DISSIPATION OF CHLORPYRIFOS IN RED LOAM SOIL AND ITS EFFECT ON SOIL ORGANISMS

REKHA, P.R.

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2005

Department of Soil Science and Agricultural Chemistry
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522

Approved by

Chairman :

Dr. THOMAS GEORGE
Assistant Professor,
Department of Soil Science and
Agricultural Chemistry,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

4.02.2006

Members :

Dr. V.K. VENUGOPAL
Professor and Head,
Department of Soil Science and
Agricultural Chemistry,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

04/02/06

Dr. SUMAM GEORGE
Associate Professor,
Department of Soil Science and
Agricultural Chemistry,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

04.02.2006

Dr. S. NASEEMA BEEVI
Associate Professor,
Department of Agricultural Entomology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

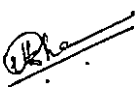
4/2/06

External Examiner : 4/2/06

DECLARATION

I hereby declare that this thesis entitled **"Dissipation of chlorpyrifos in red loam soil and its effect on soil organisms"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
7-2-2006



REKHA, P.R.
(2003-11-27)

CERTIFICATE

Certified that this thesis entitled **"Dissipation of chlorpyrifos in red loam soil and its effect on soil organisms"** is a record of research work done independently by Ms. Rekha, P.R. (2003-11-27) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
7-2-2006

Dr. THOMAS GEORGE
(Chairman, Advisory Committee)
Assistant Professor
Department of Soil Science and
Agricultural Chemistry,
College of Agriculture, Vellayani
Thiruvananthapuram.

ACKNOWLEDGEMENT

I express my sincere appreciation and deep gratitude to Dr. Thomas George, Assistant Professor, Department of Soil Science and Agricultural Chemistry and chairman of my Advisory Committee for suggesting the problems and for imparting his unceasing guidance, concrete suggestions, constant encouragement and utmost interest during the course of the present investigation and preparation of this manuscript. As a token of my heart felt gratitude I dedicate the work to him.

I am extremely grateful to Dr. Venugopal and Dr. Sumam George, esteemed members of the Advisory Committee for their valuable suggestions and assistance.

I feel immense pleasure to avail the opportunity to covey my heartfelt thanks to Dr. S. Naseema Beevi, Associate Professor, Department of Agricultural Entomology for providing necessary facility and encouragement.

I wish to record my special thanks to the generous and selfless help of Dr. (Mrs.) V.K. Girija, Associate Professor, Department of Plant Pathology, rendered to me during the course of my investigation.

I am very much obligated and grateful to all the teachers of my division for their constant support and encouragement.

I wish to express my sincere thanks to Shailaja chechi for her valuable help in the GLC analysis of the samples.

I take this opportunity to express my special thanks to my friend, Devikrishna for her constant support and encouragement.

Special acknowledgement is made to my friends, Jyolsna, V.K., Shjama Nafeesa Basheer, Poornima chechi, Jasmin chechi, Devi chechi, Beena chechi, Aparna chechi, Sheeba chechi, Simi chechi, Raji chechi,

Anie chechi, Jeena chechi, Gouripriya chechi, Kavitha chechi and Queen Flower for their immense help and co-operation during the course of the investigation.

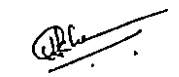
I am extremely grateful to Mr. Shivarajan for the help rendered during the course of study.

My thanks also to Shri. C.E. Ajithkumar, Programmer for the helps rendered in the analysis of data.

I express my unboundful gratitude to my parents without their support, encouragement, prayers and blessings I could never have reached this stage. I express my regards to my sister for the continued moral support.

Finally I thank Mr. Biju for nicely and promptly typing this manuscript.

The financial assistance received in the form of Junior Research Fellow awarded by Kerala Agricultural University is gratefully acknowledged.



Rekha, P.R.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

LIST OF APPENDIX

Sl. No.	Title	Appendix No.
1	Chromatogram of chlorpyrifos	1

LIST OF ABBREVIATIONS

%	–	Per cent
µg	–	Micro gram
µg g^{-1}	–	Microgram per gram
µl	–	Micro litre
@	–	At the rate of
a.i.	–	Active ingredient
cc	–	Cubic centimetre
CD	–	Critical difference
CEC	–	Cation exchange capacity
cm	–	Centimetre
dSm^{-1}	–	Deci seimens per metre
EC	–	Electrical conductivity
20 EC	–	Emulsifiable concentrate
ECD	–	Electro capture detector
et al.	–	And others
Fig.	–	Figure
g	–	Gram
GLC	–	Gas liquid chromatography
ha^{-1}	–	Per hectare
Hg	–	Mercury
Kg	–	Kilogram
KOC	–	Adsorption coefficient
LD50	–	Lethal dose 50
cmol	–	Centi mole
mg	–	Milligram
Mg m^{-3}	–	Mega gram per cubic metre
min.	–	Minutes
ml	–	Millilitre
mm	–	Millimetre
POP	–	Package of Practices
ppm	–	Parts per million
RBD	–	Randomized block design
t	–	Tonnes
UV	–	Ultraviolet
viz.	–	Namely

INTRODUCTION

1. INTRODUCTION

Use of high yielding varieties and improved agricultural technologies in the green revolution period resulted in high crop yields and pest build up. To manage the pest problem without affecting the production, synthetic organic pesticides were introduced in the field of plant protection. Now use of pesticides has become an integral part of modern agriculture and without this production from high yielding varieties would be doubtful. The intensive use of these chemicals is initially promising but in due course will create serious problems like pest resistance, secondary pest outbreak, pest resurgence, bioaccumulation, bio magnification, chronic and acute toxicity and above all contamination of non-target sites.

Regardless of the method of application large amount of pesticides find their way into the soil, which acts as a reservoir of these chemicals. In soil they are acted upon by various physical, chemical and biological process. The fate of chemicals in the soil depends on processes like adsorption, transfer and degradation.

The persistence of a pesticide depends on the rate of degradation which in turn is dependent on concentration of pesticide applied, the physical and chemical properties of pesticides and environmental and soil factors. Thus some pesticides may either remain for years or some others may easily be lost from the soil. The residues of persistent compounds can find their way in to different biological entities and get accumulated in their tissues.

The soil is the natural habitat of numerous macro, meso and microorganisms like earthworms, termites, mites, insect larvae, millipedes, centipedes, ants, fungi, bacteria and actinomycetes (Brown, 1978). These organisms play an important role in soil dynamics and in

maintaining the soil physical and chemical properties. The pesticide residue in the soil can adversely affect the population of soil organisms and the microbes can degrade the various toxic xenobiotics. (Swaminathan and Prasad, 1978).

Chlorpyrifos is the world's leading insecticide in terms of volume and is effective against a wide range of insect. The acute oral LD_{50} value of chlorpyrifos is in the range of 135-160 mg kg^{-1} (rats) and is classified as moderately hazardous. It is the only insecticide now popularly available for the effective control of soil insects due to its high persistence. When applied at the normal rates, the half lives as in the order of 1-2 months. At higher termiticidal rates, persistence of chlorpyrifos increases

Chlorpyrifos has a soil adsorption coefficient (KOC) of greater than 5000 and has a strong tendency to be adsorbed by soil and organic matter. This places chlorpyrifos in the immobile leaching category with negligible downward mobility. The strong adsorption to the soil together with rapid degradation result in limited field run off. Hydrolysis is the most important process responsible for the degradation of chlorpyrifos in soil. Chlorpyrifos can persist more in acidic soil condition and other soil conditions can affect the degradation processes. Moreover, literature on the persistence of chlorpyrifos in acidic Kerala soils are scanty inspite of its wide use in the state.

In this context an investigation was carried out to assess the dissipation of chlorpyrifos in red loam soils of Kerala with the following objectives.

1. To assess the dissipation pattern of chlorpyrifos in red loam soil at varying levels of organic matter and lime.
2. To study its effect of on soil microflora and arthropods

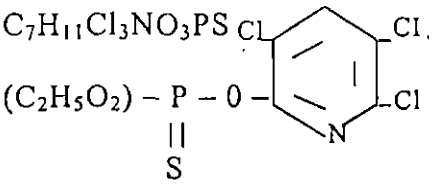
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Organophosphorus compounds constitute an important versatile group of highly active molecules widely used for pest control. More than one lakh different organophosphorus compounds have been synthesized and evaluated as pesticides of which nearly one hundred and fifty are widely used in agriculture. Substances with wide variety of biological properties like insecticides, acaricides, nematicides, herbicides, defoliants and fumigants are a few among the organophosphorus compounds. Most of the compounds are characterised by low dose and rapid insecticidal action. These compounds undergo rapid degradation from the environment and disappear after the period of pesticidal action and will not accumulate in the body of animals. Though many of the earlier compounds of this group were highly toxic to vertebrates, a large number of organophosphorus compounds with moderate to low mammalian toxicity have been synthesized recently.

Chlorpyrifos was first discovered by the Dow Chemical Company in 1962, initially designated as DOWCO 179 and later as chlorpyrifos. It is an active insecticide for the control of sucking and chewing plant pests, soil inhabiting pests and household parasites. Chlorpyrifos is a broad spectrum contact and stomach insecticide. It is highly stable in the environment and is not sensitive to UV radiation. It is non systemic in plants, moderately toxic to mammals and is readily metabolized with rapid loss of activity. It does not cause threat to environment and appears ecologically safe, thereby preferred for use in household and public health insect control as well.

2.1 SALIENT FEATURES OF CHLORPYRIFOS

Common name	-	Chlorpyrifos
Chemical name	-	O, O-diethyl (3,5,6-trichloro 2-pyridyl) phosphorothioate.
Alternate names	-	Dursban, Lorsban, Tricel
Emperical formula	-	$C_7H_{11}Cl_3NO_3PS$
Structural formula	-	
Molecular weight	-	350.6
Physical properties	-	Odourless, white chrystalline, solid
Melting point	-	41-43.5°C
Solubility	-	2 mg L^{-1} at 23°C
Vapour pressure	-	1.81 x 10^{-5} mm Hg at 25°C
Specific gravity	-	1.38 gcc^{-1} at 46°C
Stability	-	Stable in air and is not sensitive to UV radiation. Stable in neutral and weakly acidic solution, hydrolysed by strong bases. Thermally sensitive to temperature over 50°C. Violent exothermic decompositionabove100^{0}C.
Formulation	-	Emulsifiable Concentrate Wettable powder, Granule
Toxicological data	-	Less toxic to mammals and birds, oral LD_{50} for rat is 135-165 mg kg^{-1}
No effect level	-	1 mg kg^{-1} day^{-1}
Acceptable daily intake	-	0.01 mg kg^{-1} day^{-1}

2.1. 1 Solubility of chlorpyrifos

Benzene	-	7900 g kg^{-1}
Acetone	-	6500 g kg^{-1}
Chloroform	-	6300 g kg^{-1}
Carbon disulfide	-	5900 g kg^{-1}
Carbon tetrachloride	-	3100 g kg^{-1}
Diethyl ether	-	5100 g kg^{-1}
Ethanol	-	630 g kg^{-1}
Ethyl acetate	-	>2000 g kg^{-1}
ISO octane	-	790 g kg^{-1}
Methanol	-	450 g kg^{-1}
Methylene chloride	-	4000 g kg^{-1}
Propylene glycol	-	40 g kg^{-1}
Toluene	-	1500 g kg^{-1}
Trichloroethane	-	4000 g kg^{-1}
Triethylene glycol	-	50 g kg^{-1}
Xylene	-	4000 g kg^{-1}

(Drummond, 1986; Hummel and Crummet, 1964)

2.1. 2 Half Life of Chlorpyrifos in soil

pH	Temperature	T ½ (days)
5	25	63
7	15	100
7	25	35
7	35	12
8	25	23

(Drummond, 1986)

2.2 USES OF CHLORPYRIFOS

Chlorpyrifos is an organophosphorus compound effective against wide range of crop pests and soil inhabiting insects.

2.2.1 *Chlorpyrifos as Crop Prtectant*

Chlorpyrifos is an effective insecticide for the control of pests in a variety of crops.It was reported that chlorpyrifos @ 0.2 per cent caused maximum mortality of red hairy caterpillar, *Amsacta moorei* (Wlk.) Butler larvae under laboratory conditions (Tandi *et al.*, 1993).

Upadyay and Agarwal (1993) reported that chlorpyrifos when applied@0.03per cent to mustard caused complete control of the mustard aphid *Lipaphis erysimi* (Kalt.) after 24 hours of application.

Khajuria and Sherma (1995) observed that among the several insecticides tried, chlorpyrifos @ of 0.04 per cent was the most efficient in controlling infestation by the pea leaf minor *Chromatomyla horticola* (Gourau) on pea.

Chlorpyrifos when applied at 0.05 per cent reduced population of lima bean pod borer, *Etiella zinkenella* Treit in peas by 80 per cent due to a prolonged persistence till harvest (Bijur and Verma, 1997). Shijian (1997) also reported the high efficiency of chlorpyrifos against *Spodoptera litura* (Hb.) both in laboratory and field trials.

Djuwarso and Harnoto (1998) reported that infestation due to pod borer *Etiella* spp. in soya bean could be effectively controlled by spraying chlorpyrifos (@0.05 per cent) just after egg hatching during the first larval instar. Rajareddy and Divakar (1998) also reported the ovicidal action of this organophosphorus insecticide on the eggs of *Etiella* spp.

Rao and Subbaratnam (1998) observed that the ragi cutworm, *Spodoptera exigua(Hb.)* in onion could be effectively controlled by application of chlorpyrifos at 0.05 per cent.

Rajareddy and Divakar (2000) also reported the ovicidal action of chlorpyrifos on the eggs of tomato fruit borer, *Helicoverpa armigera* (Hb.). A similar finding was also reported by Takur and Vaidya (2000) the efficacy of chlorpyrifos for black cut worm, *Agrotis ipsilon* (Hfn.) in maize.

2.2.2 Chlorpyrifos as a Soil Insecticide

Chlorpyrifos is a widely used insecticide for the control of soil inhabiting insects like termites, field crickets, ants, white grubs, wire worms and black field earwig. Chlorpyrifos is a moderately stable organophosphorus insecticide widely used as a soil applied termiticide throughout the world. It was reported that application of chlorpyrifos as a termiticde resulted in initial residue of several hundred ppm and nearly 70 per cent of initially applied chlorpyrifos remained in the soil after eighteen months. It is reported that the effectiveness of chlorpyrifos for termite control in soil could be the result of long residual effect, high application rates and the inherent properties of the compound (Racke, 1993)

In another study Racke *et al.* (1994) reported that the termiticidal soil barrier treatments often resulted in initial deposits of 1000 $\mu g\ g^{-1}$ in soil. In such situation the degradation halflife of chlorpyrifosis was 175-1576 days, thus giving better soil protection.

Forscher and Townscnt (1998) studied the effectiveness of chlorpyrifos as termiticide in three different soils and showed that lethal concentration of termiticides were at least seven times lower in sand compared with sandy loam and sandy clay loam compared to agricultural concentrations of 100 and 10 mg kg^{-1}.

2.3 PERSISTENCE OF CHLORPYRIFOS IN SOIL

Persistence is the time required to reduce the pesticide concentration in soil to 75-100 per cent of the amount initially added (Kearney and Helling, 1969). The environmental fate of chlorpyrifos has been studied extensively and the reported half life in soil varied from 100-120 days with 3, 5, 6-trichloro 2 pyridinol (TCP) as the major degradation product (Getzen, 1985) (Fig.1).

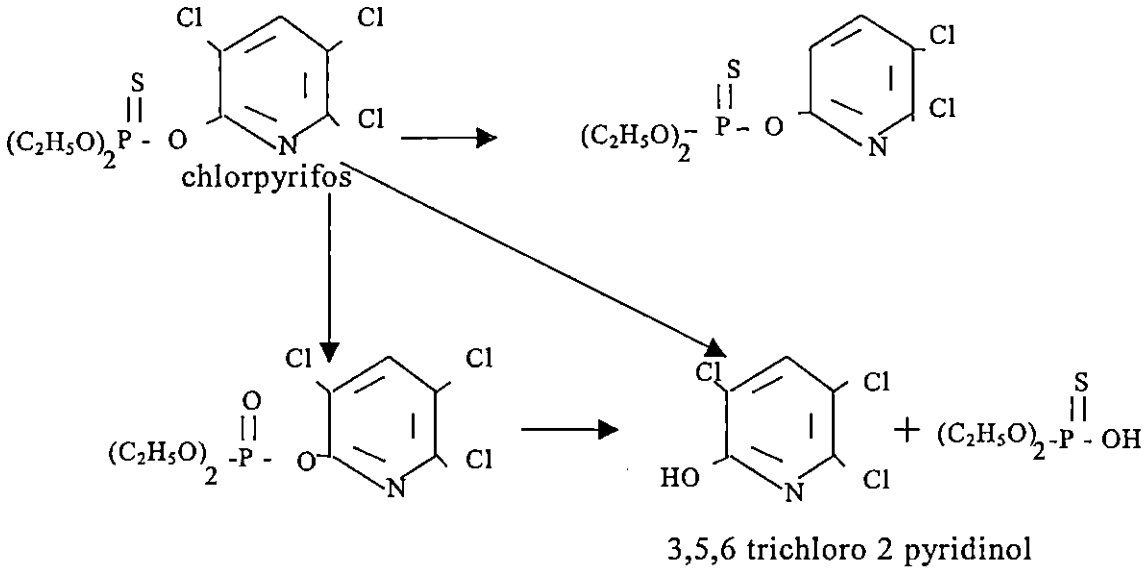
Bhatnagar and Gupta (1992) studied the persistence of chlorpyrifos in soil and groundnut seed. When applied @ 800 and 1200 g ai ha^{-1} the residues of insecticide were present in the soil upto 40 days after treatment and half life values of chlorpyrifos were found to be 11.55 and 16.96 days respectively.

Racke (1993) demonstrated that an increase in application rate of chlorpyrifos from a typical agricultural use (10 ppm) to that for urban termiticide application (1000 ppm) resulted in a dramatically decreased rate of degradation.

Racke *et al.* (1994) reported that the major metabolite formed in chlorpyrifos treated soil was 3, 5, 6 trichloro 2-pyridinol (TCP) which represented upto 61 per cent of applied amount after 13 months of incubation. Minor quantities of CO_2 (<5 per cent) and soil bound residues (12 per cent) were also present.

Kalpana *et al.* (2002) studied the persistence of chlorpyrifos in sandy loam soil and reported that seed dressing of wheat with chlorpyrifos @ 0.9 g ai kg^{-1} seed showed an initial low levels of chlorpyrifos residues in the surface soil, which gradually built up with time and reached maximum on 20^{th} day and declined thereafter. Chlorpyrifos, when applied through irrigation water dissipated very rapidly with a half life 8.3 days.

Fig. 1. Degradation pathway of chlorpyrifos in soil



3,5,6 trichloro 2 pyridinol

The persistence of chlorpyrifos in soil vary considerably depending on the soil conditions. Chapman and Chapman (1986) reported a large variation in half life of chlorpyrifos in different soils. This variation has been attributed to variation in factors such as pH, temperature, moisture content, organic carbon and type of pesticide formulation used. The degradation of chlorpyrifos is generally biphasic showing an initial faster degradation followed by a slower rate of degradation. Therefore the degradation rate during slower phase (after two months period) only followed the first order law.

2.3.1 Effect of pH and Lime

The prevailing pH conditions of the soils decide the rate of hydrolysis and degradation of pesticides. Soil pH plays a major role in deciding the extent of persistence of pesticide in soil as most of the pesticides are unstable under alkaline pH. Hydrolysis appeared to have caused a rapid loss of chlorpyrifos especially in the highly alkaline soils (Spark, 1989).

Addition of lime to soil of pH 4.7 increased the pH to 7.5 and similar addition to pH 5.7 increased its pH to 8.6. The degradation of chlorpyrifos in these modified pH soil was similar to that originally of high pH. (Somasundaram *et al.*, 1989)

Sprenkel and Hamburg (1989) studied the effect of pH on dursban and found no significant difference in chlorpyrifos degradation with differed pH and temperatures for 60 days.

Racke *et al.* (1990) reported that degradation of chlorpyrifos was high in highly alkaline soil (pH 8) and was not microbially mediated and appeared to be mainly through hydrolytic process.

Zidan *et al.* (1991) demonstrated that the degradation of chlorpyrifos and fenvalerate were rapid in sandy soil compared to clay loam soil having pH between 5.4 – 5.5.

Smith and Rust, (1992) found that chlorpyrifos residues in a highly alkaline soil (pH 8.1) disappeared after the first year of application, due to its high dissipation.

Racke *et al.* (1996) concluded that hydrolysis is the major route of degradation of chlorpyrifos in both acid and alkaline soils. Chlorpyrifos hydrolysis proceed at a low rate in acid soils (pH $\leq$ 7). This indicated that soil pH is the independent variable displaying the strongest association with hydrolytic rate.

Pulverised lime stone applications reduced the capacity of controlled release (CR) insecticide to prevent cane grub damage where soil pH was raised above (approximately) 6.0 – 6.2. Broadcast application and incorporation of 5 t ha^{-1} lime stone shortly before planting can result in insufficient chlorpyrifos insecticide in soil to prevent pest damage (Chandler and Hogarth, 1998).

Robertson *et al.* (1998) investigated the residues of chlorpyrifos in a soil from sugar cane field where the insecticide was no longer effective and reported that chlorpyrifos was readily hydrolysed to TCP in alkaline soil.

Huang-Xing Jiang *et al.* (2000) reported large difference in degradation rates of chlorpyrifos in soils, with the fastest rate observed in soil with higher pH and cation exchange capcity.

Singh *et al.* (2003) found that degradation of chlorpyrifos in an acidic soil was slow, especially in soil with pH 4.7 where the half life was 256 days. Chlorpyrifos degradation at pH 5.7 was somewhat faster with a half life of 58 days. The chlorpyrifos degradation was rapid in two alkaline soils (pH 7.7 and 8.4) with a half life of 16 days in both of them.

2.3.2 Effect of Organic Matter

Organic matter plays an important role in the persistence of many insecticides in soil especially by increasing adsorption on organic colloid and reducing the absorption of systemic insecticides by plants. Soil organic matter was reported to be the most important single factor influencing the adsorption of pesticides in soil (Ramalingam, 1989).

Benson and Long (1991) in their study on the influence of humic content on acute toxicity of organophosphate insecticides showed that in the presence of humic acid, the toxicity of chlorpyrifos were greatly reduced, whereas toxicity of parathion increased.

Racke and Robbins (1991) reported that the rate of TCP degradation varied between soils and was related with organic matter content and glucose.

Ritcey *et al.* (1991) reported that fenofos, chlorpyrifos and isofenfos were more persistent in organic soil and with the exception of chlorpyrifos all had declined to less than half the original level 96 days after application.

Rouchaud *et al.* (1991) observed that the rate of biodegradation of chlorpyrifos was slower in organic fertilizer treated soil than untreated soil.

Lemmon and Pylippiw (1992) studied the persistence of pesticides after composting with grass clippings. They did not detect residues of any pesticide shortly after application while they appeared quickly after composting.

Rouchaud *et al.* (1992) reported that insecticides like chlorpyrifos and carbofuran showed greater persistence in organic fertilizer treated plots compared to the untreated plots. This is because the organic fertilizer increased the pesticide adsorption on to the soil organic matter, protecting the insecticide against the metabolizing soil microbial activity.

Horst *et al.* (1996) observed little chlorpyrifos movement through the thatch layer to the underlying soil. In a 113 days experiment, chlorpyrifos was retained mainly in the top layers (0–2 cm thatch layer and 2–10 cm mat layer) during this period. This implies that the insecticide was retained in the thatch and mat layers because these layers have much higher organic matter content and consequently higher potential for adsorption.

Vandervoot (1997) showed a similar result of decreasing concentration of chlorpyrifos, 2,4-D, isoxaben, trichlorpyr, chlopyralid and fluprimidol after composting with grass clippings.

Huang Xing Jiang *et al.* (2000) reported that animal lagoon effluent are good sources of organic matter, however they affect the degradation and transport of soil applied pesticides. Chlorpyrifos was degraded by aerobic microbial processes in animal derived lagoon effluent.

Organic carbon content and relatively low pH of the soil greatly affected the release of residues of chlorpyrifos (Rahman and Motoyoma, 2000).

Jones and Huang (2003) demonstrated relationship between humic substances interaction with both organic and inorganic pesticides and pesticide toxicity in the natural system and they suggested that pesticide toxicity in the natural waters can be reduced by compost humic addition in the contaminated ground water and surface water.

Wu *et al.* (2002) reported that chlorpyrifos is strongly adsorbed, especially in the thatch and mat layers having high organic matter.

Singh (2003) observed that addition of organic matter increases adsorption of pesticides and decreases their subsequent mobility in the soil profile.

Benzal (2004) reported that there exists a significant positive correlation between soil organic matter content and pesticide

concentration denoting the more adsorption and persistence of pesticides by soil organic matter.

In a study Smith and Rust (1992) found that increase in clay or organic matter content in soil reduced the efficacy of chlorpyrifos.

2.3.3 Effect of Soil Moisture and Temperature

These are factors which influence the breakdown of insecticides in soil, both directly and indirectly. The direct effect results from dilution and chemical interaction and the indirect from their effects on existing soil microflora. The bacterial numbers in general increase with increasing moisture content and change in temperatures.

Moisture content is important because it controls the oxygen levels in soil. Adequate moisture and temperatures are essential for most biological and non-biological processes (Brock, 1970).

Awasthi and Prakash (1997) reported that chlorpyrifos was degraded rapidly in air dried soil and slower in soils at field capacity and under submerged conditions.

Getzen (1981) revealed that chlorpyrifos degraded four times faster at 35°C than at 15°C.

Miles *et al.* (1984) examined persistence of chlorpyrifos and chlorfenvinfos in sterile and natural mineral and organic (muck) soil at four moisture levels, *viz.*, air dry, 20, 40 and 60 per cent of moisture holding capacity at 28°C and found that chlorpyrifos was fairly stable in sterile soil with 50 per cent remaining at the end of the 24 week long experiment and dry soil chlorpyrifos disappeared more rapidly and less from three moist soils.

Getzen (1985) reported that chlorpyrifos residues in water based sprays under high mean temperatures disappeared considerably faster than those weathered under low mean temperatures.

Chlorpyrifos methyl (Reldan) residues in wheat stored at moisture contents of 10 or 13 per cent at 25, 30, 35 and 40°C showed a decline in residual concentration with the degradation most rapid at 40°C and 13 per cent moisture content and slowest at 20°C and 10 per cent moisture (Masud and Praveen, 1991).

Click and Coats (1993) found that soil moisture greatly affect mineralisation of insecticides. The highest percentage mineralisation occurred in soil maintained near field capacity (0.3 bars), while thelowest mineralisation occurred in soil maintained under drier condition (3 bars).

Racke *et al.* (1994) reported that temperature also had a marked effect on chlorpyrifos degradation rate, which approximately doubled with every 10°C increase in temperature.

Racke *et al.* (1996) reported that chlorpyrifos hydrolysis was greatly accelerated under low moisture conditions both in acidic and alkaline soil.

Rahman and Motoyama (2000) demonstrated that degradation of chlorpyrifos was slower at low temperatures and was markedly stimulated by increasing temperature.

Aylmore *et al.* (2001) studied the persistence of chlorpyrifos and chlorfenvifos in sterile and natural mineral and organic soil at 3, 15 and 28°C for 24 weeks. In sterile soil, chlorpyrifos was the least stable at 28°C with 67 per cent remaining in muck and 38 per cent in sandy loam after 24 weeks. In natural soil there was a marked difference in persistence at 3°, 15° and 28°C. Chlorpyrifos in muck soil had half lives of greater than 24, 15, and 6 weeks and in loam 16, 6 and 2.5 weeks at 3, 15 and 28°C, respectively.

2.3.4 Soil Depth

The rate of degradation in general increased with depth in the soil profile. The time for 50 per cent loss of chlorpyrifos was found to be 23-

28 days in surface soil and 7-10 days in the sub surface soil (Racke *et al.*, 1996).

Aylmore *et al.* (2001) reported that the degradation of chlorpyrifos was slower in sub soil (25-50 cm) than in the surface soil (0-25 cm). It could be due to the interactive effect of changes in soil microorganisms and organic matter in the different soil layers.

2.3.5 Effect of Microbes

Microbial degradation is the major pathway deciding the of fate of chlorpyrifos in soil. The relationship between high pH and rapid abiotic hydrolysis is poor, since high pH soils fail to hydrolyse chlorpyrifos when sterilized, suggesting an active involvement of microorganisms in degradation (Racke *et al.*, 1990).

Sikora *et al.* (1990) reported that insecticide treated soils had higher acid phosphatase activity which enhanced biodegradation by microbes.

Flavobacterium sp., ATCC 27551 isolated from diazinon-treated rice fields and *Arthrobacter* sp. isolated from flooded soil treated with methyl parathion were able rapidly to degrade 10 $\mu g\ ml^{-1}$ chlorpyrifos added to mineral salts medium as sole carbon source or to soil under flooded and unflooded condition. In the mineral salts medium degradation was complete in 24 h with Arthrobacter and 48 h with Flavobacterium (Al-Minaha, 1998).

Thirteen fungal species isolated from soil treated with pesticides were tested for their ability to mineralize and degrade three organophosphate insecticides chlorpyrifos and chlorfenviphos in liquid media free from phosphorus (P) and sulphur (S) it was seen that all fungal species grew successfully on the culture media treated with the three used doses of insecticides (10, 50 and 100 ppm ai). At 10 ppm level, insecticide degradation was the highest with all fungi tested (Omar, 1998).

The ability of *Flavobacterium* sp. to utilize organophosphorus compounds as sole carbon source was demonstrated for parathion with a degradation rate of almost 100 per cent after 30 min and for chlorpyrifos with a degradation rate of 33 per cent after 48 h of incubation. The products of hydrolysis of these compounds, P nitrophenol and 3, 5, 6 trichloro-2-pyridinol accumulated in the medium and were not used as substrates for growth by *Flavobacterium* (Gaberleit *et al.*, 2000).

Sterilization of two Andosol soils greatly reduced the rate of chlorpyrifos degradation indicating the involvement of microbial activities (Rahman and Motoyama, 2000).

Singh *et al.* (2002) while examining the role of microorganism in the degradation of chlorpyrifos in soil found a microbial population that utilized chlorpyrifos as a source of carbon in the soil. Two strains found to be associated with enhanced degradation are *Arthrobactor* and *Pseudomonas.*

A study on the degradation of chlorpyrifos using immobilized recombinant *E. coli* conducted by incubating chlorpyrifos with immobilized cells at 37°Cit was found that their was 25 per cent degradation after 1 h and 45 per cent after 8 h (Quioa *et al.*, 2003).

2.3.6 Movement in Soil

Chlorpyrifos residue (0.02 $\mu g\ g^{-1}$) was found at soil depth as far as 25 cm in 14 days of application indicating significant downward movement of this chemical through the soil (Medina, 1991).

Rachman (1994) studied the movement of chlorpyrifos in the soil and found that the compound moved very rapidly in short time after application of insecticide before the first step of adsorption. After a few hours the movement was controlled by adsorption and degradation. Adsorption of chlorpyrifos by soil constituents lowered the degradation process.

2.4 EFFECT ON SOIL MICROORGANISMS

In a lab experiment Wiles *et al.* (1996) found that residues of chlorpyrifos were toxic to four species of *Collembola candida and* with 60-80 per cent mortality on the first day after treatment on sandy and sandy clay loam soils under cropped and non-cropped situations.

Csinos (1985) studied the antifungal effect of chlorpyrifos and its products and found that the hydrolysis product of chlorpyrifos, TCP, reduced radial growth of fungus at 1 $\mu g\ ml^{-1}$, sclerotium formation at 1-25 $\mu g\ ml^{-1}$ and sclerotial germination at 1-10 $\mu g\ ml^{-1}$.

Racke *et al.* (1988) reported the property of TCP, the primary metabolite of chlorpyrifos to inhibit both bacterial and fungal population and thus microbial degradation of pesticide.

Martinez-Toledo *et al.* (1992) reported that the populations of fungi and denitrifying bacteria were not affected by normal doses of methyl pyrimofos and chlorpyrifos but doses of 10-30 $\mu g\ g^{-1}$ of insecticides lead to the reduction in aerobic nitrogen fixing bacteria and nitrogen fixation.

Pozo *et al.* (1995) showed that the presence of 2, 3.5, 5 and 10 $kg\ ha^{-1}$ of chlorpyrifos significantly decreased aerobic dinitrogen fixing bacteria and nitrogen fixation, particularly after second insecticide treatment. The total number of bacteria increased significantly at a concentration of 2-10 $kg\ ha^{-1}$. Activities of acid and alkaline phosphatase and dehydrogenase decreased initially but recovered after 14 days to a level similar to that of control soil without chlorpyrifos. Population of fungi, nitrifying and denitrifying bacteria were not affected consequent to addition of chlorpyrifos.

Pandey and Singh (2000) observed short term inhibitory effect on the total bacterial population after chlorpyrifos application in groundnut field, which recovered within 60 days of soil treatment. The fungal population was significantly enhanced after chlorpyrifos treatment.

Singh *et al.* (2002) reported that the microbial parameters (enzyme activity and total microbial biomass) were stable in the pesticide free control soil throughout 90 days of incubation period and the effect from chlorpyrifos on soil microbial characteristics were either very small or insignificant.

Study of sensitivity of instantaneous rate of population increase of Collembola to toxicants like cadmium, copper, pyrene and Chlorpyrifos showed that calculated instantaneous rate of population, LC_{50} and EC_{50} values were low for chlorpyrifos and hence it is most toxic (Herbert *et al.*, 2004).

2.5 EFFECT ON ROOT NODULATION

Prabakaran and Ramaswamy (1990) showed that seed treatment with seven pesticides including chlorpyrifos in green gram and blackgram reduced rhizobium population to 67-99 per cent in greengram and 95 – 99 per cent in blackgram and also the nodulation in these crops.

Revellin *et al.* (1992) reported that chlorpyrifos and lindane mixture did not show any detrimental effect on *Brady bacterium japonicum*. Early nodulation was not found to be impaired in soil experiments conducted using 1-2 times the normal rate of commercial formulation.

Root biomass, root density and root replacement were reduced by chlorpyrifos application. The reduced root biomass reduced the root nodulation as well (Dawson *et al.*, 2001).

2.6 EFFECT ON SOIL FERTILITY

Sud *et al.* (1998) in their study on potato using phorate and chlorpyrifos in acidic soil showed a higher residual bray phosphorus (2-20 ppm) and available sulphur (1-8 ppm) content in pesticide treated soil than control at 10-20 days after application.

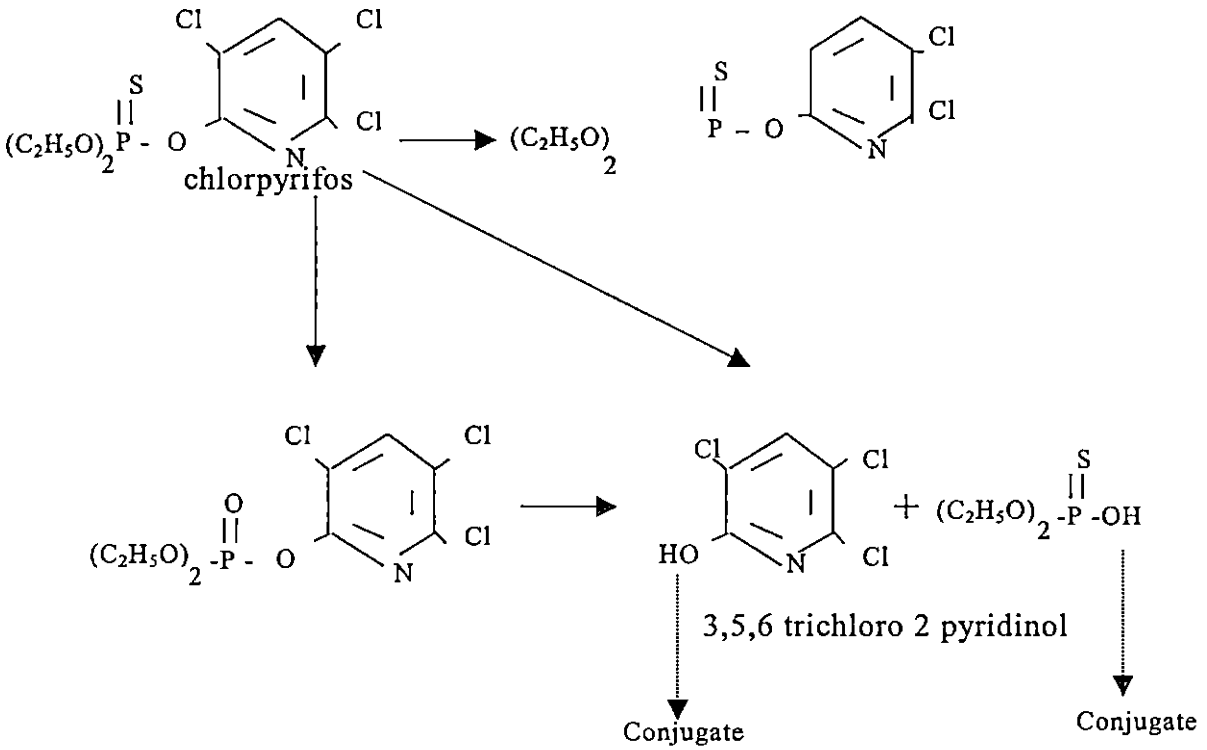
The urea mineralisation was found to be has significantly inhibited, (3.15-24.5 per cent) by the application of pesticides and it is comparatively higher in acid soil (Chandra and Priyander, 2002).

2.7 PERSISTENCE OF CHLORPYRIFOS IN PLANTS

Ritcey *et al.* (1991) showed residues of chlorpyrifos in immature onion bulbs (64-76 days after seeding) with the level of residue being much higher in the roots and outer skin. Ninety six days after seeding, insecticides residues in the bulb were below the level of detection.Metabolic pathway of chlorpyrifos in plant is presented in the Fig.2

Singh and Battu (2003) estimated the residue of chlorpyrifos in different components of paddy ie, polished rice grain, rice bran, rice husk and also straw. The result revealed high concentration of chlorpyrifos in rice husk as compared to rice bran.

Fig. 2. Metabolic pathway of chlorpyrifos in plant



MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation entitled "Dissipation of chlorpyrifos in red loam soil and its effect on soil organisms" has been carried out to study the dissipation pattern of chlorpyrifos in red loam soil at varying levels of organic matter and lime in presence of a legume crop and also the effect of chlorpyrifos on soil microflora and arthropods. The study also envisaged the effect of chlorpyrifos on nodulation in legumes.

3.1 EXPERIMENTAL DETAILS

3.1.1 Location and Soil

The experiment was conducted at the fourth block of Instructional Farm, attached to the College of Agriculture, Vellayani. The site is situated at 8° 30' N latitude, 76° 54' E longitude and at an altitude of 29 m above mean sea level. The soil was "fine loamy kaolinitic isohyperthermic, typic Kandiustult".

3.1.2 Preparation and Layout

The field was ploughed and divided into 30 plots. The plots were separated with inner bunds of 30 cm thickness and outer bunds of 60 cm thickness. The plots were ploughed well and levelled before planting. The crop planted for the experiment was cowpea (grain type). Layout of experimental plot is given in the Fig. 3.

Design	-	RBD
Treatment	-	10
Replication	-	3
Net plot size	-	1.5 m x 1.5 m
Crop	-	Cowpea
Variety	-	Bhagyalekshmi
Spacing	-	30 x 15 cm

R_1T_8	R_1T_4	R_1T_2	R_1T_7	R_1T_9	R_1T_1	R_1T_5	R_1T_3	R_1T_6	R_1T_{10}
R_2T_{10}	R_2T_1	R_2T_6	R_2T_4	R_2T_8	R_2T_3	R_2T_2	R_2T_7	R_2T_9	R_2T_5
R_3T_1	R_3T_9	R_3T_5	R_3T_3	R_3T_6	R_3T_4	R_3T_7	R_3T_8	R_3T_2	R_3T_{10}

Treatment -10 Replication -3 Design -RBD

Fig. Layout plan of the experimental field

3.1.3 Treatment

The treatment consisted of chlorpyrifos applied at the rate of 3 ml l^{-1} in various combinations as detailed below.

T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}

T_2 – T_1 + organic manure @ 20 t ha^{-1}

T_3 – T_1 + organic manure @ 10 t ha^{-1}

T_4 - T_1 + lime @ 600 kg ha^{-1}

T_5 – T_2 + lime @ 600 kg ha^{-1}

T_6 – T_3 + lime @ 600 kg ha^{-1}

T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}

T_8 – Crop + organic manure @ 20 t ha^{-1} + Chlorpyrifos 20 EC @ 3 ml l^{-1}

T_9- chlorpyrifos 20 EC @ 3 ml l^{-1}

T_{10}- Control

The treatments were replicated thrice. Farm Yard Manure was used as the organic manure. Urea (46 per cent N), Rock phosphate (20 per cent P_2O_5) and Murate of Potash (60 per cent K_2O) were used as chemical nutrient sources in treatments T_1, T_2, T_3, T_4, T_5 and T_6 as per POP recommendation. Chlorpyrifos 20 EC @ 3 ml l^{-1} was applied in all plots except control. Each plot was treated with 15 L of pesticide solution prepared from chlorpyrifos 20 EC formulation. The plots were irrigated two days prior to treatment application and maintained at field capacity.

3.1.3.1 Standardisation of Treatment Volume

Quantity of treatment volume needed to wet 15 cm soil depth was determined by drenching 30 cm x 30 cm area with known quantity of water.

It was found that 600 ml water was required to wet 15 cm soil when applied in an area of 30 cm x 30 cm. Accordingly, the volume of pesticide solution required per plot in an area of 2.25 m^2 was estimated as 15 L.

200 ml - 5 cm

400 ml - 10 cm

600 ml - 15 cm

ie, 0.09 m^2 - 600 ml

for 2.25 m^2 - $\frac{0.6 \times 2.25}{0.09} = 15$ L

3.1.4 Sampling

Samples of soil were taken at 0, 5, 10, 20, 40 and 60 days after treatment of pesticide and also after the harvest of crop (75 days), at 0-15 and 15-30 cm depth by using a core sampler. The collected samples were homogenised and used immediately for the estimation of soil arthropods and microbes. The samples intended for residue estimation were stored in a deep freezer at sub- zero temperatures.

3.2 RECOVERY EXPERIMENT

A recovery experiment was carried out to determine the efficiency of the analytical procedures adopted during the experiment.

3.2.1 Preparation of Standard Spray Solution

Measured volume of chlorpyrifos 20 EC formulation supplied by Excel Crop Care Ltd., Mumbai (Tricel 20 EC) was dissolved in water so as to obtain 1000 ppm solution. An aliquot of 10 ml was diluted to 100 ml with water to get 100 ppm solution. Both of these stocks were kept in refrigerator for further use.

3.2.2 Fortification of Soil with Chlorpyrifos

Fifty gram of soil was taken in a 250 ml conical flask and spiked separately with 0.5 and 1.0 ml of 100 ppm solution to get the deposit of 1

and 2 μg g^{-1} of chlorpyrifos in soil. The flask containing spiked soil was homogenised for few minutes, extracted and analysed by selected procedures for choosing the best one for the estimations of the study.

3.2.3 Preparation of Analytical Standard

A weighed amount of technical grade chlorpyrifos (95.8 %) supplied by Excel Crop Care Ltd., Mumbai was dissolved in distilled acetone and made up with distilled hexane to obtain 1000 ppm solution. An aliquot of 10 ml was diluted to 100 ml with distilled hexane to get a 100 ppm stock solution. From this 1 ppm standard solution was prepared for use as reference standard during analysis.

3.2.4 Recovery of Chlorpyrifos

A recovery experiment was conducted to standardize the procedure for extraction and cleanup process of the experiment. The experiment was conducted by adding a known quantity of chlorpyrifos to soil and trying the extraction process using different solvents / solvent system.

A 25 g sample of the air dry soil was spiked with 2.5 ml of standard solution containing 10 μg ml^{-1} of chlorpyrifos in triplicate. A similar sample was taken and spiked with 5 ml of the same standard triplicate. These treatments represent a concentration of 1 and 2 μg g^{-1} in soil. These samples were then extracted by using different solvents to assess the extraction efficiency and thereby standardizing the procedure for extraction.

The soil samples (three sets in triplicate) were extracted separately using three solvents *viz.*, acetone, acetone + hexane (10 per cent) and acetone + Dichloro methane (10 per cent). The samples were extracted thrice, combined, concentrated using vacuum flash evaporator and after the complete removal of solvents, the residue were partitioned to n-hexane, concentrated at low temperature and subjected to cleanup for

removal of co-extractives which otherwise might interfere in the estimation.

3.2.4.1 Clean up

The cleanup of concentrated extract was tried using three different adsorbents. The materials used as adsorbents *viz.*, alumina, activated charcoal and florisil were activated at 105° C for 2 hours and cooled under moisture free environment. A 10 g of the activated adsorbent was loaded on a column (60cm x 1.5cm) above a layer of activated sodium sulphate (Na_2SO_4). The concentrated hexane extract was loaded on top of the adsorbent and was then eluted with distilled hexane till 30 ml eluate was collected. It was then made upto 25 ml and the residues were estimated in GLC (ECD). The solvent system and adsorbent which gave satisfactory recovery of >80 per cent was adopted for all analytical purpose of the study.

3.3 ESTIMATION OF CHLORPYRIFOS RESIDUE IN SOIL

The estimation of chlorpyrifos residue in soil was carried out in two steps.

3.3.1 Extraction

A 15 g soil sample was taken in a conical flask to which 50 ml of acetone was added and shaken for 1 hour in a rotary shaker. The acetone extract was collected in a beaker and the process was repeated three times, the extracts combined and concentrated at low temperature in a rotary vacuum evaporator.

The aqueous residue obtained was diluted using 50 ml of distilled water containing 5 g sodium chloride (NaCl) and transferred to a separating funnel (250 ml). The organic residue was extracted using 25 ml n-hexane. The residues were partitioned three times and the hexane extracts were collected through anhydrous sodium sulphate ($Na_2 SO_4$) into a conical flask. The combined extracts were concentrated to dryness at

low temperature and made upto 15 ml using distilled hexane and subjected to clean up for removing the co- extractives. Extractions were also tried using dichloro methane (CH_2Cl_2) and n- hexane and the per cent recovery obtained in each case was assessed.

3.3.1.1 Clean up

The co-extractives present in the hexane extract were removed by passing the concentrated extracts through a column (60 cm x 1.5 cm) packed with activated acidic alumina overlying a layer of anhydrous sodium sulphate (Na_2SO_4). The column was then eluted using 25 ml distilled hexane. It was then concentrated to 15 ml and used for estimation of residue.

3.3.2 Estimation

Estimation of residue was done in a Gas Liquid Chromatograph (Shimadzu-2010) equipped with $^{63}N_i$ ECD. The following parameters were found optimum for estimation of chlorpyrifos.

Column (capillary)	-	BP-1
Length	-	30 m
Temperature	-	230° C
Detector temperature	-	300° C
N_2 (IOLAR) flow rate	-	1.08 ml min^{-1}
Volume injected	-	1 µl
Retention time	-	3.30 min

3.4 RESIDUE QUANTIFICATION

$$\text{Residue} = \frac{\text{Peak area of sample} \times \text{concentration of Std} \times \text{Final volume of extract}}{\text{Peak area of Std} \times \text{Weight of sample in gram} \times \text{Volume injected in µl}}$$

3.5 CALCULATION OF HALF LIFE PERIOD

Theoretically residues should decrease logarithmically since the amount lost per unit time should be proportional to the total present at any time provided all were equally exposed to weathering, degradation reaction etc. (Hoskins, 1981). When log of residue was plotted against time elapsed a linear trend could be observed. This means that log D can be represented as a liner function of 't' where D is the residue in ppm at time 't', 't' being expressed in week or days. Thus the model is log $D = K_1E + \log K_2$, which means that $D = k_2$. Thus k_2 estimates the initial deposits. The time taken to reduce the deposit to D/2, which is defined as the time required for half of the given quantity of material to react or dissipate is calculated on $t½ = \log 2/k_1$.

3.6 PESTICIDE RESIDUE IN THE POD

The pods harvested on 30th and 45th day after treatment were dried, powdered and a sample of 10 g was used for estimation of residue. The sample was thoroughly extracted using 25 ml acetone, 10 ml acetone + 15 ml dichloromethane followed by 25 ml dichloromethane. The extracts were combined, concentrated at low temperature and the aqueous residue was diluted with 25 ml distilled water containing 2.5 g NaCl. It was extracted sequentially thrice using 25 ml distilled hexane each time, combined, concentrated and cleaned by column chromatography as mentioned in section 3.3.1.1. The cleaned extracts were made up to 10 ml and the residues were estimated in GLC (ECD) as mentioned in section 3.3.2.

3.7 SOIL ANALYSIS

Soil sample collected before treatment application and after harvest of the crop were analysed for pH, electrical conductivity, cation exchange capacity, bulk density, particle density, water holding capacity, field

Table 1 Analytical methods followed in soil analysis

Sl. No.	Parameter	Method	Reference
1	Texture	Interntioanl pipette method	Piper (1966)
2	Bulk density	Core method	Gupta and Dakshnamoorthy (1980)
3	Particle density	Pycnometer method	Gupta and Dakshnamoorthy (1980)
4	Water holding capacity	Core method	Gupta and Dakshnamoorth (1980)
5	Field capacity	Core method	Gupta and Dakshnamoorth (1980)
6	pH (soil : water) 1 : 2.5	pH meter with glass electrode	Jackson (1973)
7	EC (soil : water) 1 : 2.5	Conductivity meter	Jackson (1973)
8	CEC	N ammonium acetate method	Jackson (1973)
9	Organic carbon	Walkley and Black method	Jackson (1973)
10	Total nitrogen	Digestion with H_2SO_4 and Microkjeldhal distillation method	Jackson (1973)
11	Total phosphorus	Nitric – Perchloric digestion + colorimetry	Jackson (1973)
12	Total potassium	Nitric – Perchloric digestion + Flame photometry	Jackson (1973)
13	Total calcium	Nitric – Perchloric digestion and estimation with versenate method	Jackson (1973)
14	Total magnesium	Nitric – perchloric digestion and estimation with versenate method	Jackson (1973)
4.	Available N ($KMnO_4$ – N)	Alkaline permanganate method	Subbiah and Asija (1956)
5.	Available P	Bray No. 1 extraction and photoelectric colorimetry	Jackson (1973)
6.	Available K	Neutral normal ammonium acetate extraction and flame photometry	Jackson (1973)

capacity moisture content and soil nutrients such as organic carbon, total contents of nitrogen, phosphorus, potassium, calcium and magnesium following standard analytical procedures. Analytical method followed in soil analysis are presented in the Table 1.

3.8 ESTIMATION OF MICROBIAL POPULATION

3.8.1 Soil Collection

Pre- treatment soil samples were taken two hours before insecticide treatment and post- treatment samples at 5, 10, 20, 40, 60 and 75 (harvest) days after treatment and also before treatment at 0-15 and 15-30 cm depth using core sampler.

3.8.2 Enumeration of Bacteria and Fungi

The total counts of bacteria and fungi were assessed by serial dilution plate technique (Johnson and Curl, 1972). The soil extract agar (Lochhead, 1940) and Rose Bengal Agar (Martin, 1950) were used for isolation of bacteria and fungi respectively.

3.8.2.1 The Composition of Media Used for Isolation

Rose Bengal Agar

Dextrose	–	10 g
Peptone	–	1 g
KH_2PO_4	–	1 g
$MgSO_4.7H_2O$	–	0.5 g
Rose Bengal	–	33 mg
Agar	–	15 g
Distilled water	–	1000 ml
Streptomycin	–	30 mg

Soil Extract Agar

Soil extract	–	100 ml
Glucose	–	1 g
$KH PO_4$	–	0.5 g
Agar	–	15 g
Tap water	–	900 ml
pH	–	6.8

One kg of sieved garden soil was mixed with 1000 ml of tap water and steamed in an autoclave for 30 min. A small amount of $CaCO_3$ was added and filtered through a double layered filter paper. The agar (15g) was dissolved in 900 ml of water to which 100ml of stock soil extract solution was added. Then glucose and KH_2PO_4 were added before dispersing in flask. After mixing the components it was autoclaved at 125°C at 25 psi for one hour and preserved for use.

3.8.2.2 Serial Dilution

One gram soil added to 99 ml of sterile distilled water in a conical flask under aseptic conditions and mixed well so as to get 10^{-2} dilution. With a fresh sterile pipette, transferred 1 ml of 10^{-2} dilution into a 99 ml dilution blank and mixed well to obtain a 10^{-4} dilution. Pipette 1 ml of this to a sterile petri plate. Using a sterile pipette transferred 1 ml of 10^{-4} dilution to 99 ml dilution blank and mixed well to obtain a 10^{-6} dilution. 1 ml of this 10^{-6} dilution was transferred to a sterile petridish. The 10^{-4} and 10^{-6} dilutions were used for estimation of fungi and bacteria, respectively. Poured approximately 25 ml of media (Rose bengal agar and soil extract agar) which have been autoclaved and cooled to 45° C in a water bath into these petri plates. Then rotate each plate clockwise and anticlockwise to mix the inoculum and the medium. After the medium has completely solidified, the plates were inverted and kept for incubation at

30° C. The colonies were enumerated after 24 hours in the case of bacteria and 72 hours for fungi.

3.8.3 Estimation of Arthropods

The population of macro arthropods in soil were assessed by counting the number in 1000 g surface soil after spreading as a thin layer on a white paper.

Micro arthropods like collembola and mites were counted using Berlese-Tullgren funnel method (Macfadyen, 1961). For this, a one kg soil sample along with litter materials was taken with minimum disturbance and placed on a wire gauze over a steep sided funnel and the soil was heated gently using 40 watts electric bulb. Heating was continued for a day and the soil arthropods moved down and eventually got collected in a collecting vial kept at the tail of the funnel containing water. This provision was to create a gradient in the relative humidity for soil arthropods to move down. The content in the collecting vial was directly transferred to a counting dish and the population of the collembola and mites were counted under a binocular microscope.

3.9 NODULE COUNT

The effect of pesticide treatment on nodulation was determined by taking nodule count at two and four weeks after treatment. The plants were uprooted carefully by removing the adhering soil using a jet of water without damaging the root hairs and the effective nodules were counted.

3.10 STATISTICAL ANALYSIS

Data relating to each character were analysed by applying the analysis of variance. The significance was tested by F test (Snedecor and Cochran, 1975).

Plate 1. General view of the experimental field

RESULTS

4. RESULTS

A field experiment was conducted to study the dissipation of chlorpyrifos in red loam soil in combination with organic matter and lime in presence of legume crop. The study also envisaged the effect of chlorpyrifos on soil microorganisms and nodulation in cowpea. The results and observations are presented under the following heads.

4.1 PRETREATMENT ANALYSIS

Physical and chemical characteristics of soil used for the study was done as per standard procedures and are presented in the Table 2

The soil was sandy clay loam composed of 49.20 per cent coarse sand, 13.65 per cent fine sand, 7.50 per cent silt and 27.50 per cent clay. The organic carbon content of the soil was 0.61 per cent. The initial soil pH was found to be 5.10 and EC 0.20 dS m^{-1}. The soil was of low fertility.

4.2 RECOVERY OF CHLORPYRIFOS FROM SOIL

The percentage recovery of chlorpyrifos with different extractants have been presented in Table3. Among the different solvents tried, extraction using acetone was found superior to the other two solvent system. The percent recovery with acetone was 89 per cent while that with acetone + hexane (10 per cent) was 82 per cent and with acetone + Dichloro methane (10 per cent) 84 per cent

Among the different adsorbents tried, the efficiency of all of them were found to be almost on par. Among the different solvent system and adsorbents tried, extraction using acetone followed by florisil cleanup was found to be best as evident from Table 5. Recovery with acetone : hexane (9 : 1) followed by activated charcoal cleanup was the lowest. However, considering satisfactory good performance, low cost and easy availability, alumina cleanup was preferred for the study.

Table 2 Physico-chemical properties of soil

1	Texture	Sandy clay loam
2	Mechanical analysis	
	Coarse sand (%)	49.0
	Fine sand(%)	13.65
	Silt (%)	7.50
	Clay (%)	27.50
3	Bulk density (Mg m^{-3})	1.16
4	Particle density (Mg m^{-3})	2.61
6	Water holding capacity (%)	39.33
7	Field capacity (%)	20.76
8	Soil pH (soil : H_2O - 1 : 2.5)	5.10
9	EC (soil : H_2O – 1 : 2.5)	0.21
10	CEC (cmole kg^{-1})	2.90
11	Organic carbon (%)	0.61
12	Total nitrogen (%)	0.07
13	Total phosphorus (%)	0.02
14	Total potassium (%)	0.03
15	Total calcium (%)	0.28
16	Total Magnesium (%)	0.04
17	Available Nitrogen (kg ha^{-1})	150
18	Available phosphorus (kg ha^{-1})	30
16	Available potassium (kg ha^{-1})	75

Table 3. Recovery of chlorpyrifos by different solvents

Solvent / adsorbent system used	Recovery amount added (µg)	Recovery (µg)	Recovery (%)
Acetone + Alumina	12.5	11.0	88
	25.0	22.5	90
Acetone + activated characoal	12.5	10.5	84
	25.0	12.5	86
Acetone + Florisil	12.5	11.5	92
	25.0	23.5	94
Acetone + hexane + Alumina	12.5	10.0	80
	25.0	21.0	84
Acetone + hexane + Activated charcoal	12.5	9.5	76
	25.0	20.0	80
Acetone + hexane + Florisil	12.5	10.5	84
	25.0	22.0	88
Acetone + dichloromethane + Alumina	12.5	10.5	84
	25.0	21.0	84
Acetone + dichloromethane + Activated charcoal	12.5	10.0	80
	25.0	20.0	80
Acetone + dichlormethane + Florisil	12.5	11.0	88
	25.0	22.0	86

4.3 EFFECT OF TREATMENTS ON RESIDUES OF CHLORPYRIFOS IN SOIL

4.3.1 Residues of chlorpyrifos in 0-15 cm soil Depth

The data on the residues of chlorpyrifos persisting upto 15 cm soil in different treatments are presented in Table 4.

In all the treatments the residues of chlorpyrifos in soil declined with time. Maximum residue was observed in treatment T_9, with only 22.7 per cent of the initial residue getting dissipated. The half life of chlorpyrifos observed in the un cropped situation (bare soil) as in T_9 was 235 days and with NPK application alone as in T_1, in presence of a legume crop the half life was reduced to 111 days and 62.6 per cent of the initial residue still remained in soil after 75 days. When it was supplemented with the application of organic manure at the normal rate of 20 t ha^{-1}, the half life got further reduced to 100 days and 44.5 per cent of initial residue got dissipated in 75 days. When organic manure in the form of farmyard manure supplied was reduced to 10 t ha^{-1}, 51 per cent of the residue were remaining after 75 days and only 49 per cent got dissipated with a half life of 90 days. When lime @ 600 kg ha^{-1} was added to the soil, the dissipation got enhanced and 49 per cent of the residue got dissipated in 75 days with a half life of 61.5 days.

In the case of T_5, when lime @ 600 kg ha^{-1} was applied in T_2, the half life of chlorpyrifos obtained was 69.9 days while in T_6, where lime was applied to T_3, the half life observed was 66.9 days. In treatments T_4, T_5, T_6 where lime was applied, 61-69.9 per cent of the initial residues got dissipated in 75 days.

When no materials like organic manure, NPK or lime were added, it was found that only 35.76 per cent of the initial residue got dissipated in 75 days with a half life of 126.3 days (T_7). When NPK application was not

Table 4 Mean residue of chlorpyrifos in soil at 0-15 cm depth ($\mu g\ g^{-1}$)

Treatments	Residue of chlorpyrifos at intervals(Days)							
	0	5	10	20	40	60	75	$t_{½}$
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	44.7	43.3 (3.1)	41.3 (7.6)	37.0 (17.2)	34.0 (23.9)	30.0 (32.8)	28.0 (37.4)	111.19
$T_2 – T_1$ + organic manure @ 20 t ha^{-1}	45.1	41.7 (4.5)	37.3 (17.2)	34.0 (24.6)	32.0 (29.1)	28.7 (36.4)	25 (44.6)	100.13
$T_3 – T_1$ + organic manure @ 10 t ha^{-1}	45.1	40.3 (10.6)	35.3 (21.7)	31.0 (31.3)	29.3 (35.0)	26.7 (40.8)	23 (49.0)	90.04
T_4 - T_1 + lime @ 600 kg ha^{-1}	44.0	36.7 (16.6)	32.0 (27.3)	27.3 (38.0)	23.7 (46.1)	20.0 (54.5)	17 (61.4)	61.46
$T_5 – T_2$ + lime @ 600 kg ha^{-1}	44.3	39.7 (10.4)	35.3 (20.3)	30.3 (31.6)	26.0 (41.3)	22.7 (48.7)	20 (54.9)	69.88
$T_6 – T_3$ + lime @ 600 kg ha^{-1}	45.0	39.7 (11.7)	34.7 (22.9)	29.3 (34.0)	25.0 (44.4)	21.3 (52.6)	20 (55.6)	66.90
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	46.7	44.0 (5.8)	41.3 (5.8)	38.7 (17.1)	34.0 (27.2)	33.0 (29.3)	30 (35.8)	126.46
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	44.7	41.3 (7.6)	34.7 (22.4)	31.0 (30.6)	30.0 (32.9)	28.7 (35.8)	26 (41.8)	113.03
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	45.3	44.0 (2.9)	43.0 (5.1)	41.0 (9.5)	39.7 (12.3)	38.7 (14.6)	35 (22.7)	235.17
T_{10} – Control	-	-	-	-	-	-	-	-

* Figures in parenthesis are percentage decrease in chlorpyrifos residue in soil

done and organic manure @ 20 t ha^{-1} was applied as in T_8, 41.8 per cent of residue got dissipated with a half life of 113.5 days.

4.3.2 Residues of chlorpyrifos at 15-30 cm soil Depth

The data on residue of chlorpyrifos persisting in 15-30 cm soil in different treatments are presented in Table 5

In general, the residues of chlorpyrifos found in the sub soil layer (15-30 cm) were much lower than that found in the upper layer. It could be seen that the dissipation was slow in T_2 where organic matter was applied with only 25.39 per cent of initial residue getting dissipated with a half life of 235.7 days. When organic matter was reduced to 10 t ha^{-1} as in T_3, 35.7 per cent of residues got dissipated with a half life of 152 days.

When lime @ 600 kg ha^{-1} was applied to the soil as in T_4 the dissipation got hastened and 60 per cent of the initial residue got dissipated in 75 days with a half life of 69.26 days. In the case of T_5, when lime was applied in T_2, the half life of chlorpyrifos was reduced to 63.9 days with 60.4 per cent of the residue getting dissipated, while in T_6, where lime was applied T_3, the half life observed was 53.7 days with 64.4 per cent of initial residue got dissipated in 75 days.

When chlorpyrifos alone was applied as in T_7, it was found that 35.89 per cent of residue got dissipated with a half life of 149.2 days. When NPK alone was applied as in T_1 only 29.55 per cent of the residue got dissipated. When organic manure @ 20 t ha^{-1} with out NPK was applied (T_8) 58.3 per cent of residue got dissipated in 75 days with a half life of 55.89 days.

4.4 EFFECT OF CHLORPYRIFOS ON MICROBIAL POPULATION

4.4.1 Bacterial Population at 0-15 cm soil Depth

The effect of chlorpyrifos application on population of soil bacteria at 0-15 cm depth are presented in Table 6

Table 5 Mean residue of chlorpyrifos in soil at 15-30 cm depth (μg g^{-1})

Treatments	Residue of chlorpyrifos at intervals (Days)							
	0	5	10	20	40	60	75	$t_{½}$
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	8.8	7.5 (14.8)	7.3 (17.0)	7.3 (17.6)	7.0 (20.5)	6.50 (26.0)	6.2 (29.6)	199.64
T_2 – T_1 + organic manure @ 20 t ha^{-1}	6.3	5.8 (7.9)	5.5 (12.7)	5.0 (20.6)	5.25 (16.7)	5.00 (20.6)	4.70 (25.4)	235.77
T_3 – T_1 + organic manure @ 10 t ha^{-1}	7.0	6.0 (16.6)	5.3 (24.3)	5.3 (24.3)	5.00 (28.6)	4.70 (32.8)	4.5 (35.7)	152.21
T_4 - T_1 + lime @ 600 kg ha^{-1}	4.0	3.25 (18.8)	2.8 (30.0)	2.5 (37.5)	2.30 (42.5)	2.0 (50.0)	1.6 (68.0)	69.26
T_5 – T_2 + lime @ 600 kg ha^{-1}	4.8	4.0 (16.7)	3.5 (27.1)	3.0 (37.5)	2.50 (47.9)	2.30 (52.1)	1.9 (60.4)	63.96
T_6 – T_3 + lime @ 600 kg ha^{-1}	4.5	4.0 (11.1)	3.5 (22.2)	2.5 (44.4)	2.0 (55.6)	2.0 (55.6)	1.6 (64.4)	53.75
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	7.8	7.0 (10.3)	6.8 (12.8)	6.0 (23.1)	6.0 (23.1)	5.8 (25.6)	5.0 (35.9)	149.27
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	4.8	4.3 (10.4)	3.3 (31.3)	3.0 (37.1)	2.8 (41.7)	2.5 (47.9)	2.0 (58.3	55.87
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	11.3	10.5 (7.0)	9.8 (11.3)	9.5 (15.3)	9.0 (17.7)	8.8 (22.1)	7.3 (35.4)	154.26
T_{10} – Control	-	-	-	-	-	-	-	-

* Figures in parenthesis represents percentage decrease in chlorpyrifos residue in soil

The effect of the application of chlorpyrifos in different treatments on bacterial population in soil revealed that irrespective of the treatments there was a decline in population upto 10 days and an increased there after. In all the treatments the bacterial population reached the original level after about 75 days.

Among the different treatments significant inhibition of bacterial population at five days after application of chlorpyrifos was observed in the case of treatments T_1, T_2, T_3, T_4, T_6 and T_8. while in T_5 and T_7 it was not significant. The percentage decrease in bacterial population showed a marked decline upto 10 days after application and there after reequipped and reached the initial level in 75-80 days. Among the different treatments maximum inhibition was pronounced in treatments T_2, T_3, T_7 and T_8 while in other cases, it was moderate.

At 40 days after application none of the treatments showed any significant difference with regard to bacterial population. The per cent increase in bacterial population was maximum in treatment T_2 (55 per cent) and the least was observed in the treatment T_4 (42.74 per cent). At 75 days after application of chlorpyrifos the bacterial population in top 15 cm soil became equal to the initial population as indicated by an equivalent number of colony forming units.

4.4.2 Bacterial Population at 15-30 cm soil Depth

The effect of chlorpyrifos application on population of soil bacteria in 15-30 cm depth are presented in Table 7

At 15-30 cm soil depth all the treatments showed a significant inhibition of bacterial population at five days after application of chlorpyrifos. The per cent decrease in bacterial population showed a marked decline upto ten days thereafter enhanced and reached the initial level in 75 days. The percentage decrease in bacterial population was high in treatments T_1, T_2, T_3, T_7 and T_8, while in other cases it was moderate.

Table 6 Mean population of bacteria in soil at 0-15 cm depth (x 10^6 Cfu g^{-1} soil

Treatments	population at intervals(Days)						
	0	5	10	20	40	60	75
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	47.67	24.67 (48.25)	29.67 (37.75)	38.33 (19.59)	45.33 (4.90)	49.00 (-2.79)	48.00 (-0.69)
T_2 – T_1 + organic manure @ 20 t ha^{-1}	37.00	16.33 (55.86)	23.66 (36.05)	32.33 (12.62)	37.67 (-1.81)	37.33 (-0.89)	36.33 (1.80)
T_3 – T_1 + organic manure @ 10 t ha^{-1}	44.00	21.00 (52.27)	28.33 (35.61)	36.3 (17.4)	43.67 (0.75)	44.33 (-0.8)	44.67 (-1.5)
T_4 - T_1 + lime @ 600 kg ha^{-1}	40.00	25.00 (47.5)	29.67 (25.8)	35.33 (11.7)	39.67 (0.83)	42.67 (-6.7)	43.67 (-9.2)
T_5 – T_2 + lime @ 600 kg ha^{-1}	48.00	25.33 (47.2)	31.33 (34.7)	34.67 (27.8)	45.00 (6.3)	47.67 (0.7)	48.00 (-)
T_6 – T_3 + lime @ 600 kg ha^{-1}	31.67	17.67 (44.2)	20.33 (35.8)	24.67 (22.1)	29.67 (6.3)	32.00 (-1.1)	32.66 (-3.1)
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	55.00	27.67 (49.7)	34.00 (38.2)	40.00 (27.3)	47.33 (13.9)	53.67 (2.4)	54.33 (1.2)
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	28.33	13.33 (52.9)	18.00 (36.5)	24.33 (14.1)	25.67 (9.4)	29.00 (-2.4)	28.67 (-1.2)
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	32.67	17.33 (46.9)	22.60 (30.8)	28.67 (12.2)	30.33 (7.2)	32.00 (2.1)	32.67 (-)
Control	36.00	37.00	34.33	36.67	35.00	37.33	34.67
CD	12.9689	11.6867	9.68646	10.0987	11.2679	12.3952	13.667

• Figures in parenthesis represents percentage decrease in bacterial population

Table 7 Mean population of bacteria in soil at 15-30 cm depth (x 10^6 Cfu g^{-1} soil)

Treatments	population at intervals (Days)						
	0	5	10	20	40	60	75
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	31.67	16.33 (48. 4)	18.00 (43.2)	22.33 (29.5)	27.33 (13.7)	31.67 (-)	32.00 (-1.0)
T_2 – T_1 + organic manure @ 20 t ha^{-1}	26.00	13.00 (50.0)	16.33 (37.2)	19.00 (26.9)	23.67 (9.0)	26.00 (-)	26.33 (-1.2)
T_3 – T_1 + organic manure @ 10 t ha^{-1}	31.00	16.00 (48.4)	18.33 (40.9)	22.67 (19.8)	27.67 (10.7)	30.33 (2.2)	31.00 (-)
T_4 - T_1 + lime @ 600 kg ha^{-1}	28.67	18.67 (34.9)	21.00 (26.8)	23.00 (26.9)	24.6 (14.0)	28.00 (2.3)	29.00 (-1.2)
T_5 – T_2 + lime @ 600 kg ha^{-1}	31.33	19.00 (39.4)	21.33 (31.9)	23.67 (24.4)	27.67 (11.7)	30.67 (2.1)	32.00 (-2.1)
T_6 – T_3 + lime @ 600 kg ha^{-1}	18.00	11.33 (37.0)	11.67 (37.1)	23.67 (22.2)	15.00 (16.7)	17.00 (5.6)	18.67 (-3.6)
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	36.67	18.67 (49.1)	22.00 (40.0)	24.67 (32.7)	29.00 (21.0)	34.00 (7.3)	36.67 (-)
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	14.67	7.67 (47.7)	8.67 (41.0)	11.00 (25.0)	12.67 (13.6)	14.00 (15.6)	15.00 (-2.3)
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	30.00	14.33 (52.2)	16.00 (46.7)	19.33 (35.6)	22.00 (26.7)	25.33 (4.6)	30.33 (-1.1)
T_{10} – Control	28.67	27.67	28.33	27.67	28.33	28.67	28.33
CD	9.1199	5.5891	6.8266	7.5689	7.3410	8.3829	8.7099

* Figures in parenthesis represents percentage decrease in bacterial population

Among the treatments, maximum inhibition was observed in T_2 with 50.00 per cent of the population getting reduced and in T_3 where 48.30 per cent of the population getting reduced, which was on par with T_1 (48.42 per cent). The lowest inhibition was observed in treatment T_4 with only 34.85 per cent of the population getting reduced. At 75 days after application none of the treatment showed any significant difference with regard to bacterial population inhibition.

4.4.3 Fungal Population

Effect of chlorpyrifos on fungal population in 0-15 cm depth in soil is presented in Table 8 and in 15-30 cm depth is presented in Table 9.

The effect of chlorpyrifos on fungal population in different treatments revealed that there was no significant reduction in the fungal population. Throughout the experimental period the fungal population was almost the same (16.00 - 22.56 x 10^4 cfu g^{-1}). The fungal population at 15-30 cm depth was less compared to 0-15 cm depth. Thus it could be inferred that different treatments and soil depth have no significant effect on the fungal population in the soil by chlorpyrifos application.

4.4.4 Arthropod Population

The effect of chlorpyrifos application on population of soil arthropods are presented in the Table10

The effect of application of chlorpyrifos in different treatments on arthropod population revealed that irrespective of the treatments there was a sudden decline in population upto 10 days and showed slight but gradual increase upto 75 days of application.

All the treatments showed a significant inhibition of arthropod population till five days after application and continued upto ten days and thereafter a slow increase. Among the treatment maximum inhibition was observed in T_2, T_3, T_5, T_7 and the lowest in the case of treatment T_4. In other cases it was moderate. Among these treatments the per cent decrease

Table 9. Mean population of fungi in soil at 0-15 cm depth (x 10^4 Cfu g^{-1} soil)

Treatments	Population at intervals (Days)						
	0	5	10	20	40	60	75
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	18.00	17.67	18.33	17.33	17.33	19.33	18.33
T_2 – T_1 + organic manure @ 20 t ha^{-1}	19.00	19.33	19.67	19.33	19.67	19.67	19.00
T_3 – T_1 + organic manure @ 10 t ha^{-1}	21.00	20.33	20.67	21.00	20.00	21.00	20.00
T_4 - T_1 + lime @ 600 kg ha^{-1}	21.67	22.00	21.33	21.00	21.00	22.67	22.00
T_5 – T_2 + lime @ 600 kg ha^{-1}	18.67	19.00	18.67	19.33	18.00	19.33	19.33
T_6 – T_3 + lime @ 600 kg ha^{-1}	17.00	17.67	18.00	17.67	18.67	18.67	17.67
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	20.67	21.00	21.67	21.00	20.33	22.00	20.00
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	16.00	16.67	16.67	16.33	17.00	16.67	17.00
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	19.67	20.33	19.33	19.00	20.00	18.67	20.67
T_{10} – Control	18.00	18.30	17.67	18.67	18.33	18.00	18.67
CD	4.0629	4.1155	3.9434	5.7843	6.1572	4.3548	6.2373

* figures in parenthesis represents percentage decrease in fungal population

Table 8 Mean population of fungi in soil at 15-30 cm depth (x 10^4 Cfu g^{-1} soil)

Treatments	population at intervals(days)						
	0	5	10	20	40	60	75
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	8.67	9.00	9.33	9.33	9.00	9.00	8.67
T_2 – T_1 + organic manure @ 20 t ha^{-1}	11.00	10.33	11.33	11.33	10.33	11.67	11.67
T_3 – T_1 + organic manure @ 10 t ha^{-1}	11.33	11.67	11.33	11.33	11.67	11.67	11.67
T_4 - T_1 + lime @ 600 kg ha^{-1}	6.33	6.00	7.00	7.00	6.67	7.00	7.33
T_5 – T_2 + lime @ 600 kg ha^{-1}	8.00	8.67	8.67	8.00	7.67	8.33	9.00
T_6 – T_3 + lime @ 600 kg ha^{-1}	11.00	11.67	11.67	11.33	11.67	12.00	11.00
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	6.33	6.00	6.00	7.00	7.67	7.00	7.00
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	8.67	8.00	8.00	8.00	9.33	9.00	8.00
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	11.67	11.67	11.67	11.67	12.33	12.67	12.00
T_{10} – Control	10.00	10.67	9.67	10.00	10.33	9.67	10.67
CD	4.7667	3.9922	3.9922	4.8015	4.6369	3.7095	3.7095

* figures in parenthesis represents percentage decrease in fungal population

Table 10 Mean population of arthropods in soil (kg^{-1} soil)

Treatments	Population at intervals (Days)						
	0	5	10	20	40	60	75
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	19.00	8.33 (56.2)	8.67 (54.4)	8.33 (56.2)	8.67 (54.4)	11.67 (38.6)	12.67 (33.3)
T_2 – T_1 + organic manure @ 20 t ha^{-1}	18.67	6.00 (67.9)	8.33 (55.4)	8.33 (55.4)	10.33 (44.7)	10.00 (46.4)	11.67 (37.5)
T_3 – T_1 + organic manure @ 10 t ha^{-1}	20.33	7.33 (63.9)	8.00 (60.6)	8.67 (57.4)	10.00 (50.8)	11.00 (45.9)	12.00 (41.0)
T_4 - T_1 + lime @ 600 kg ha^{-1}	18.67	11.33 (39.3)	10.67 (42.8)	11.67 (37.5)	12.33 (34.0)	11.00 (41.1)	14.00 (25.0)
T_5 – T_2 + lime @ 600 kg ha^{-1}	19.67	9.33 (52.6)	10.33 (47.5)	10.33 (47.5)	11.67 (40.7)	13.33 (32.2)	14.00 (28.8)
T_6 – T_3 + lime @ 600 kg ha^{-1}	15.00	8.00 (46.7)	8.67 (42.2)	9.00 (40.0)	10.00 (33.3)	11.00 (26.7)	12.00 (20.0)
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	19.67	8.33 (57.7)	8.67 (55.9)	9.00 (54.3)	10.00 (49.2)	11.00 (44.1)	13.00 (33.9)
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	14.33	7.00 (51.2)	7.67 (46.5)	7.33 (48.8)	9.00 (37.2)	10.00 (30.2)	10.67 (25.5)
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	16.60	8.00 (51.8)	8.667 (47.8)	9.00 (45.8)	9.67 (41.7)	10.33 (37.8)	11.11 (30.1)
T_{10} – Control	19.00	18.67	19.00	18.33	20.33	18.00	20.00
CD	3.9062	2.7945	3.2125	2.9981	3.1449	3.1338	3.4978

* Figures in parenthesis represents percentage decrease in arthropod population

in bacterial population was maximum in T_2 with 67.86 per cent of the population getting reduced and minimum reduction was observed in T_4 where 60.69 per cent of the population got affected. After the initial decrease the arthropod population did not recover till 75 days after chlorpyrifos application. At 75 days after application none of the treatment showed significant difference in the arthropod population. The per cent increase in arthropod population was maximum in T_2 with 48.85 per cent population recovering from the application and the least increase was observed in treatment T_4 when only 19 per cent of the population was able to recover.

4.4 EFFECT OF CHLORPYRIFOS ON NODULATION IN COWPEA

The effect of chlorpyrifos on nodulation in cowpea is presented in the Table11.

The effect of application of chlorpyrifos in different treatments on nodulation in cowpea revealed that irrespective of the treatments there was only a slight decline in nodule count compared to control.

4.4.1 Two weeks after chlorpyrifos application

The treatments T_1, T_2, T_3, T_7 and T_8 showed a significant inhibition of nodule count and in other cases it was not significant. The per cent decrease in nodule count was maximum in T_1 with a reduction of 25 per cent and was on par with treatments T_7, T_8, T_3. The lowest inhibition was in treatment T_6 (9.86 per cent) and was on par with T_4 and T_5.

4.4.2 Four weeks after chlorpyrifos application

The treatments T_1, T_3, T_7 and T_8 showed significant inhibition on the nodule count and in the other cases it was not significant. The maximum inhibition was noticed in treatment T_1 (35.14 per cent) and the lowest inhibition was in treatment T_6 with a decrease of 1.34 per cent.

Table 11 Mean nodule count (numbers plant^{-1})

Treatments	Nodule count at intervals (week)	
	2nd week	4th week
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	14.67 (25.2)	16.00 (35.1)
T_2 – T_1 + organic manure @ 20 t ha^{-1}	16.00 (18.4)	21.00 (14.8)
T_3 – T_1 + organic manure @ 10 t ha^{-1}	15.67 (20.4)	19.33 (21.6)
T_4 - T_1 + lime @ 600 kg ha^{-1}	17.00 (13.3)	24.00 (2.7)
T_5 – T_2 + lime @ 600 kg ha^{-1}	17.33 (11.6)	23.33 (5.4)
T_6 – T_3 + lime @ 600 kg ha^{-1}	17.67 (9.9)	24.33 (1.3)
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	15.00 (23.5)	19.00 (23.0)
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	15.00 (23.5)	19.67 (20.2)
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	-	-
T_{10} – Control	19.60	24.66
CD	3.1713	3.4358

* Figures in parenthesis represents percentage decrease in nodulation

4.5 EFFECT OF TREATMENTS ON SOIL FERTILITY

The effect of chlorpyrifos on different soil parameters are presented in Table12.

The sampling for soil analysis was done after the crop harvest. The effect of application of chlorpyrifos in different treatments on the soil parameters revealed that there was only slight increase in the analytical value for different soil parameters as compared to control.

The values for cation exchange capacity, organic carbon and total phosphorus were highest in the case of treatment T_5 and those for total nitrogen and total magnesium were highest for the treatment T_6. Maximum Water Holding Capacity and moisture at field capacity were observed in treatment T_2. Treatment T_4 was found to have the highest total calcium and pH. Electrical conductivity and bulk density were the highest in treatments T_3 and T_8.

4.6 RESIDUES OF CHLORPYRIFOS IN PODS AND GRAINS

The pods and dried seeds of cowpea were thoroughly extracted for estimating the residue of chlorpyrifos present in them and the estimation of residues was done as discussed in section 3.6. The residues present in all the samples were below detectable limits and hence inferred to be free of residues.

Table 12 Effect of treatments on soil properties

Treatments	pH	Electrical Conductivity (dS m^{-1})	Cation ExchangeCapacity (cmol kg^{-1})	Bulk Density (Mg m^{-3})	Water Holding Capacity (%)	Field Capacity (%)
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	5.27	0.22	3.10	1.16	39.30	21.30
T_2 – T_1 + organic manure @ 20 t ha^{-1}	5.20	0.29	3.37	1.23	44.77	24.77
T_3 – T_1 + organic manure @ 10 t ha^{-1}	5.47	0.33	3.47	1.14	42.70	22.50
T_4 - T_1 + lime @ 600 kg ha^{-1}	5.77	0.24	3.43	1.17	40.63	22.46
T_5 – T_2 + lime @ 600 kg ha^{-1}	5.53	0.25	3.48	1.30	44.27	24.03
T_6 – T_3 + lime @ 600 kg ha^{-1}	5.70	0.23	3.43	1.21	41.57	23.63
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	5.20	0.26	2.90	1.20	39.96	21.73
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	5.30	0.27	2.90	1.37	42.50	22.30
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	5.28	0.26	2.85	1.35	42.10	22.00
T_{10} – Control	5.10	0.25	3.00	1.25	39.33	20.00
CD	0.228	0.0589	0.4016	0.1037	2.625	1.3264

Table 12 Effect of treatments on soil properties (contd.)

Treatments	Organic carbon (%)	Total nitrogen (%)	Total phosphorus (%)	Total potassium (%)	Total calcium (%)	Total magnesium (%)
T_1 – Crop + NPK @ 20 : 30 : 10 kg ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	0.795	0.078	0.041	0.026	0.285	0.046
T_2 – T_1 + organic manure @ 20 t ha^{-1}	0.864	0.080	0.043	0.034	0.285	0.051
T_3 – T_1 + organic manure @ 10 t ha^{-1}	0.816	0.084	0.043	0.035	0.363	0.055
T_4 - T_1 + lime @ 600 kg ha^{-1}	0.768	0.072	0.044	0.037	0.384	0.057
T_5 – T_2 + lime @ 600 kg ha^{-1}	1.190	0.081	0.047	0..39	0.357	0.057
T_6 – T_3 + lime @ 600 kg ha^{-1}	1.100	0.104	0.045	0.040	0.316	0.062
T_7 – Crop + Chlorpyrifos 20 EC @ 3 ml l^{-1}	0.704	0.080	0.030	0.030	0.272	0.042
T_8 – Crop + organic manure @ 20 t ha^{-1} + chlorpyrifos 20 EC @ 3 ml l^{-1}	0.892	0.081	0.024	0.033	0.274	0.047
T_9 – Chlorpyrifos 20 EC @ 3 ml l^{-1}	0.850	0.079	0.020	0.030	0.252	0.042
T_{10} – Control	0.850	0.070	0.020	0.029	0.281	0.044
CD	0.2834	0.0135	0.0085	0.00784	0.0963	0.0111

DISCUSSION

5. DISCUSSION

Chlorpyrifos is the most widely used insecticide in India and world. It is mainly used as a soil insecticide for the control of soil inhabiting pests. It is used in soil both under cropped as well as non cropped conditions. The concentration of the insecticide used depends on the situation under which it is used. In Kerala it is recommended to use 20 per cent EC formulation @ 3 ml l^{-1} in order to wet the top 15 cm layer of soil. People resort to the use of several times higher concentration for the control of termites in non-cropped situation. With this background an experiment relating to the persistence of chlorpyrifos in red loam soil was conducted under different situations and the result obtained from the experiment are discussed under the following heads.

5.1 PHYSICO-CHEMICAL PROPERITES OF SOIL

The physico-chemical properties of soil were estimated as per standard procedures. The soil used for the study moderately acidic having a pH of 5.10 and with an electrical conductivity of 0.20 dS m^{-1}. It can be categorized under the textural class sandy clay loam with a water holding capacity of 39.30 per cent and can be identified as fine loamy kaolinitic isohyperthermic, Typic kandiustult. The contents of organic carbon, total nitrogen, total phosphorus, total potassium, total calcium and total magnesium were estimated and it was found that the soil is low in total nitrogen (0.07 per cent), total phosphorus (0.20 per cent) and total potassium (0.03 per cent) and medium in organic carbon (0.6 per cent). The total calcium and magnesium were also found to be low (0.28 per cent and 0.04 per cent, respectively). The moisture percentage at field capacity of this soil under study was 20.76 per cent. Bulk density and particle density were 1.16 and 2.61 Mg m^{-3}. The CEC of the soil was 2.90 c moles kg^{-1}.

5.3 STANDARDIZATION OF ANALYTICAL PROCEDURE

The procedure for the estimation of chlorpyrifos residues in soil were standardized by conducting a recovery experiment using known quantity of standard chlorpyrifos. The extraction and clean up procedures were tried using different solvents and adsorbents and it was found that extraction using acetone followed by clean up using activated alumina was the most suitable for the extraction of chlorpyrifos from soil. This procedure was hence adopted for all residue estimations during the study. The chromatographic conditions standardized were.

	Temperature (°C)
Injector	-250
Column	-230
Detector	-300
N_2 flow rate	-1.08 ml min^{-1}
Retention time	- 3.3 min

The population of bacteria, fungus, arthropods in soil were assessed by serial dilution technique and Berlese-Tullgren funnel method.

5.4 PERSISTENCE OF CHLORPYRIFOS IN SOIL UNDER DIFFERENT TREATMENTS

The effect of different treatments on the persistence of chlorpyrifos in soil was studied at two soil depths namely 0-15 and 15-30 cm.

5.4.1 Persistence of Chlorpyrifos in 0-15 cm Soil Depth

The data on the residues of chlorpyrifos persisting in top 15 cm soil layer (Fig 4) under the different treatments revealed that the persistence was maximum in non cropped soil having half life of 235.17 days. The high persistence in bare soil can presumably be due to the acidic pH of the soil, relatively high proportion of clay and absence of a rhizosphere effect.

The degradation of chlorpyrifos was reported to be mediated mainly by biotic agents (Singh *et al.*, 2002) as well as alkaline soil condition (Singh *et al.*, 2003) which were lacking in soil under study and could be the reason for high persistence observed in T_9 (bare soil). When a legume crop was introduced as a treatment as in T_7 the dissipation got enhanced and the half life obtained was 126.45 days. A faster degradation observed in this case could presumably be mainly due to rhizosphere effect of the legume crop. When compared to other crops legumes can harbor a wide variety of bacteria in association with the root system which by their enzymatic activity caused an enhanced degradation of the compound. When NPK was added to T_7 as in T_1 the degradation was more than that of T_7 which did not receive NPK presumably due to better root growth resulting in a more effective and active root system. When organic matter was added to the soil the degradation got further enhanced. When applied @ 20 t ha^{-1}, the half life obtained was 100.129 days and @ 10 t ha^{-1} the half life obtained was 90.03 days. The organic matter added might have provided a more favourable environment for proliferation of the microorganism or aeration of the soil and both these would have together contributed to a still faster degradation. This observation is contrary to the reports by Huang Xin Jiag *et al.* (2000) on the increased persistence of chlorpyrifos in presence of high organic matter. The enhanced degradation observed in the present study could be the combined effect of the legume crop, its root system, high enzyme activity, high microbial activity and a better aeration of the soil wherein chlorpyrifos would have degraded along with organic matter. No significant difference was obtained in half life between two levels of organic matter addition.

The normal practice of addition of lime to legume crop further enhanced the degradation of chlorpyrifos. In treatments T_4, T_5 and T_6, lime @ 600 kg ha^{-1} was incorporated to the soil which resulted in the reduction in half lives to 61.5, 69.8 and 66.9 days respectively. Further enhancement of dissipation of chlorpyrifos in presence of lime could be attributed

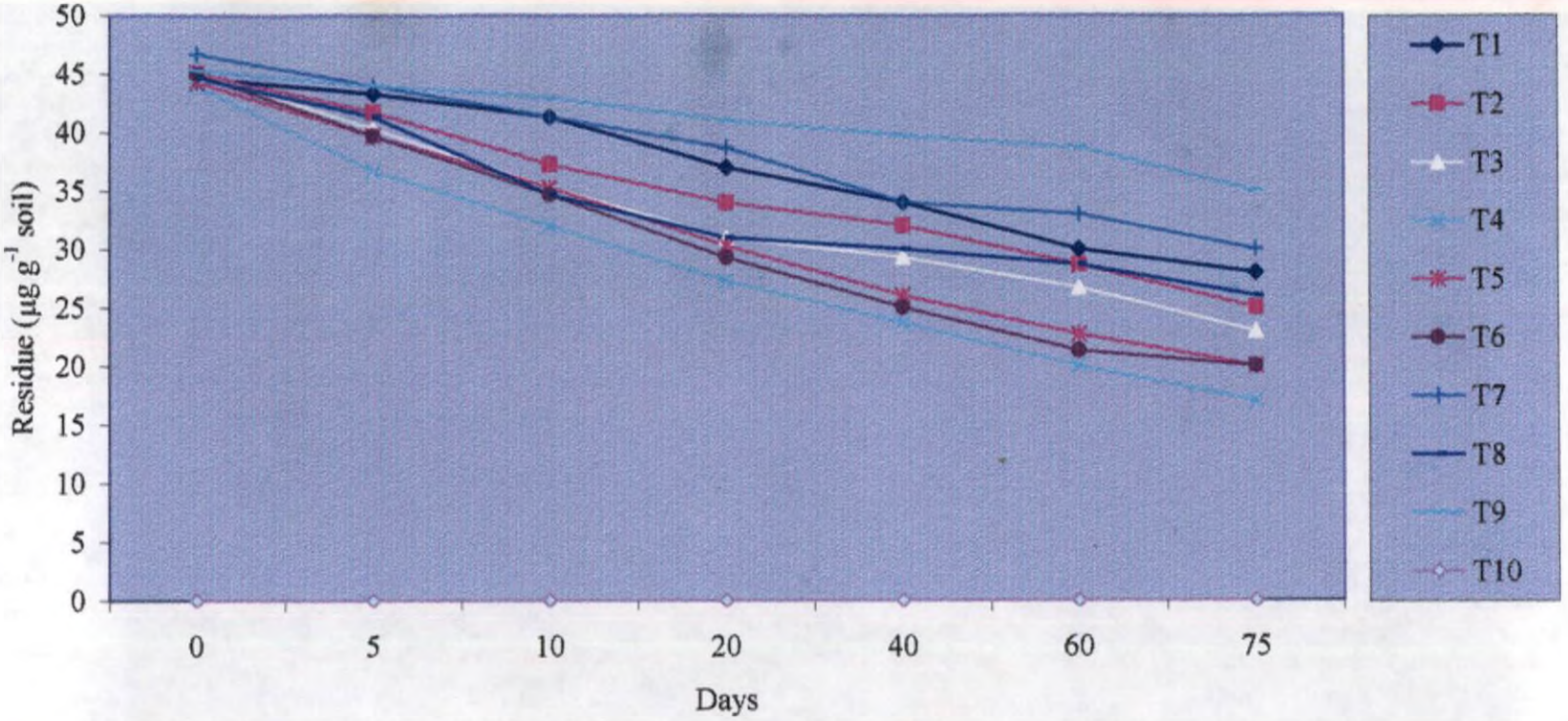


Fig. 4. Dissipation of chlorpyrifos in soil at 0-15 cm depth

directly to the effect of lime, an alkaline material. Most of the pesticides are unstable in presence of alkali and there are several reports on the use of lime to decontaminate the disposal sites of outdated pesticides and other disposal pits (Smith and Rust, 1992). Among these three treatments fastest degradation of chlorpyrifos observed in T_4 might presumably be due to the direct effect of lime without any buffering effect. In treatments T_5 and T_6 organic matter was added @ 20 t ha^{-1} and 10 t ha^{-1} respectively, which could have caused sufficient buffering effect on the action of lime thereby reducing the potency of the effect of lime. When NPK was excluded from the treatment T_2 as in T_8, the degradation declined and the half life of chlorpyrifos got enhanced from 100 to 113. The increased persistence of chlorpyrifos in the absence of NPK could be due to a decline in the plant growth and vigour resulting in a less healthy root system with low activity and decreased rhizosphere effect.

The samples of control plots *ie*, soil without the application of chlorpyrifos showed no residues of it and were totally free of co-extractives also, when analysed in GLC.

From the forgoing discussion it can be summarized that the presence of a legume crop can significantly enhance the degradation of chlorpyrifos in soil. The degradation can be further hastened by the application of NPK, organic matter and lime each separately or together. The maximum effect on the degradation of chlorpyrifos was observed with regard to the application of lime.

5.4.2 Persistence of Chlorpyrifos in 15-30 cm Soil Depth

The residues of chlorpyrifos found in the lower layer of soil (15-30 cm) were much lower than that found in the upper layer (Fig 5). It could be seen that the residues though low, persisted for a longer period in treatments where organic matter @ 20 t ha^{-1} was added with a half life of 235.7 days presumably due to a higher adsorption on to organic matter. When organic matter content was reduced to 10 t ha^{-1} as in T_2 the

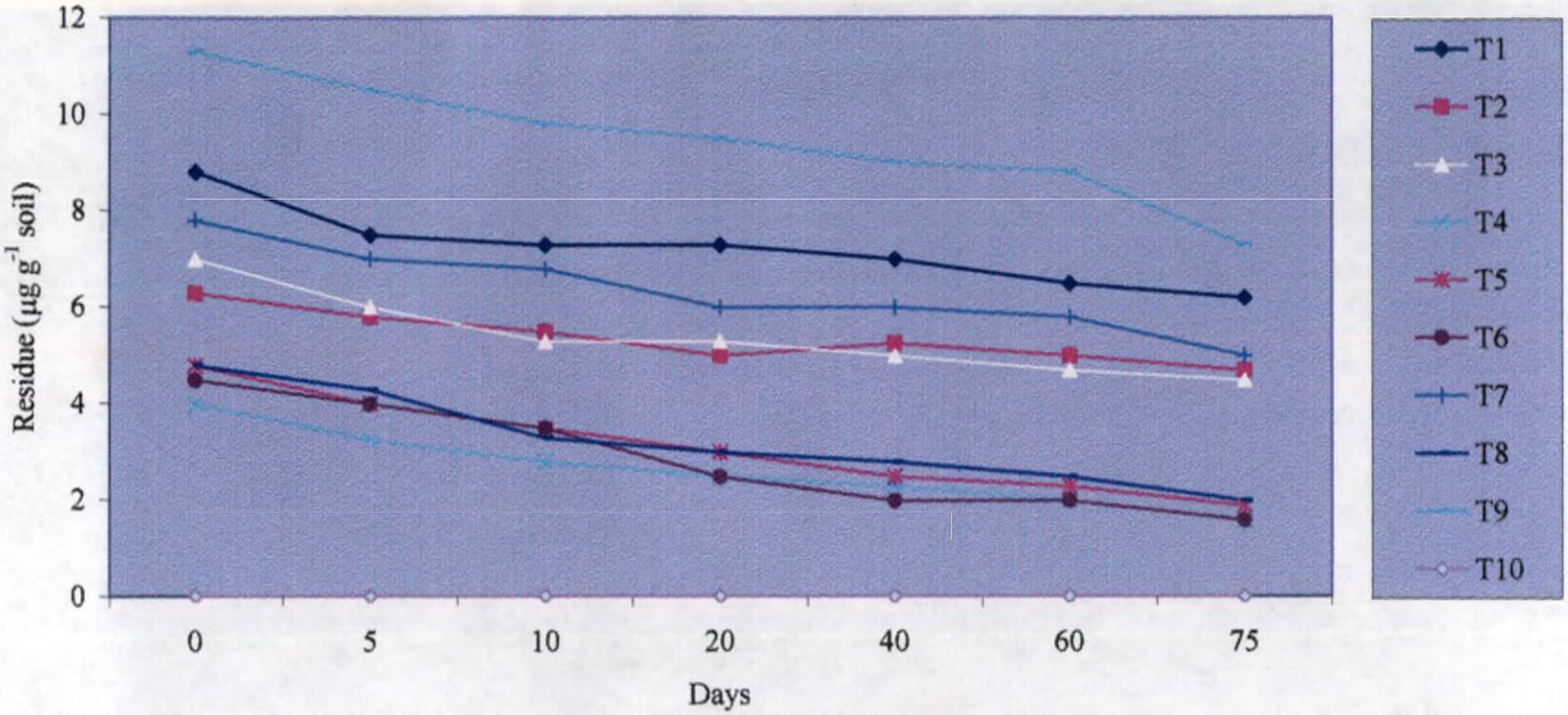


Fig. 5. Dissipation of chlorpyrifos in soil at 15-30 cm depth

degradation got slightly enhanced with a half life of 152.2 days. This enhanced degradation may be the result of low adsorption and high microbial activity. Several reports are available on the high persistence of chlorpyrifos with addition of organic matter (Bensal, 2004). In the absence of organic matter with only NPK application as in T_1 the chlorpyrifos degradation got reduced due to lowered microbial activity in the absence of organic matter with a half life of 199.6 days. When lime was added to the soil @ 600 kg ha^{-1} as in T_4, T_5, T_6, the degradation got enhanced and the half lives observed were 69.2, 63.9 and 53.7 days respectively. The enhancement in the degradation in the presence of lime in treatments T_4, T_5, T_6 were due to faster hydrolysis of chlorpyrifos in alkaline soil. In alkaline condition majority of the pesticides are unstable. Among the treatments maximum degradation was observed in the treatments T_6. The level of degradation in T_8 was on par with T_6.

It can be summarized that in lower soil layers, half life of chlorpyrifos was more indicating a slow rate of degradation. The half life was maximum in treatments where organic matter was added. Addition of lime brought down the half life considerably by enhancing degradation.

5.5 EFFECT OF TREATMENTS ON MICROBIAL POPULATION

5.5.1 Bacterial Population at 0-15 cm Soil Depth

The results of the effect of chlorpyrifos treatment on bacterial population under different soil conditions revealed in the top 15 cm soil layer indicated a marked decline in the population of bacteria immediately after treatment (Fig.6). However after around 75 days the initial population of bacteria was regained. The treatments T_1, T_2, T_3, T_4, T_6 and T_8 showed a significant inhibition on bacterial population upto five days of application of chlorpyrifos, while in T_5 and T_7 it was not significant. The inhibition of bacterial population in the treatments due to chlorpyrifos application could be due to the direct effect of chlorpyrifos on the growth and multiplication of bacteria. A relatively high level of inhibition in T_2

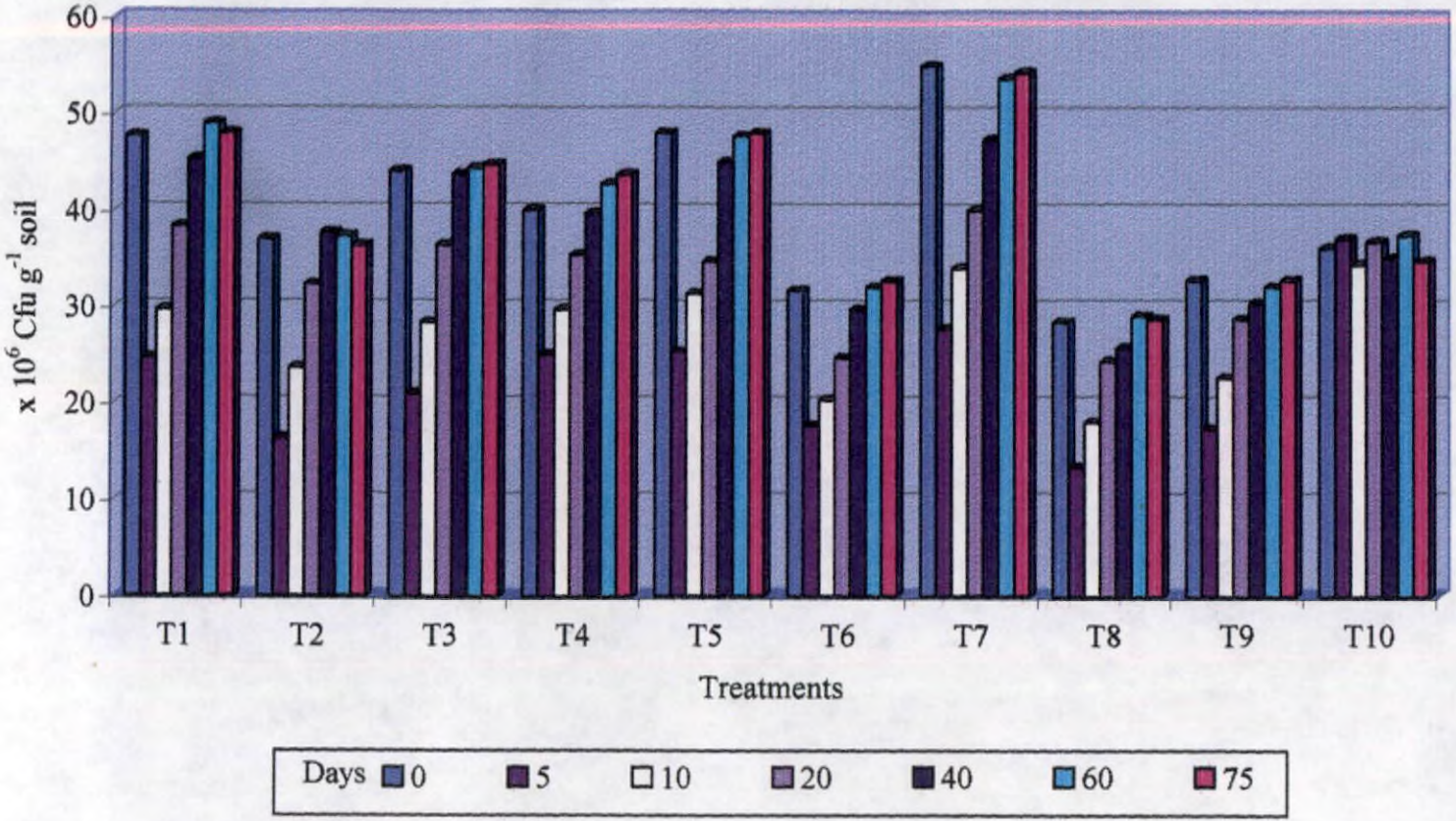


Fig. 6. Population dynamics of bacteria in soil as influenced by chlorpyrifos at 0-15cm depth

and T_3 in the initial stages could be due to a higher adsorption of chlorpyrifos on organic matter fraction. In T_4 and T_5 the inhibition was less due to the presence of lime which caused rapid degradation of chlorpyrifos. However in T_6 there was a very high level of inhibition in the presence of organic matter and lime, presumably due to a lower proliferation of bacteria because of a lower level of organic matter addition. In T_8 a maximum inhibition of bacterial population was observed presumably due to a very high level of adsorption of chlorpyrifos on to organic matter, coupled with the absence of NPK which restricted the root growth this might have resulted in a very poor survival of the bacteria in the soil.

In T_9 also a very high level of bacterial population inhibition was observed similar to T_8. In T_9 the treatment compared of chlorpyrifos application in an uncropped soil to the retention of chlorpyrifos will be less and the absence of crop will make the condition unsuitable for bacterial growth. However the level of inhibition that was on par with T_8 might presumably be due to poor retention of the compound in the soil due to absence of organic matter. The population of bacteria in this soil layer showed a progressive increase with time and attained initial level in 60-75 days time. After an initial decline the bacteria would have acclimatized to the harmful effect of chlorpyrifos or could have started utilizing it as a carbon source leading to a general reduction in the level of residues. The population of bacteria thus got enhanced and reached the initial value.

5.5.2 Bacterial Population at 15-30 cm soil Depth

The results of the effect of chlorpyrifos treatment on bacterial population under different soil conditions revealed that the bacterial population at 15-30 cm soil depth was much lower than that found at 0-15 cm soil depth (Fig.7) and could be due to low aeration and low root activity in the lower layers of the soil. The result showed a marked decline in the population of bacteria immediately after treatment and in 75 days the

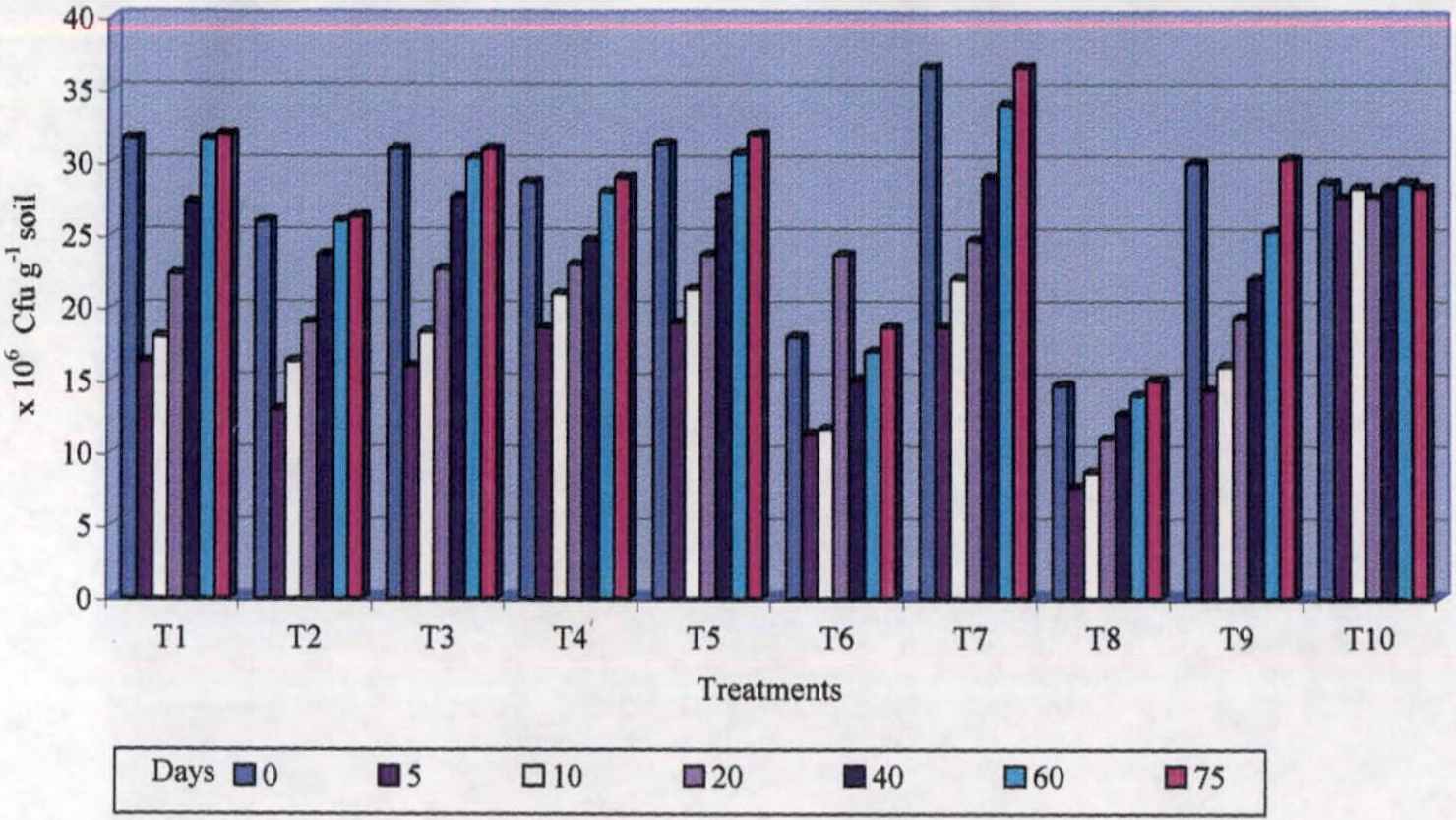


Fig. 7. Population dynamics of bacteria in soil as influenced by chlorpyrifos at 15-30cm depth

initial population of bacteria was regained. Among the treatments high inhibition was pronounced in T_1, T_2, T_3, T_7 and T_8 while in other cases it was medium. The inhibition on bacterial population may presumably be due to the direct effect of chlorpyrifos on the growth and multiplication of bacterial population. The relatively high level of inhibition in T_2, T_3 and T_8 in the initial stages could be due to higher adsorption of chlorpyrifos on organic matter. It was on par with treatments T_1, T_7 and T_9. When lime @ 600 kg ha^{-1} was applied as in T_4, T_5 and T_6, the inhibition got reduced presumably due to the enhanced degradation of chlorpyrifos in the presence of lime. The factors like soil depth, aeration and root proliferation do contribute to the degradation of chlorpyrifos in soil in addition to pH.

5.5.3 Fungal Population

The results on the effect of chlorpyrifos treatment on fungal population under different soil condition revealed that there was no significant reduction in the fungal population by its application (Fig.8 and 9). Similar results were reported by Martinez-Toledo *et al.* (1992) on the effect of chlorpyrifos on fungi. Throughout the experimental period, the fungal population remained static. The fungal population at 15-30 cm depth was less compared to 0-15 cm depth. The study showed that the different treatments and soil depth have no significant effect on fungal population.

5.5.4 Soil Arthropods

The results of the effect of chlorpyrifos treatment on the arthropod population under different soil condition revealed a sudden decline in the arthropod population immediately after treatment and thereafter a slow but gradual increase (Fig.10). The initial population was not attained even after 75 days. A similar behaviour of arthropod population by chlorpyrifos application was reported by Pandey and Singh (2000). All the treatments showed a significant inhibition of arthropod population upto 10 days.

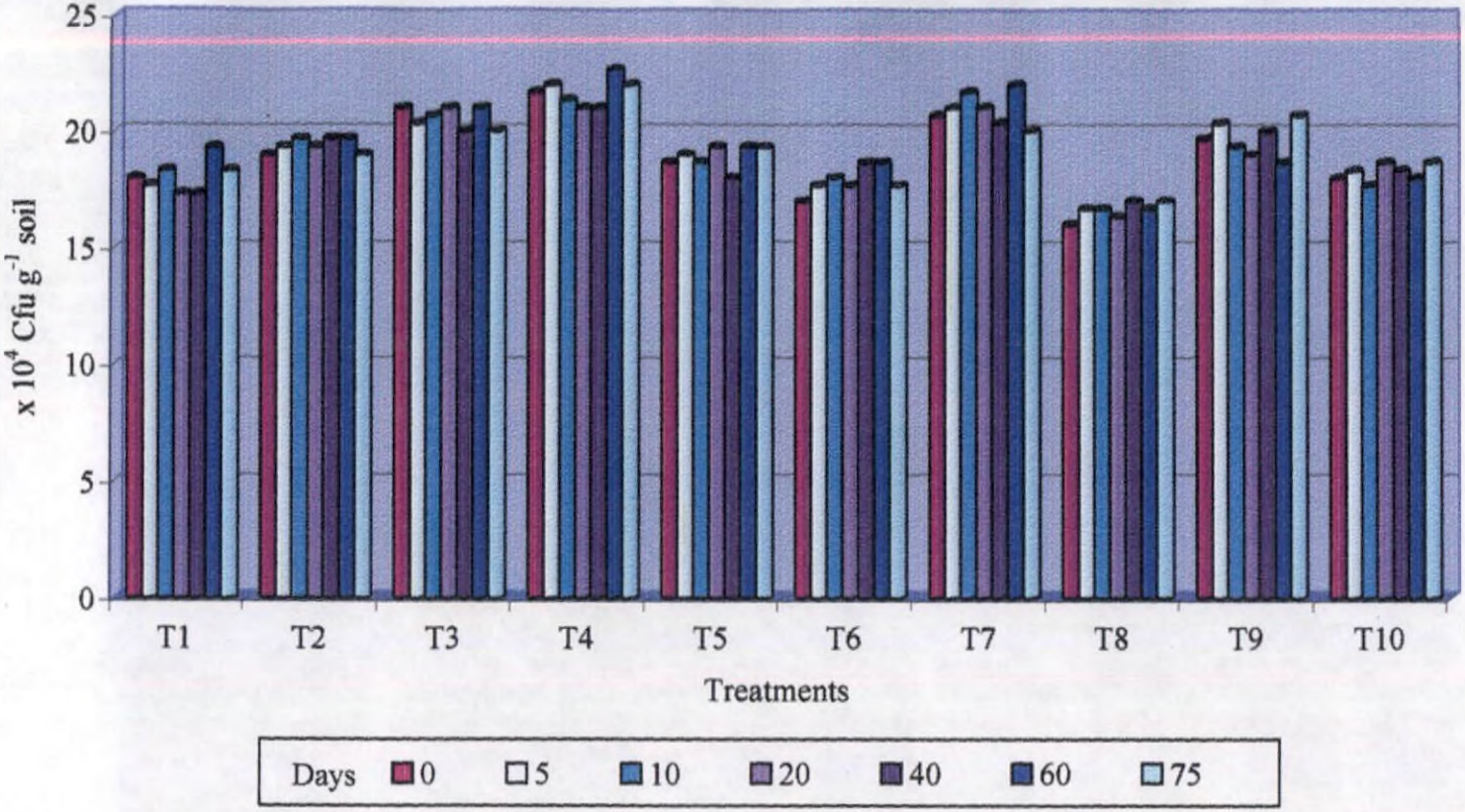


Fig. 8. Population dynamics of fungi in soil as influenced by chlorpyrifos at 0-15cm depth

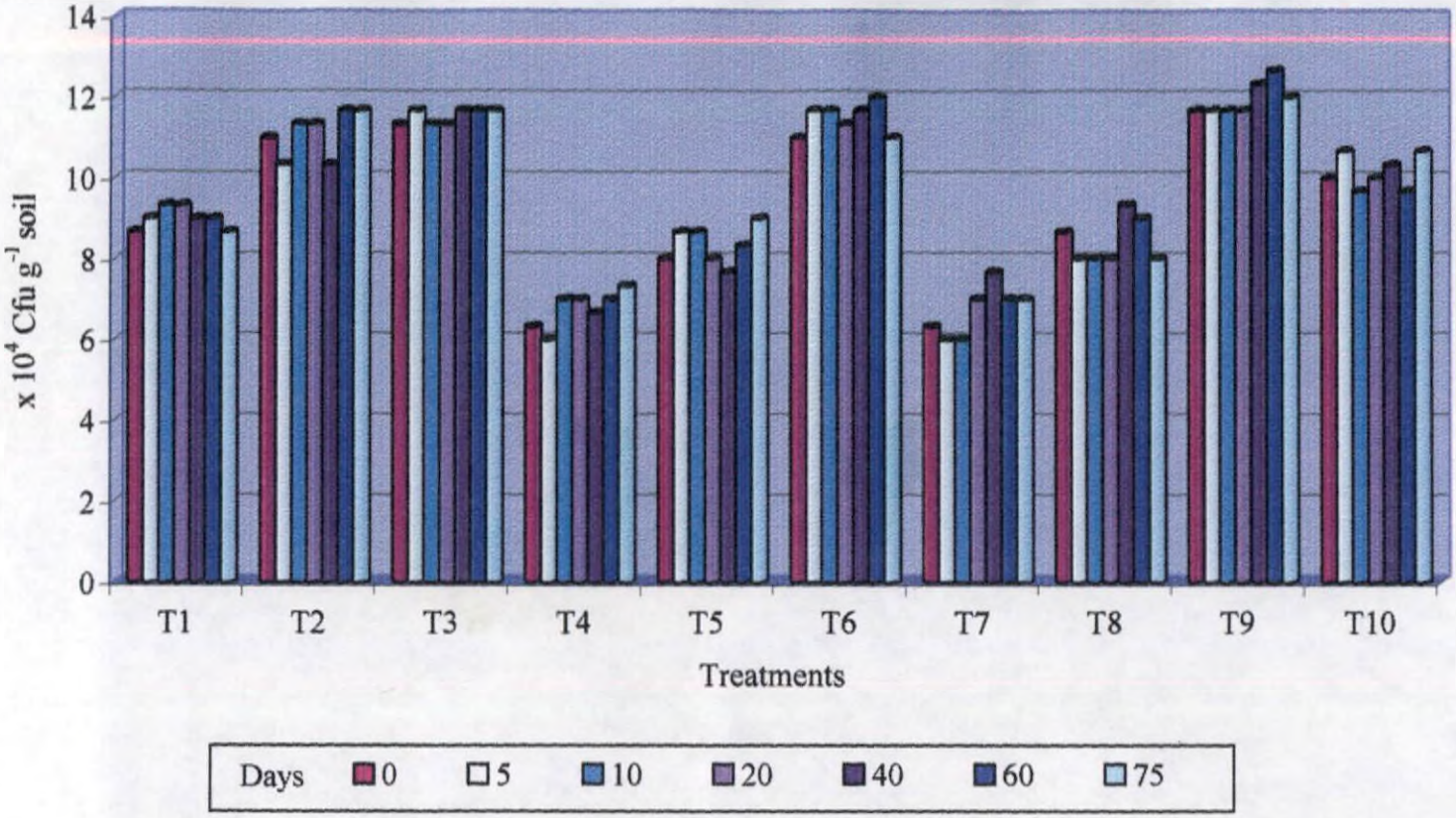


Fig. 9. Population dynamics of fungi in soil as influenced by chlorpyrifos at 15-30cm depth

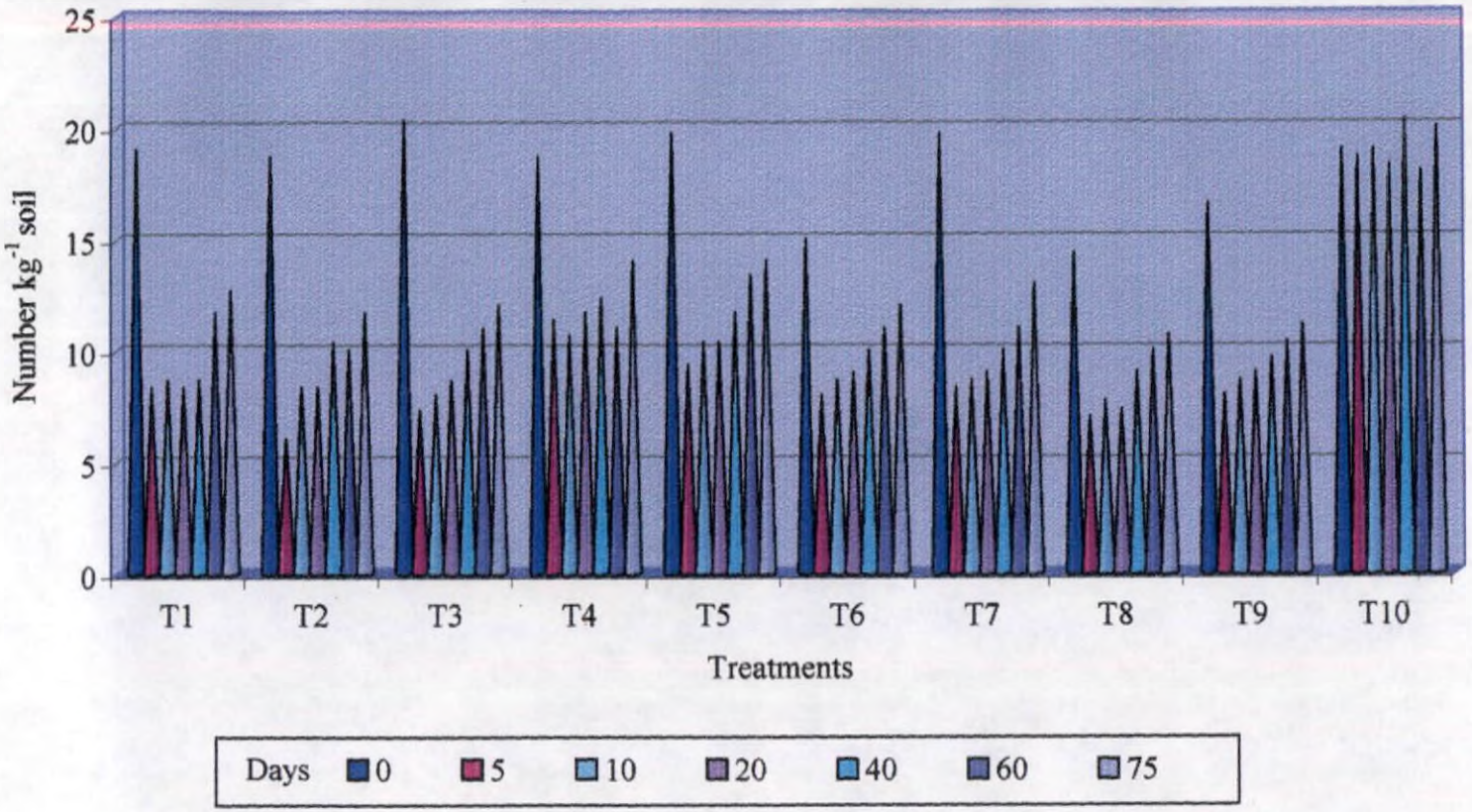


Fig. 10. Population dynamics of arthropod in soil as influenced by chlorpyrifos

Among the treatments maximum inhibition was observed in T_2 and T_3 presumably due to high persistence of chlorpyrifos in the presence of organic matter. This was on par with T_1 and T_7. The lowest inhibition was observed in T_4 and T_6 due to the presence of lime which resulted in the hydrolysis of chlorpyrifos. In T_5 the inhibition was high even in the presence of lime presumably due to the high adsorption on the organic matter. In T_8 a high level of inhibition of arthropod population was presumably due to a very high level of adsorption of chlorpyrifos on to organic matter coupled with the absence of NPK which restricted root growth resulting in very poor survival of arthropods. The per cent inhibition was on par with T_9 which could presumably be due to poor retention of compound in the soil due to absence of organic matter. The population of arthropod in the soil showed a gradual increase but was not reinstated till 75 days time.

5.6 EFFECT OF CHLORPYRIFOS ON NODULATION IN COWPEA

The result of the effect of chlorpyrifos on nodulation in cowpea under different soil conditions revealed that there was decline in nodule count at second and fourth week after application as compared to control (Fig.11). A significant inhibition of nodulation was observed in T_1, T_2, T_3, T_7 and T_8. This could be due to higher persistence and a slow rate of dissipation of chlorpyrifos in these treatments resulting in an inhibition of nodulating bacteria in soil. Among the treatments, the highest per cent inhibition was found in T_1 and the lowest in T_6.

5.7 EFFECT OF CHLORPYRIFOS ON SOIL FERTILITY

The results of the effect of chlorpyrifos on different soil parameters revealed that there was slight increase in the analytical values as compared to control (Fig 12 and 13). This may presumably be due to the addition of different amendments.

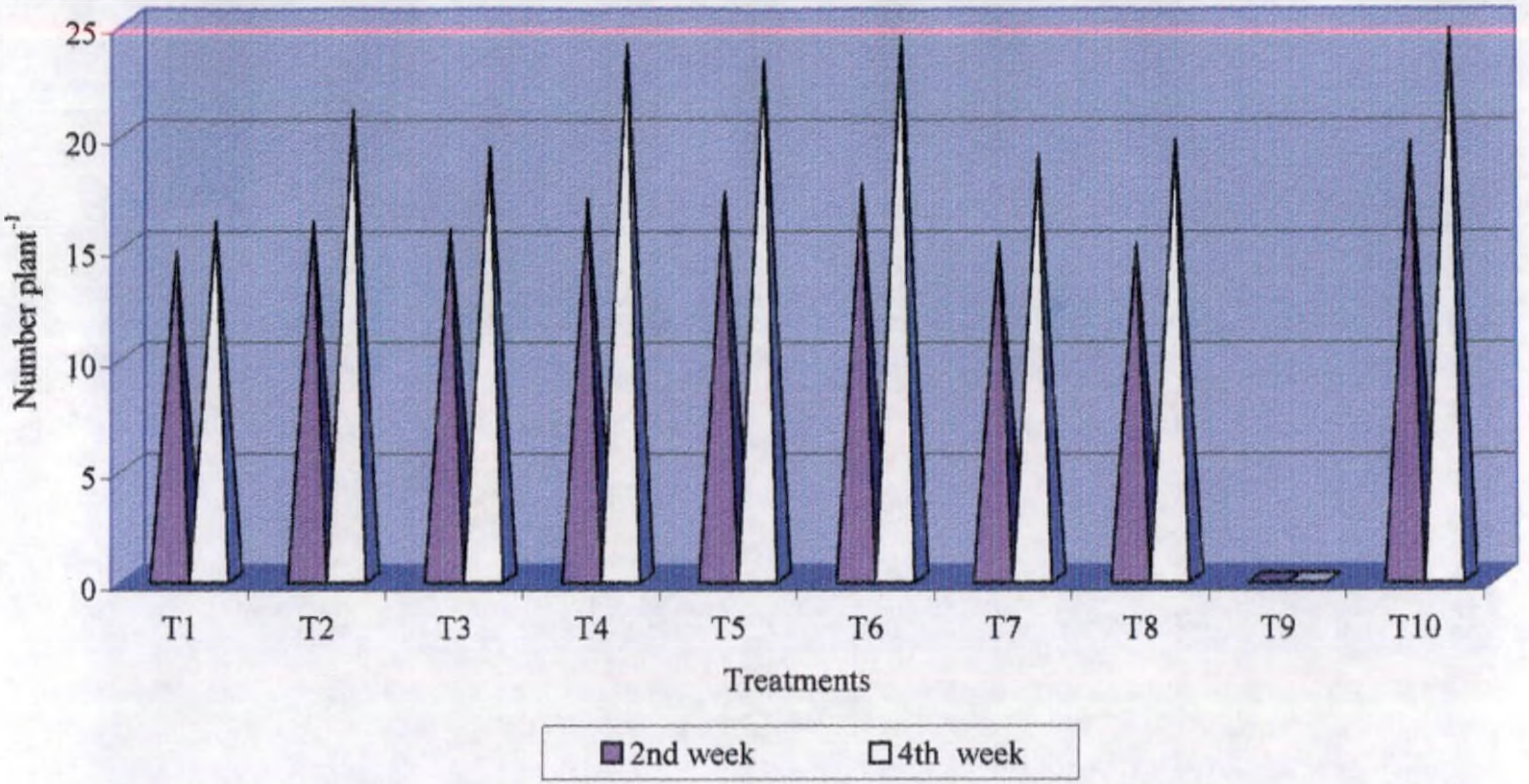


Fig. 11. Effect of chlorpyrifos on nodulation

Among the treatments CEC, total phosphorus and organic carbon contents were the highest in the case of treatment T_5 which could be due to the addition of organic matter @ 20 t ha^{-1} andlime@600 kg ha^{-1}. The values for total nitrogen, total magnesium and total phosphorus were observed to be the highest in T_6 and this may be due to the effect of organic matter and lime. Maximum calcium and pH were observed in treatment T_4, presumably due to liming @ 600 kg ha^{-1}. T_2 showed highest water holding capacity, EC and field capacity moisture presumably due to high organic matter content.

5.8 RESIDUES OF CHLORPYRIFOS IN PLANT

The harvested green pods as well as dried grains of cowpea were subjected to different treatments of chlorpyrifos as mentioned in section 3.6, were analysed for the residues of the chlorpyrifos. None of the samples showed residues of chlorpyrifos. Hence it can be inferred that no absorption and translocation of chlorpyrifos occurred from the soil to the crop system.

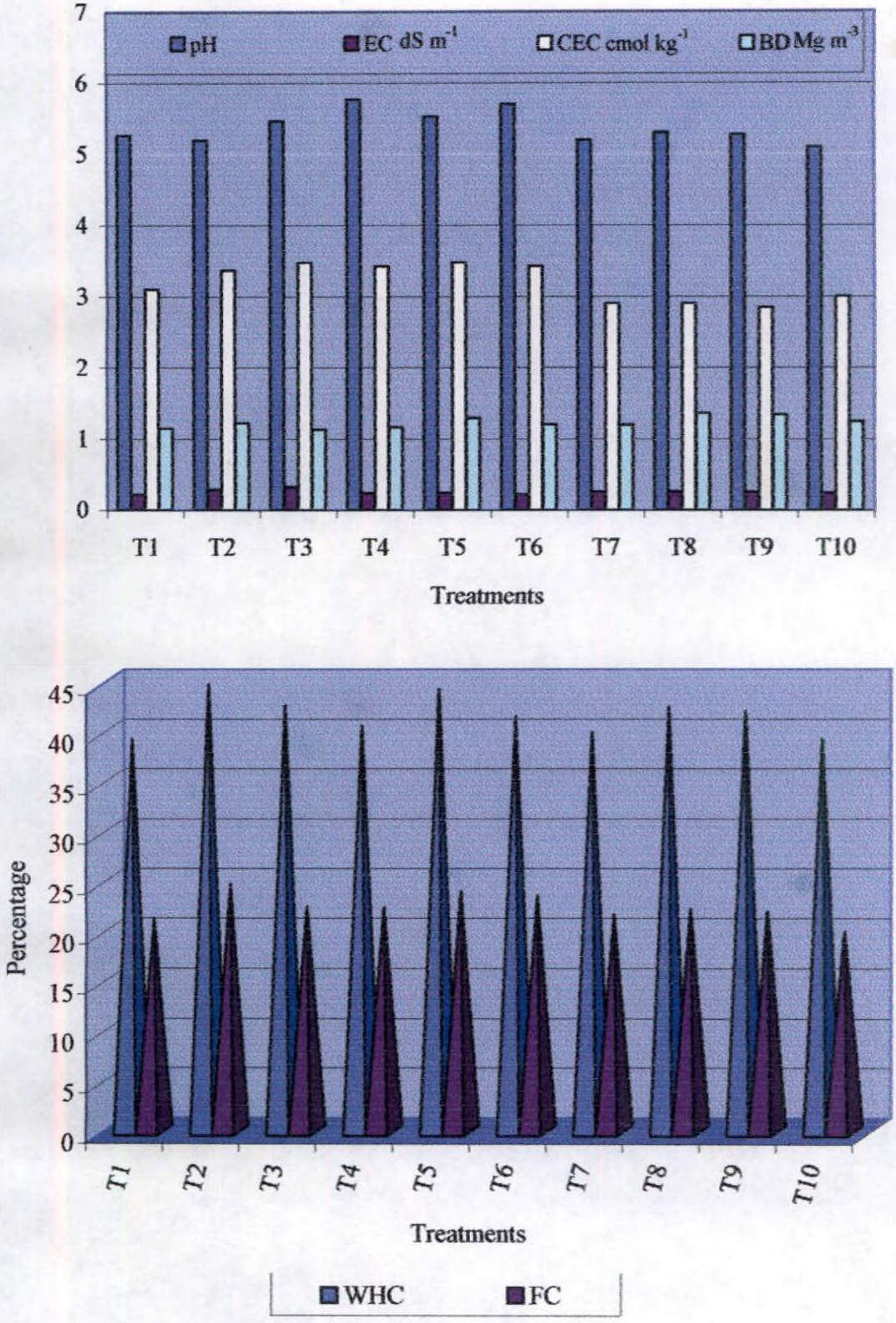


Fig. 12. Effect of treatment on soil properties

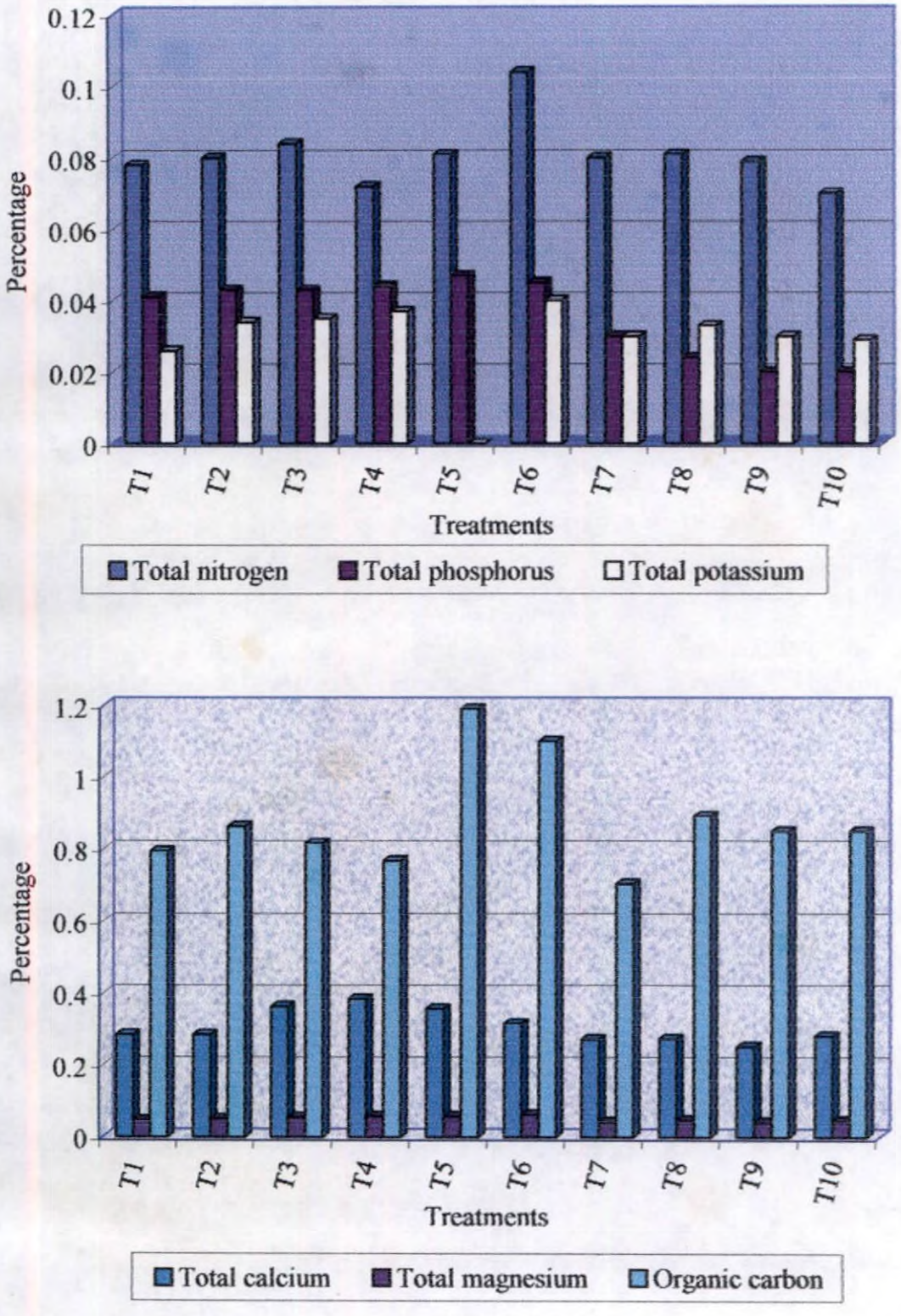


Fig. 13. Effect of treatment on soil properties

SUMMARY

6. SUMMARY

Pesticides are indispensable in modern agriculture using high yielding varieties of crops, which get infested by a variety of pests. This is also true with regard to the control of various soil inhabiting pests. Among the different methods of pest control, farmers resort to the use of chemicals for their control. A chemical used in agriculture or public health purposes should disappear from the environment after the desired period of pesticidal action and should not cause any adverse effect on soil flora or fauna or processes. An insecticide for soil application should have low mammalian toxicity and moderate stability in the environment.

Chlorpyrifos is the world's leading insecticide in volume terms and effective against wide range of insects. It is widely used for the control of soil inhabiting insects due to high persistence in soil. Literature on persistence of chlorpyrifos in acid soils of Kerala is scanty inspite of its wide use in the state. In this context an investigation was carried out to assess the dissipation pattern of chlorpyrifos in red loam soil at varying levels of organic matter and lime and to study its effect on soil microflora and arthropods.

A field trial was conducted at the Instructional Farm, College of Agriculture, Vellayani using cowpea variety Bhagyalekshmi. The experiment was laid out adopting Randomised Block Design in plots of 1.5 m x 1.5 m. Chlorpyrifos (20 % EC) was applied @ 3 ml l^{-1} and FYM in two levels @ 20 kg ha^{-1} and 10 kg ha^{-1}. Lime was applied @ 600 kg ha^{-1}. The treatments were replicated thrice. Soil samples were drawn at 0, 5, 10, 20, 40, 60 and at crop harvest (75 days after treatment). Insecticide residues were estimated using GLC. The data were statistically analysed and results were summarized below.

1. The soil is sandy clay loam with pH 5.1, organic matter 1.06 per cent and available N, P and K were 150, 30 and 75 kg ha^{-1} respectively and the soil is of low fertility.

2. The efficiency of extraction of chlorpyrifos, clean up and estimation of chlorpyrifos from soil were standardized through recovery experiment. Acetone extraction followed by clean up with alumina was found to be best.

3. At 0-15 cm soil depth initial residues of chlorpyrifos was found to be 44.00-46.70 $\mu g\ g^{-1}$ which reduced to 20.00-35.00 $\mu g\ g^{-1}$ after 75 days of application, which corresponds to 22.76-61.36 per cent reduction. Maximum persistence was observed when chlorpyrifos was applied in bare soil (T_9) with a half life of 235.17 days. The dissipation followed a biphasic pattern in soil with an initial phase of rapid dissipation followed by a phase of slow and steady dissipation.

4. Significant difference in dissipation was observed among different treatments. In the presence of lime (alkaline material) chlorpyrifos was highly unstable and dissipated rapidly. In treatments T_4, T_5 and T_6 , where lime was applied @ 600kg ha^{-1}half life of chlorpyrifos were 61.46, 69.88 and 66.90 days, respectively.

5. The application of organic manure (@ 20 and 10 t ha^{-1}), resulted in a high level of residues initially. In treatments T_2 and T_3 chlorpyrifos got dissipated with a half life of 100.13 and 90.04 days, respectively.

6. The presence of a legume crop and NPK @ 20: 30: 10: kg ha^{-1}as in T_1 also had significant effect on the degradation of chlorpyrifos and the half life observed in T_1 was 111.19 days. So, treatments like addition of organic manure, lime, presence of a legume crop and

even NPK were found to hasten the dissipation of chlorpyrifos in the upper layers of soil.

7. The residues persist for a longer period in the lower layers of soil with half lives ranging from 53.75 to 235.77 days with only 25.39-64.44 per cent of residues getting dissipated. Maximum persistence was observed in treatments with organic matter (T_2). A higher degradation was observed in presence of lime with 60.00-64.44 per cent of the chemical got dissipated while the addition of organic matter increased the persistence of the chemical with half life of 152.20 – 235.77 days. The degradation was rapid in the surface layer while in the lower layer, it was slow. The study revealed that degradation of chlorpyrifos in soil is governed by soil pH, organic matter, presence of alkaline materials, soil depth and the rhizosphere effect of the crop

8. Chlorpyrifos application @ 3 ml l^{-1} had significant effect on the multiplication of soil organisms. The bacterial population showed a declining trend upto 10 days of application with 34.88 – 55.86 per cent of the initial population getting reduced which thereafter increased and reached initial level at 75 days after application. Maximum inhibition of bacterial population by chlorpyrifos was observed in the presence of organic manure and minimum when lime was applied. The high persistence of chlorpyrifos due to adsorption on organic matter significantly reduced the bacterial population upto 40 days after application.

9. Chlorpyrifos application @ 3 ml l^{-1} do not have any influence on fungal population in all the treatments and the population of fungus remained steady throughout the period of observation.

10. An adverse effect on soil arthropods was observed as a result of chlorpyrifos application with 39.30 – 67.86 per cent of the initial

population got reduced within 10 days of application and not recovered till 75 days.

11. Application of chlorpyrifos reduced the nodulation in cowpea with 2.67 – 35.12 per cent reduction in nodule count. The nodulation was significantly reduced in T_1, T_2, T_3. T_7 and T_8, while it was slightly reduced in the case of T_4, T_5 and T_6. This could be due to the inhibition in the growth of nodulating bacteria by chlorpyrifos.

12. An increase in the analytical values of different soil properties were observed after crop harvest due to the effect of treatments.

13. The residues present in the cowpea pod and grains were below detectable limits and no translocation of residues occurred from soil to the crop.

REFERENCES

7. REFERENCES

Al–Minaha, A. A; Salama, A. K. and Abdalla, M. Y. 1998. Biodegradation of chlorpyrifos by either single or combined cultures of some soil borne plant pathogenic fungi. *J. Environ. Sci. Health* 33 (6): 693-704

Awasthi, M.D. and Prakash, N.B. 1997. Persistence of chlorpyrifos in soils at different moisture regimes. *Pestic. Sci.* 50: 1-4

Aylmore, J. A., Di, H. J. and Kookana, R. S. 2001. Degradation rates of eight pesticides in the surface and subsurface soil under laboratory and field condition. *Soil Sci.* 163(5): 404-411

Bensal, O.P. 2004. Pesticides and heavy metals in soils of Aligarh district. Accumulation of heavy metals in plant species. *Pestic. Res. J.* 16 (1): 71-78

Benson, W. H. and Long, S. F. 1991.Evaluation of humic pesticide interaction on the acute toxicity of selected organophosphate and carbamate insecticides. *J. Ecotoxicol. Environ. Safty* 21 (3): 301-307

Bhatnagar, A. and Gupta, A. 1992. Persistence of chlorpyrifos residue in soil and groundnut seed. *Int. Arachis Newsl.* 12: 16-17

Bijjur, S. and Verma, S. 1997. Persistence and efficacy of insecticides against pest complex of pea crop. *Pestic. Res. J.* 9:25-3

Brock, T.D. 1970. *Biology of Microorganisms*. Prentice Hall, Engle Wood Clifts NI, 282 p.

Brown, A. L. 1978. *Ecology of Soil Organisms*. Hexomann Educational Book Ltd., London. 228 p.

Chandler, K. J. and Hogarth, D. M. 1998. Limestone can prevent cane grub control with controlled release insecticide. *Proc. Conf. Aust. Soc. Sugarcane Technol. 28th April to 1st May*. Ballina, N. S. W., Australia, pp. 270-275

Chandra, B. G. and Priyadar, R. 2002. Effect of pesticides on Enzymatic activities and nitrogen mineralization in soils. *Indian J. agric. Chem.* 36 (2): 71-79

Chapman, R.A. and Chapman, P.C. 1986. Persistence of granular and EC formulation of chlorpyrifos in a mineral and an organic soil incubated in open and closed containers. *J. Environ. Sci. Health* 21: 447-456

*Click, J. H. and Coats, J. R. 1993. Effects of concentration temperature and soil moisture of chlorpyrifos in an urban lowa soil. *A. C. S. Sym. Ser. Am. Chem. Soc.* 522: 62-69

Csinos, A. S. 1985. Non target activity of chlorpyrifos and hydrolysis products on *Sclerotium rolfsi. Plant Des.* 69 (3): 254-256

Dawson, I.A., Grayston, S.J., Murray, P.J. and Cook, R. 2001. Special issue : The dynamic interface between plants and the earth. *Sixth Symp. Int. Soc. Root Res. Nagoya, Japan* 255 (1): 121-130

Djuwarso, T. and Harnoto, V. 1998. Strategy for controlling the soyabean pod borer, *Etiella* spp. (En). *J. Denelitian Dengemvongam Pertanian* 17: 90-98

*Drummond, J.N. 1986. *Solubility of Chlorpyrifos in Various Solvents.* DowElanco, Indianapolis, NI, 160 p.

Forscher, B.T. and Townscnd, M.L. 1998. Mortality of eastern subterranean termites (Isoptera-Rhintemitidae) Exposed to four soils treated with termiticides. *J. Econ. Ent.* 12: 38-40

Gaberleit, S., Spener, F. and Zaborosch, C. 2000. Microbial and cytoplasmic membrane based potentiometric biosensors for direct determination of organophosphorus insecticides. *Appl. Microbiol. Biotechnol.* 54 (3): 652-658

Getzen, L.W. 1981. Degradation of chlorpyrifos in soil : influence of autoclaving, soil moisture and temperature. *J. Econ. Ent.* 74: 158-162

Getzen, L.W. 1985. Factors influencing the persistence and effectiveness of chlorpyrifos in soil. *J. Econ. Ent.* 78 (2): 412-418

Gupta, R. P. and Dakshnamoorthi, C. 1980. *Procedures for Physical Analysis of Agrometeorological Data.* Indian Agricultural Research Institute, New Delhi, 635 p.

Herbert, I.M., Svendsen, C., Hankard, P.K. and Spurgeon, D.J. 2004. Comparison of instantoneon rate of population increase and critical effect estimates in *Folsomia candida* exposed to four toxicants. *Ecotoxicol. Environ. Safety* 57 (2): 175-183

Horst, G.L., Shea, P.J., Christian, N.E., Miller, D.R., Stuefer-Powerl, C. and Starett, S.K. 1996. Pesticide dissipation under golf course fairway condition. *Crop Sci.* 36: 362-370

Hoskins, M.L. 1981. Mathematical treatment of loss of pesticide residue. *Pl. Prot. Bull. FAO* 9: 163-169

Huang-Xing Jiang, Lee, L.S., Nakatsu, C. and Huang, X.J. 2000. Impact of animal waste lagoon effluents on chlorpyrifos degradation in soils. *Environ. Tiocol. Chem.* 19 (12): 2864-2870

*Hummel, R.A. and Crummet, W.B. 1964. *Solubility of O, O-diethyl O-(3,5,6-trichloro-2pyridly) phosphorothioate in various solvents.* Dow Elanco, Indianapolis, NI, 138 p.

Jackson, M.L. 1973. *Soil Chemical Analysis.* Second edition. Prentice Hall of India (Pvt.) Ltd., New Delhi, 498 p.

Johnson, L.F. and Curl, E.A. 1972. *Isolation of Group of Microorganisms from Soil: Methods for Research on the Ecology for Soil Borne Plant Pathogens*. M/S Burgus Publishing Company, 212 p.

Jones, K.D. and Haung, W.H. 2003. Evaluation of toxicity of pesticides, chlorpyrifos and arsenic in the presence of compost humic substances in aqueous system. *J. Hazardous Material* 103: 93-105

Kalpana, D., Patel, B.A., Raj, M.F., Shah, P.G., Patel, J.A. and Patel, B.K. 2002. Dissipation of chlorpyrifos and endosulfan in soil and their residues in wheat. *Pestic. Res. J.* 14 (1): 107-112

Kearney, P.C. and Helling, C.S. 1969. Factors influencing the persistence of organophosphorus insecticide in soil. *Residue Rev.* 25: 25-44

Khajuria, D.R. and Sherma, J.P. 1995. Efficiency of insecticides on controlling pea leaf minor (*Chromatomyia horticola Gourau*) on seed crop of pea (*Pisum sativum*). *Indian J. agric. Sci.* 65: 381-384

Lochhead, A.G. 1940. Quantitative studies of soil microorganism III, influence of plant growth as characters of bacterial flora. *Can. J. Res.* 18: 42-53

Lemmon, C.R. and Pylippiw, H.M. Jr. 1992. Degradation of Diazinon, chlorpyrifos, Isofenphos and Pendimethalin in grass and compost. *Bull. Environ. Contamination Toxicol.* 48: 409-415

*Mac Fadyen, A. 1961. Improved funnel type extraction for soil arthropods. *J. Anim. Ecol.* 30: 171-186

Martin, J. D. 1950. Use of acid Rose Bengal and Streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69:215-233

Martinez-Toledo, M.V., Salmeron, V. and Genzalez-Lopez, J. 1992. Effect of the insecticides methyl pyrimifos and chlorpyrifos on soil microflora in an agricultural loam. *Pl. Soil.* 147 (1): 25-30

Masud, S. Z. and Praveen, Z. 1991. Persistence of permethrin, pirimiphos methyl and chlorpyrifos methyl insecticides in wheat stored under stimulated environmental conditions. *Pakist. J. Scient. Ind. Res.* 34(6): 240-24

Medina, M.T. 1991. Persistence and movement of chlorpyrifos in a paddy soil. *Toxicol. Environ. Chem.* 16: 95

Miles, J. R. W; Harris, C. R. and Tu, C. M.1984. Influence of moisture on persistence of chlorpyrifos and chlorfenviphos in sterile and natural mineral and organic soils. *J. Environ. Sci. Health* 9 (2): 237-243

Omar, S. A. 1998. Availability of phosphorus and sulphur of insecticide origin by fungi. *Biodegradation* 9 (5): 327-336

Pandey, S. and Singh, D.K. 2000. Total bacterial and fungal population after chlorpyrifos and quinalphos treatment in groundnut (*Arachis hypogea* L.). *Soil J. Hazard Mater* 80: 9-13

Piper, C. S. 1966. *Soil and Plant Analysis.* Asija Publishing House, Bombay, 368 p.

Pozo, C; Martinez- Toledo, M. V; Salmeron, V; Rodelas, B. and Gonzalez- Lopez, J. 1995. Effect of chlorpyrifos on soil microbial activity. *Environ. Toxicol. Chem.* 14(2): 187-192

Prabakaran, J. and Ramaswamy, K. 1990. Effect of seed dressing chemicals on the survival of Rhizobium sp and growth, nodulation and grain yield of green gram and black gram. *Madras agric. J.* 77: 285-290

Quiao, C. L; Yan, Y. C; Shang, H. Y; Zhou, X. T. and Zang, Y. 2003. Biodegradation of pesticides by immobilized recombinant *Escherichia coli. Bull. Environ. Contamination Toxicol.* 72 (2): 370-379

Racke, K. D. 1993. Environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* 131: 1-154

Racke, K. D. and Robbins, S. J. 1991. Factors affecting degradation of 3, 5, 6, trichloro 2 pyridinol in soil. *A.C. S. Symp. Ser.* 459:93-107

Racke, K. D., Coats, J. R and Titus, K. R. 1988. Degradation of chlorpyrifos and its hydrolysis product 3,5,6-trchloro 2 pyridinol in soil. *J. Environ. Sci. Health* 23: 527-539

Racke, K. D., Fontaine, D. D; Yoder, R. N. and Miller, J. R. 1994. Chlorpyrifos degradation in soil at termiticidal application rates. *Pestic. Sci.* 42(1): 42-43

Racke, K. D., Laskowski, D. D. and Scultz, M. R. 1990.Chlorpyrifos degradation as influenced by pH. *J. agric. Fd Chem.* 38(6): 1430-1436

Racke, K. D; Steele, K. D; Yoder, R. N; Dick, W. A. and Avidove, E. 1996. Factors affecting hydrolytic degradation of chlorpyrifos in soil. *J. agric. Fd Chem.* 44: 1582-1592

Rachman, L.M. 1994. Movement of chlorpyrifos insecticide in soil. *Acta Hort.* 369: 144-148

Rahman, G.K., and Motoyama, N. 2000. Determination of chlorpyrifos residue in andosol upland soils using methanol-phosphoric acid extraction. *J. Pestic. Sci.* 25 (4): 387-391

Rajareddy, A. Divakar, B.J. 1998. Ovicidal action of certain insecticides on *Spodoptera litura* (Hb). *Indian J. Pl. Prot.* 26: 78-79

Rajareddy, A., Divakar, B.J. 2000. Ovicidal action of certain insecticides on *Helicoverpa armigera* (Hb.). *Pestology* 24 (11): 37-38

Ramalingam, S. 1989. Studies on sorption desorption, degradation and persistence of HCH isomers in soil and the residue in rice and groundnut. MSc (Ag.) thesis. Tamil Nadu Agricultural University, Coimbatore, 112 p.

Rao, D. V. S. and Subbaratnam, G. V. 1998. Management of the ragi cut worm *Spodoptera exigua* (Hb.) in onion. *Pest Mgmt Econ. Zool.* 6: 87-91

Revellin, C., Canson, B. and Carlroux, G. 1992. Effect of mixture of chlorpyrifos and lindane on the symbiosis of *Bradyrhizobium japonicum* and soyabena (*Glycine max* (L.) Merril). *Pestic. Sci.* 36 (1): 69-74

Ritcey, G., Mc Ewen, F.L., Braun, H.E. and Frank, R. 1991. Persistence and biological activity of residues of granular insecticides in organic soil and onions with furrow treatment for control of the onion maggot (Diptera: Anthomyiidea). *J. Econ. Ent.* 84 (4): 1339-1343

Robertson, L.N., Chandler, K.J., Stickle, B.D.A., Cocco, R.F. and Abmetagic, M. 1998. Enhanced microbial degradation implicated in rapid loss of chlorpyrifos from the controlled released formulation SuSucon (R) Blue in soil. *Crop Prot.* 17: 29-33

Rouchard, J., Gustin, F., Bennet, F. Ceustermas, N. and Gillel, J. 1991. Influence of slurry, cowmanure, city refuse,mushroomcultivation compost fertilizers onto chlorpyrifos and chlorfenviphos and carbofuran soil and plant metabolism in cauliflower crops. *Mededilingen-Van-Detacultext-Land bouw wet Schappen.* 56 (3): 915-924

Rouchad, J., Gustin, F., Metsue, M. and Touillaux, R. 1992. Insecticide soil persistence and efficiency in cauliflower field crops: Influence of organic fertilizers and organic matter properties. *Toxicol. Environ. Chem.*15: 52-54

Shijian, G., Huiming, S. and Wencai, N. 1997. Efficiency of different kinds of insecticides to *Spodoptera litura* (Hb). *China Vegetables* 4: 1-3

*Sikora, L. T; Kaufman, D. D. and Hornog, L. C.1990. Enzyme activity in soils showing enhanced degradation of organophosphate insecticides. *Biol. Fertility soils* 9 (1): 14-18

Singh, B. and Battu, R.S. 2003. Persistence of chlorpyrifos in paddy. *Pestic. Res. J.* 15 (2): 175-178

Singh, B. K., Walker, A; and Wright, D. J. 2002. Degradation of chlorpyrifos, fenamifos and chlorothalonil alone and combination and their effect on soil microbial activity. *Environ. Toxicol. Chem.* 21 (12): 2600-2605

Singh, B. K., Walker, A., Morgan, J. A. and Deris W. J. 2003. Effect of pH on biodegradation of chlorpyrifos and isolation of a chlorpyrifos degrading bacteria. *Environ. Toxicol. Chem.* 21: 2650-2605

Singh, N. 2003. Organic manure and urea, effect on metalachlor transport through packed soil column. *J. Environ. Qual.* 32: 1742-1749

Smith, J. L. and Rust, M. K. 1992. Cellulose and clay in sand affects termiticide treatment. *J. Econ. Entomol.* 86 (1): 53-60

Snedecor, G.W. and Cochran, W.G. 1975. *Statistical Method.* Oxford and IBH Publishing Company, New Delhi, 593 p.

Somasundaram, J., Coats, J. R. and Racke, K. D. 1989. Degradation of pesticide in soil as influenced by the presence of hydrolysis metabolites. *J. Environ. Sci. Health* 25 (5) 457-478

Spark, D. L. 1989. *Kinetics of Pesticides and Organic Pollutant Reaction. In Kinetics of Soil Chemical Process.* Academic Press Inc. 224, p.

Sprenkel, R. and Hamburg, F. 1989. The effect of pH on Dursban insecticide. *Pest Control Technol.* 17(2): 94

Sud, K. C; Verma, B. C; Lal, S. S. and Chanda, V. K.1998. Influence of organophosphate pesticides on potato nutrition in Shimla hills. *J. Indian Potato Ass.* 25: 46-49

Swaminathan, R. and Prasad, N. N. 1978. Screening the efficient rhizobial isolates for tolerance to certain pesticides. *Pesticide* 12 (11): 30-33

Takur, S. S. and Vaidya, D. N. 2000. Management of cut worm, *Agrottis epsilon* (Hfn) in maize. *Insect Environ.* 6: 91-92

Tandi, B. L., Yadava, C. P. S. and Bhatnagar, K. N. 1993. Bioefficiency of various insecticides against red hairy caterpillar, *Amsacta moorei* (Wlk). *Indian J. Ent.* 55:317-324

Upadyay, S. and Agarwal, R. K. 1993. Persistence of some insecticides against *Lipaphis erysimi* (Kalt) on mustard. *Indian J. Pl. Prot.* 21: 104-105

Vandervoort, C. 1997. Fate of selected pesticides applied to Turfgrass: Effect of composting on residue. *Bull. Environ. Contamination Toxicol.* 58: 38-45

Wiles, J. A. and Frampton, G. K. 1996. A field bioassay approach to assess the toxicity of insecticide residues on soil to collembola. *Pestic. Sci.* 47 (3): 273-285

Wu, L., Green, R.L., Liue, G., Yalesa, M.V. and Pachicob, P. 2002. Partitioning and persistence of chlorpyrifos in the creeping bend grass putting green. *J. Environ. Quality* 31: 889-895

*Zidan, Z. H., Selim, A. A; Afifi, F. A. and Abdel-Aziz, K. 1991. Persistence of chlorpyrifos and fenvalerate insecticides in different soil and water types in laboratory. *Bull. Entomol. Soc.* 19: 121-130

---

*Original not seen

DISSIPATION OF CHLORPYRIFOS IN RED LOAM SOIL AND ITS EFFECT ON SOIL ORGANISMS

REKHA, P.R.

Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2005

Department of Soil Science and Agricultural Chemistry
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522

ABSTRACT

The persistence and degradation of chlorpyrifos, a contact insecticide, widely used for the control of soil inhabiting insects were studied in relation to the application of organic manure and lime and also its effect on soil organisms. The experiment was done in field plots at Instructional Farm, College of Agriculture, Vellayani.

The insecticide (chlorpyrifos 20 % EC) was applied @ 3 ml l^{-1} in different treatments. Soil samples were drawn 0, 5, 10, 20, 40 and 60 days after application and after the crop harvest. The residues were estimated in a gas liquid chromatograph using ECD.

The results showed that dissipation of chlorpyrifos followed a biphasic pattern with an initial phase of rapid dissipation followed by a phase of slow dissipation. Significant difference in dissipation was observed among different treatments. In the presence of lime chlorpyrifos was found to be highly unstable and dissipated easily by alkaline hydrolysis. Organic manure applied plots the persistence of chlorpyrifos was high due to the adsorption of insecticide in organic matter. The combined application of lime and organic manure cause significant difference in the degradation. The pattern of degradation of chlorpyrifos at lower depth of 15-30 cm soil were similar to surface layer except the initial survey of the insecticide at lower surface layer was less compared to upper layer (0-15 cm).

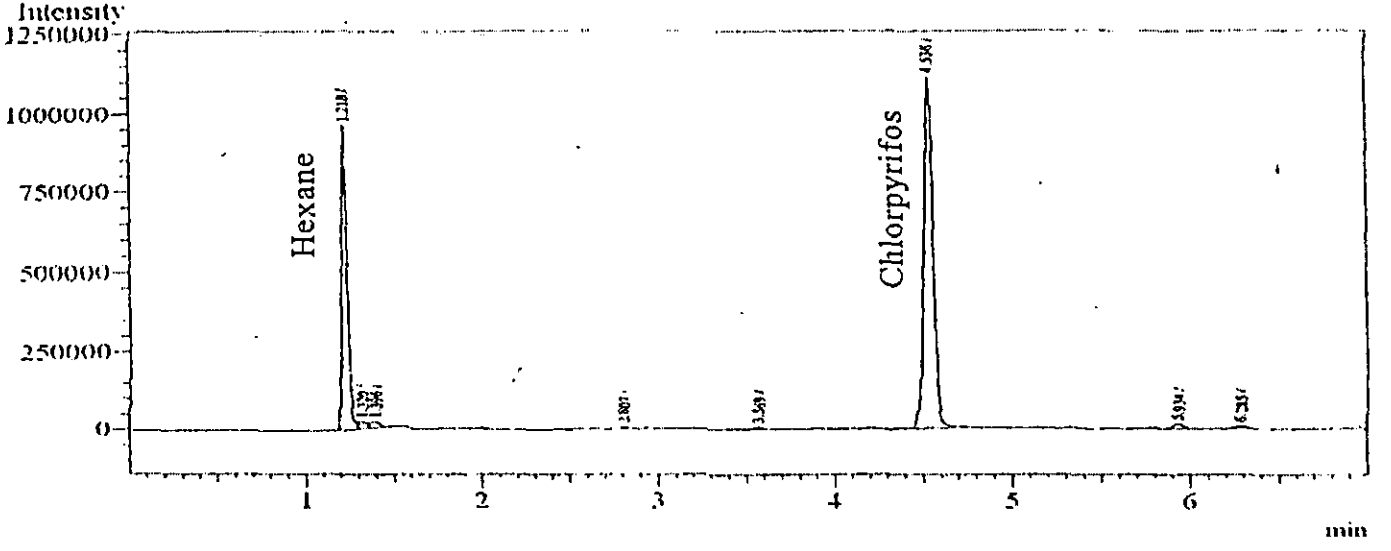
Application of chlorpyrifos inhibited the population of bacteria and arthropods, irrespective of the treatment. The bacterial population showed a significant decrease upto 10 days of application of chlorpyrifos and the original count was regained in 75 days. The result also showed that chlorpyrifos application did not affect fungal population. Chlorpyrifos was found to be highly toxic to soil arthropods upto 10 days of application

and were not regained till 75 days of application. There was a slight decrease in nodulation in cowpea as compared to control. No residue of chlorpyrifos was detected in the plant parts (pods and grain) from any of the treatment after harvest. Nutrient content on the soil showed an increase in the analytical values due to the treatment effects.

APPENDIX

APPENDIX – II

Chromatogram of chlorpyrifos



Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Name	Units
1	1.218	2013025	949714	0.000		
2	1.320	80373	23746	0.000		
3	1.396	69184	19557	0.000		
4	2.807	13046	5088	0.000		
5	3.569	19367	6584	0.000		
6	4.536	3965566	1106969	0.000		
7	5.934	58888	14729	0.000		
8	6.285	27775	7764	0.000		
Total		6247224	2134151			