were affected by application of glyphosate and chlorpyriphos. Higher concentration of Round up (12 mL L^{-1}) showed the maximum decline at both sampling intervals whereas Glycel at lower concentration showed the minimal decline at both sampling intervals.

5. In the field study, application of glyphosate caused short term inhibitory effect on soil fungus up to 30 days after spraying only. Soil bacteria count and dehydrogenase enzyme activity was unaffected by glyphosate application.

6. Application of glyphosate in the banana field did not produce any significant effects on chemical characteristics of the soil such as pH, organic carbon and available nutrients.

7. It could be observed that application of chlorpyriphos caused only a short term inhibitory effect on soil bacteria (at 30 DAS). No effects were observed on soil fungi and dehydrogenase activity.

8. Application of chlorpyriphos in lawns did not produce any significant difference on any of the chemical characteristics of the soil like pH, organic carbon, available N, P, K Ca, Mg, Fe, Zn, Cu and Mn.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*Originals not seen

Appendix I. Meteorological data during the soil sample collection

Week	Interval	Temperature		Rainfall	Rainy days	Sunshine hours	Relative humidity	
		Max.	Min.				Morning	Evening
1	(29 - 4/Feb)	34.2	23.5	0.0	0	66.8	70	32
2	Feb (5 - 11)	35.0	23.6	0.0	0	59.2	81	40
3	(12 - 18)	34.6	24.4	17.0	1	48.5	77	44
4	(19 - 25)	33.9	23.0	67.4	1	61.5	77	39
5	(26 - 4/Mar)	36.3	21.8	0.0	0	70.5	65	19
6	Mar (5 - 11)	35.1	25.4	7.8	1	34.6	74	47
7	(12 - 18)	35.1	24.3	6.8	1	48	87	51
8	(19 - 25)	36.2	24.7	0.0	0	58.3	92	50
9	(26 - 1/Apr)	34.6	24.9	0.0	0	51.2	91	53
10	Apr (2 - 8)	34.2	25.3	0.0	0	35.2	92	58
11	(9 - 15)	35.4	25.7	0.0	0	48	89	57
12	(16 - 22)	34.5	24.5	0.0	0	52.9	84	52
13	(23 - 29)	35.2	25.1	0.0	0	45.6	85	54
14	(30 - 6/May)	34.7	26.0	0.0	0	27.3	91	60
15	May (7 - 13)	34.3	25.8	0.0	0	26.2	92	58
16	(14 - 20)	34.4	25.1	6.4	1	38.2	89	55
17	(21 - 27)	34.0	24.6	5.7	1	36.8	92	61
18	(28 - 3/Jun)	29.9	23.5	210.8	5	11.3	96	79
19	Jun (4 - 10)	29.4	22.8	149.2	6	8.4	95	79
20	(11 - 17)	28.6	22.3	302.5	7	7.6	97	87
21	(18 - 24)	27.5	23.0	284.1	7	3.4	98	86

Appendix II. Media used for enumeration of Soil microorganisms

Microorganism assayed	Name of the growth media	Composition of the growth media
Bacteria	Soil extract agar media	Glucose:1.0g KH_2PO_4:0.5g Soil extract :100mL Distilled water:1000 mL
Fungi	Martin's rose Bengal agar media	Dextrose:10.0g Peptone:5.0g KH_2PO_4:1.0g $MgSO_4$:0.5g Agar:20.0g Rose Bengal:0.003g Distilled water :1000 mL

IMPACT OF GLYPHOSATE AND CHLORPYRIPHOS ON CHEMICAL AND BIOLOGICAL PROPERTIES OF LATERITIC SOIL

By

SHITHA C. R.

ABSTRACT OF THE THESIS

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Kerala Agricultural University

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR-680656

KERALA, INDIA

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Abstract

The thesis entitled "Impact of glyphosate and chlorpyriphos on chemical and biological properties of lateritic soil" was done in the year 2012-2013 in the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Vellanikara. The programme consisted of laboratory experiment *viz.*, avoidance behaviour of earthworm , pot culture study on survival of earthworm and soil microflora under the different pesticide treatments and two field experiments *viz.*, application of (1) glyphosate and (2) chlorpyriphos in cropped area and lawn respectively.

The laboratory and pot culture studies consisted of seven treatments. The treatments included two levels of application (recommended and double the recommended dose) of two formulations of glyphosate (1.2 and 2.4 kg a.i. ha^{-1}) and single chlorpyriphos formulation (400 and 800 g a.i. ha^{-1}) and an absolute control. Glyphosate formulations namely Roundup® and Glycel® were applied at 6 and 12 mL L^{-1} respectively and chlorpyriphos formulation Dursban® was applied at 4 and 8 mL L^{-1}. The main observations included in this research programme were biological properties of soil *viz.*, earthworm count, total microbial count, dehydrogenase activity and chemical properties of soil viz., pH, organic carbon, available N, P, K Ca, Mg, Fe, Zn, Cu and Mn.

Both laboratory and pot culture experiments were done with native earthworms. The laboratory experiment was done to understand the avoidance behavior of earthworm to the two glyphosate formulations and chlorpyriphos. This study showed that all the chemicals were avoided by earthworms and the maximum avoidance was obtained with the application of Roundup® (12 mL L^{-1}) followed by Glycel® (12 mL L^{-1}) and Dursban® (8 mL L^{-1}). Earthworm avoidance test done by two methods of application (mixing as well as spraying) showed similar trends. However, higher net response was obtained by mixing the pesticides in soil. Spraying on the surface imitated the field situation, in which the avoidance response ranged from 20.0 to 46.7 %, while in the case of mixing the pesticides with the soil, the avoidance response ranged from 33.3 to 73.3 %. When the soil was mixed with the chemicals, the earthworms had direct contact with the pesticides throughout the treated soil section and thus they had shown greater tendency to avoid the area.

Pot culture studies on the survival of earthworms revealed that neither glyphosate nor chlorpyriphos had deleterious effects on their multiplication. The study also showed that both soil fungi and bacteria were affected by application of glyphosate and chlorpyriphos. The adverse effect of chemicals on the soil fungi followed the order Roundup 12 mL L^{-1} > Dursban 8 mL L^{-1} > Glycel 12 mL L^{-1} > Dursban 4 mL L^{-1} > Roundup 6 mL L^{-1} > Glycel 6 mL L^{-1}. Percent decline in the fungal population was 10.1 to 70.5 at one week and 5.8 to 62.9 at one month after application. It was noticed that Roundup and Glycel at the recommended rates of application did not cause adverse effect on soil fungi at one month after spraying, indicating that the effect was temporary. However, chlorpyriphos formulation caused a significant decline in the fungal population at both the sampling intervals.

In the case of bacteria, percentage decline over control followed the order Roundup 12 mL L^{-1} > Glycel 12 mL L^{-1} > Roundup 6 mL L^{-1} > Dursban 8 mL L^{-1} > Glycel 6 mL L^{-1} > Dursban 4 mL L^{-1}, at one week after spraying and the extent of decline ranged from 14.3 to 71.6%. At one month, the effect of chlorpyriphos (Dursban) on bacteria was comparatively lower than that of glyphosate formulations at both the concentrations.

Two field studies were conducted to find out the effect of application of glyphosate in cropped area (banana field) and chlorpyriphos in the lawn. Experiment in the banana field was conducted with five treatments namely Roundup® and Glycel® each at 6 and 12 mL L^{-1} and an absolute control. The treatments were replicated four times. Studies with chlorpyriphos were carried out in the established lawns of the College of Horticulture, Vellanikkara with three treatments viz., Dursban® at 4 and 8 mL $L^{-1}$and seven replications. Both biological and chemical properties were studied. It was found that application of glyphosate caused short term inhibitory effect on soil fungus up to 30 days after spraying, where as application of chlorpyriphos caused short term inhibitory effect on soil bacteria (upto 30 DAS). Population of soil microflora did not vary between treatments at 60 days after spraying glyphosate and chlorpyriphos formulations. Dehydrogenase activity and available nutrients in the soil were unaffected by the application of glyphosate and chlorpyriphos formulations used in the study.