“APPROACHES TO ASSESS CHLORPYRIFOS DEGRADATION IN NORTHERN LATERITE SOILS OF KASARAGOD (AEU 11)”

by

ARYA P. R.

(2019 - 11 - 173)

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF AGRICULTURE

PADANNAKKAD, KASARAGOD – 671314

KERALA, INDIA

2022

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THESIS

Submitted in partial fulfilment of the

Requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF AGRICULTURE

PADANNAKKAD, KASARAGOD – 671314

KERALA, INDIA

2022

DECLARATION

I, hereby declare that this thesis entitled **"Approaches to assess chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Place: Padannakkad

Date: 08/04/2022

Arya P. R.

2019-11-173

CERTIFICATE

Certified that this thesis entitled "**Approaches to assess chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)**" is a record of research work done independently by Ms. Arya P. R. (2019-11-173) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place: Padannakkad

Date: 8/4/22

Dr. N.K Binitha

(Major Advisor, Advisory Committee)

Assistant Professor and Head

Dept. of Soil Science and Agricultural Chemistry

College of Agriculture, Padannakkad

Kasaragod.

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Arya P. R. (2019-11-173), a candidate for the degree of **Master of Science in Agriculture** with major in Soil Science and Agricultural Chemistry, agree that the thesis entitled **"Approaches to assess chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)"** may be submitted by Ms Arya P. R. (2019-11-173), in partial fulfilment of the requirement for the degree.

Dr. N.K. Binitha
(Chairperson, Advisory Committee)
Assistant Professor and Head
Dept. of SSAC
College of Agriculture, Padannakkad

Dr. Nideesh P
(Member, Advisory Committee)
Assistant Professor
Dept. of SSAC
College of Agriculture, Padannakkad

Dr. Sreekumar K. M.
(Member, Advisory Committee)
Professor and Head
Department of Agrl. Entomology
College of Agriculture, Padannakkad

Dr. Boby V Unnikrishnan
(Member, Advisory Committee)
Assistant Professor
Dept. of Agrl. Microbiology
College of Agriculture, Padannakkad

ACKNOWLEDGEMENT

First and foremost, I bow my head before the almighty for enlightening and bountiful blessings showered on me with confidence, will power and courage to complete thesis work successfully.

With deep reverence, I express my deep sense of whole hearted gratitude and indebtedness to ***Dr. N.K. Binitha,*** *Assistant Professor and Head, Department of Soil Science and Agricultural Chemistry and the chairperson of my Advisory committee for her affectionate advice, meticulous guidance, transcendent suggestions, constructive criticisms, constant encouragement and patience right from the conceptualization of the research work to preparation of the thesis. I proudly acknowledge that this manuscript was gained its completeness under the kind supervision and whole hearted help rendered by her.*

I express my sincere gratitude to ***Dr. Nideesh P,*** *Assistant Professor, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Padannakkad and Member, Advisory committee for his timely suggestions, critical assessment and kind guidance throughout the course programme.*

I express my heartfelt gratitude to ***Dr. Sreekumar K. M.,*** *Professor and Head, Department of Agrl. Entomology, College of Agriculture, Padannakkad, and Member, Advisory committee for his sustained and inspiring guidance, encouragement and valuable help rendered during the period of investigation.*

I express my sincere gratitude to ***Dr. Boby V Unnikrishnan,*** *Assistant professor, Department of Agrl. Microbiology, College of Agriculture, Padannakkad and Member, Advisory committee for his valuable suggestions, technical guidance and esteemed advice throughout the research work.*

I express my heartfelt gratitude to ***Dr. P. R. Suresh,*** *Former Dean and Professor and Head, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Padannakkad, for constant support, technical guidance and encouragement during the entire post graduate programme.*

I place a deep sense of obligation to teachers ***Sri Shameer Mohammed. E and Sri Mubarak O.P.,*** *for their unwavering encouragement, candid suggestions, well timed support and help rendered throughout the research work.*

I gratefully acknowledge the invaluable help I received from ***Ramya chechi, Deepthi chechi, Joona chechi, Aswathy, Sreelakshmi, Manju chettan, Mohanetan*** *for the successful completion of my research work.*

From the bottom of my heart, I extend extreme thanks to my dear batch mates ***Anji, Ramya, Amaya, Archana, Pranavya, Aiswarya, Sree, Swathy, Veena, Haritha, Sai Parvathy, Krishna Keerthy, Akshay, Akhil, Alan, Aswin, Vishnu, Thejas and Ramesh*** *and my Senior* ***Fousy,*** *and my Junior* ***Avinash*** *for their moral support, constant help and encouragement that made me confident in difficult situations.*

Words cannot really express the help that I realized from my friends ***Reshma*** *and* ***Athira*** *for the heartfelt help and remarkable support through different phases of study.*

I wish to acknowledge with gratitude the award of fellowship by the Kerala Agricultural University during the tenure of the M.Sc. (Ag.) programme.

I am in dearth to express my love towards my beloved father ***Mr. Radhakrishnan,*** *mother* ***Smt. Remadevi,*** *brother* ***Mr. Pranav,*** *aunty* ***Smt. Rathi*** *and my relatives for all their boundless affection, moral support, eternal love, constant encouragement, blessings, motivation and prayers without which this endeavor would never have become reality.*

I express my immense and whole hearted thanks to all those people who have supported me during my post graduate programme and helped for the successful completion of this thesis.

Arya P. R.

Dedicated to my
Parents

CONTENTS

Sl. No.	Particulars	Page No.
1.	INTRODUCTION	1-3
2.	REVIEW OF LITERATURE	4-23
3.	MATERIALS AND METHODS	24-32
4.	RESULTS	33-62
5.	DISCUSSION	63-79
6.	SUMMARY	80-84
7.	REFERENCES	85-104
	ABSTRACT	105-106
	APPENDIX I	
	APPENDIX II	

LIST OF TABLES

Sl. No.	Title	Page No.
1	Analytical procedure of physical, chemical and biological parameters	25
2	Properties of the experimental soil	34
3	Effect of chlorpyrifos application on soil pH at weekly intervals in incubation experiment No.1	36
4	Effect of chlorpyrifos application on soil chloride content (mg kg^{-1}) at weekly intervals in incubation experiment No.1	36
5	Effect of chlorpyrifos application on soil phosphates content (mg kg^{-1}) at weekly intervals in incubation experiment No.1	38
6	Effect of chlorpyrifos application on soil microbial biomass carbon (µg g^{-1}) at weekly intervals in incubation experiment No.1	38
7	Effect of chlorpyrifos application on EC (dS/m) and moisture content of soil at monthly intervals in incubation experiment No.1	39
8	Effect of chlorpyrifos application on exchangeable K and Na ions in soil at monthly intervals in incubation experiment No.1	39
9	Effect of chlorpyrifos application on exchangeable Ca, Mg and ammonium ions in soil at monthly intervals in incubation experiment No.1	42
10	Effect of chlorpyrifos application on phosphate and chloride ions in soil at monthly intervals in incubation experiment No.1	42
11	Effect of chlorpyrifos application on sulphate, nitrate and bicarbonate ions in soil at monthly intervals in incubation experiment No.1	43
12	Table 12: Effect of chlorpyrifos application on exchangeable cations, anions and total ions in soil at monthly intervals in incubation experiment No.1	43
13	Degradation of chlorpyrifos in soil (mg kg^{-1}) at weekly intervals in incubation experiment No.1	44
14	Percentage variation in the soil parameters after the application of chlorpyrifos to the soil	44

15	Effect of treatments on pH of soil at biweekly intervals in the incubation experiment No.2	46
16	Effect of treatments on chloride content of the soil at biweekly intervals in the incubation experiment No.2	46
17	Effect of treatments on phosphates content of the soil at biweekly intervals in the incubation experiment No.2	48
18	Effect of treatments on degradation of chlorpyrifos in soil at biweekly intervals in the incubation experiment No.2	48
19	Effect of treatments on EC and organic matter content in soil at monthly intervals in the incubation experiment No.2	49
20	Effect of treatments on exchangeable K and Na ions in soil at monthly intervals in the incubation experiment No.2	51
21	Effect of treatments on exchangeable Ca, Mg and ammonium ions in soil at monthly intervals in the incubation experiment No.2	51
22	Effect of treatments on phosphate and chloride ions in soil at monthly intervals in the incubation experiment No.2	54
23	Effect of treatments on sulphate, nitrate and bicarbonate ions in soil at monthly intervals in the incubation experiment No.2	54
24	Effect of treatments on exchangeable cations, anions and total ions in soil at monthly intervals in the incubation experiment No.2	56
25	Effect of treatments on soil moisture content in soil at monthly intervals in the incubation experiment No.2	56
26	Effect of treatments on soil dehydrogenase activity (µg TPFg^{-1} soil day^{-1}) in soil at biweekly intervals in the incubation experiment No.2	58
27	Effect of treatments on soil phosphatase activity (µg PNP g^{-1} soil h^{-1}) in soil at biweekly intervals in the incubation experiment No.2	58
28	Effect of treatments on soil urease activity (µg NH_4^+-N g^{-1} soil h^{-1}) in soil at biweekly intervals in the incubation experiment No.2	60
29	Effect of treatments on microbial biomass carbon in soil at biweekly intervals in the incubation experiment No.2	60
30	Effect of treatments on pH and EC of water (leachate) under submerged condition at biweekly intervals in the incubation experiment No.2	62

31	Effect of treatments on chloride and phosphate ions in water (leachate) under submerged condition at biweekly intervals in the incubation experiment No.2	62
32	Effect of submerged condition on degradation of chlorpyrifos at biweekly intervals in the incubation experiment No.2	62

LIST OF FIGURES

Sl. No.	Title	Pages between
1	Effect of treatments on pH of soil	68-69
2	Effect of treatments on chloride content of soil	68-69
3	Effect of treatments on phosphate content of soil	70-71
4	Effect of treatments on chlorpyrifos residue in soil	70-71
5	Effect of treatments on dehydrogenase activity of soil	76-77
6	Effect of treatments on phosphatase activity of soil	76-77
7	Effect of treatments on urease activity of soil	78-79
8	Effect of treatments on soil microbial biomass carbon of soil	78-79

LIST OF PLATES

Plate No.	Title	Pages between
1	Layout of the incubation experiment No. 2	29-30
2	Incubation experiment No. 1	32-33
3	Chemical treatments for incubation experiment No. 2	32-33
4	Biological treatments for incubation experiment No. 2	32-33
5	Application of treatments to the soil	32-33
6	Treatments in the incubation experiment No.2	32-33
7	Decaying of azolla under submerged condition	62-63

LIST OF ABBREVIATIONS

%	-	Per cent
AEU	-	Agro Ecological Unit
B	-	Boron
^{0}C	-	Degree celcius
Ca	-	Calcium
CD	-	Critical difference
cm	-	Centimeter
COA	-	College of Agriculture
Cu	-	Copper
dS m^{-1}	-	deci Seimens per meter
^{0}E	-	East
EC	-	Electrical conductivity
et al	-	And others
Fe	-	Iron
Fig.	-	Figure
g	-	Gram
g ai ha^{-1}	-	Gram per active ingredient per hectare
g cm^{-3}	-	Gram per centimetre cube
g ha^{-1}	-	Gram per hectare
g kg^{-1}	-	Gram per kilogram
g L^{-1}	-	Gram per litre
ha^{-1}	-	per hectare
hr	-	hour
K	-	Potassium
KAU	-	Kerala Agricultural University
kg	-	Kilogram
kg ai ha^{-1}	-	Kilogram active ingredient per hectare
kg ha^{-1}	-	Kilogram per hectare
l	-	Litre

m	-	Meter
meq $100g^{-1}$	-	Milliequivalent per hundred gram
Mg	-	Magnesium
µg g^{-1}	-	Microgram per gram
µg L^{-1}	-	Microgram per litre
µg NH_4^+-N g^{-1} soil h^{-1}	-	Microgram ammoniacal nitrogen per gram soil per hour
µg PNP g^{-1} soil h^{-1}	-	p nitrophenyl phosphate per gram soil per hour
µg TPF g^{-1} soil day^{-1}	-	Microgram triphenyl formazan per gram soil per day
mg kg^{-1}	-	Milligram per kilogram
mg L^{-1}	-	Milligram per litre
ml	-	Millilitre
ml L^{-1}	-	Millilitre per litre
ml ha^{-1}	-	Millilitre per hectare
mm	-	Millimetre
mmol L^{-1}	-	Milli mole per litre
Mn	-	Manganese
Mo	-	Molybdenum
N	-	Nitrogen
^{0}N	-	North
N	-	Not Significant
Na	-	Sodium
nm	-	Nano meter
OC	-	Organic carbon
P	-	Phosphorus
pH	-	Soil reaction
S	-	Sulphur
SE(m)	-	Standard error mean
UV	-	Ultraviolet
viz	-	Namely
Zn	-	Zinc

Introduction

1. INTRODUCTION

There has been increase in the usage of pesticides in order to meet the enhanced demand of crop production in the era of modern agriculture (Bose *et al*., 2021a). As the world population is increasing, food requirement is also increasing, which can be achieved by cultivating crops in more areas. There are different factors which limit the crop production like weeds, insects, plant diseases and nutrient deficiencies. In the global scenario, the insect pests caused 40 per cent crop losses. In order to protect the food supply and meet the needs of growing population, farmers need comprehensive pest management tools to combat the pest infestation. Pesticides play an important role in Indian economy and it fetches billions of dollars generating huge income. Pesticides generated a total revenue of 84.5 billion dollars during 2019 and would be expected to achieve 137 billion dollars by 2023 (Bose *et al*., 2021b). Global scenario of annual pesticide consumption was estimated to be two million tonnes and would reach 3.5 million tonnes by 2021 (Sharma *et al.,* 2019).

The consumption of insecticides was found to be highest among the different pesticides used in India, as compared to fungicides and herbicides. Organophosphorus insecticides are one of the major groups of compounds used all over the world (Kanekar *et al.,* 2004). Organophosphorus compounds are used as substitute for organochlorines because of broad spectrum activity and less persistence. Some of the organophosphorus insecticides widely used are chlorpyrifos, quinalphos, monocrotophos and malathion. In India, chlorpyrifos dominates among the different insecticides (KaviKarunya and Reetha, 2012). Nayak and Solanki (2021) reported that usage of chlorpyrifos was highest in the year 2019- 2020 in India. Its usage in India has increased from 471 million tonnes in 2014-15 to 1431 million tonnes in 2019-20 (Nayak and Solanki, 2021).

Chlorpyrifos is an organophosphorus insecticide with non- systemic, contact and respiratory action (Testai *et al.,* 2010). Chlorpyrifos acts as a nerve poison for both humans and insects. It inhibits the acetyl choline esterase activity in the central nervous system (Xu *et al.,* 2008). It was introduced by Dow Chemical Company in United States of America in 1965 (Sardar and Kole, 2005), initially designated as DOWCO 179 and later as chlorpyrifos (Rekha, 2005). It is a highly toxic compound having colour code, yellow and it has long persistence due to which it will remain in environment for a long

period of time. Chlorpyrifos is widely used in agriculture, horticulture and forestry to control wide range of pests including root worms, borers, soil dwelling grubs and subterranean termites. Its commercial brand names are Lorsban, Dursban, Dhanwan, Omexan, Agromil, Dorson etc. (Bhagobaty *et al*., 2007). Emulsifiable concentrate, granules, wettable powder and dustable powder are the commercial formulations of chlorpyrifos. Excessive use of chlorpyrifos has lead to surface water contamination. Its unscientific usage has led to accumulation of residues in soils, crops and the ecosystems, which disturbs the environment (Qiao *et al.,* 2003).

Usage of chlorpyrifos has minimized the crop losses and enhanced the agricultural production while its excessive usage would lead to toxicity and pollution. According to World Health Organization (WHO) only 2 to 3 per cent of applied pesticides are efficiently used for preventing or killing pests, while the rest accumulated in the soil (Galloway and Handy, 2003). Pesticide residues are reported to exist even at a distance of 24 km away from the application sites (Bootharaju and Pradeep, 2012). Surface water bodies such as lakes and ponds are being contaminated by the unscientific use of pesticides (Hui *et al.,* 2021). Spray drift and runoff of this insecticide from the agricultural field also contaminate the surface water bodies (Chekroun *et al.* 2014). Chlorpyrifos residues cause serious human health issues such as carcinogenicity, reproductive disorders, neurological disorders and various health problems (Rani *et al.,* 2021). Chlorpyrifos is spontaneously hydrolyzed and hence, an acute exposure to the chemical would cause neurotoxicity in mammals. The continuous use of chlorpyrifos causes serious environmental issues due to their toxic nature and hence there is a need to eliminate these toxic chemicals from the environment.

As the need of the hour, it is important to develop a strategy to degrade and eliminate the chlorpyrifos residues from the soil and environment. Degradation is the process of breakdown of complex substances into smaller molecules. Breakdown of pesticide compounds is usually advantageous but adverse when it is broken down before the target organism has been killed. Three types of degradation of pesticides found in nature are physical, chemical and biological degradation. Degradation of pesticides in soil depends on various environmental factors such as pH, moisture, organic carbon content and the type of pesticide formulations (Racke *et al.,* 1988).

Photodegradation, a means of physical degradation of pesticides would be achieved by direct photolysis of the compound, in which pesticide molecule absorbs light energy, gets excited and transformed into different intermediates depending on the availability of light. Photodegradation is the most destructive degradation method when its end products are released to the environment (Katagi, 2004). Pesticides undergo photodegradation in presence of UV light and sunlight.

Chemical degradation is one of the important phenomena in degradation process. Oxidative reagents such as hydrogen peroxide, Fenton reagent, titanium dioxide, zinc peroxide etc. are commonly adopted in the chemical degradation of pesticides. It serves as an advanced promising technology for the removal of organic pollutants from the environment through the generation of hydroxyl radicals. Chemical methods are labour intensive and also disturbing to the environment (Harvey *et al.*, 2002).

Biodegradation is the conversion of complex substances into simpler substances with the help of microorganism and this process is catalysed by a particular enzyme, this is efficient and eco-friendly method to detoxify the environment. Microbes like bacteria, algae and fungi plays an important role in breakdown of pesticides (Nawaz *et al.,* 2011). Bioremediation is a biotechnological approach used to remediate the pollutant from the environment. In this process, microorganisms convert toxic compounds into non-toxic compounds (Mihelcic and Luthy, 1988). Biodegradation is a cost effective, efficient approach and would result in formation of nontoxic compounds.

In this context, an investigation entitled “Approaches to assess chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)” was conducted with the objective to evaluate and assess the impact of chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11) by using physical, chemical and biological methods.

Review of literature

2. REVIEW OF LITERATURE

The present investigation was carried out to evaluate different approaches of chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11). Organophosphorus compounds are broad spectrum insecticides used for the pest control as alternative to organochlorines. Chlorpyrifos (O, O-diethyl O-(3,5,6-trichloro-2-pyridyl phosphorothioate) is organophosphate insecticide cum acaricide and it was commercially produced by reacting 3,5,6-trichloro-2-pyridinol (TCP) with O, O-diethyl phosphorochloridothioate.

2.1 STATUS OF PESTICIDE USAGE IN INDIA

In the year 2019, India had invested 70 billion INR on pesticide production. The productivity of pesticides was 93000 metric tonnes in 2020. India occupies fourth position in the export of pesticide after USA, China and Japan. Indian agrochemical industry is the leading one in Asia but is ranked twelfth in the world (Bose *et al.,* 2021). Average pesticide consumption in India is 600 g ha^{-1} (Bose *et al.,* 2021). Consumption of pesticide increased by 50 per cent from 2010 to 2015 (Bose *et al.,* 2021) and the expected annual increase during 2019 to 2024 would be 8.1 per cent (Nayak and Solanki, 2021). Nayak and Solanki (2021) also reported that a total of 293 pesticides were registered for use in India and at the same time 40 pesticides were banned in India for manufacture, import and use. The per hectare pesticide consumption was reported to be highest in Punjab (0.74 kg) followed by Haryana (0.62 kg) and Maharashtra (0.57 kg) while North-eastern states like Sikkim, Assam, Manipur and Nagaland showed lowest pesticide consumption status (Nayak and Solanki, 2021), while the total pesticide consumption in India was highest in Maharashtra followed by Uttar Pradesh and Punjab (Nayak and Solanki, 2021). In India, pesticide usage pattern differs from those in the world, as insecticides were used in highest amount compared to fungicides and herbicides whereas in the world, highest consumption was that of herbicides. Out of the total insecticide consumption in India, 50 per cent usage was reported for the management cotton pest (Nayak and Solanki, 2021).

2.2 CHLORPYRIFOS

Organophosphorus compounds are neutral ester or amide derivatives of phosphoric acid carrying phosphoryl or thiophosphoryl group. They are generally the most toxic of all insecticides to insects and vertebrates as they inhibits cholineesterases. Because of its broad-spectrum activity they were replaced by organochlorine pesticides. Pesticides like chlordane, DDT and aldrin banned by Indian government enhanced the usage of organophosphorus pesticides like chlorpyrifos (Bootharaju and Pradeep, 2012). Among the organophosphorus compounds chlorpyrifos is the most important and toxic insecticides used in India (Bootharaju and Pradeep, 2012).

Chlorpyrifos also known as chlorpyrifos ethyl, has non- systemic, contact and respiratory action (Testai, 2010)). Chlorpyrifos is colourless to white crystalline solid and slightly soluble in water (2 mg/L) but soluble in organic solvents (Testai *et al*., 2010). Minimum purity of technical chlorpyrifos is between 940- 990 g kg^{-1} (Shalaby *et al.,* 2021). It is a nerve poison for both human and insects. It inhibits the acetyl choline esterase activity in the central nervous system (Xu *et al.,* 2008). It was widely used in agriculture, horticulture and forestry to control the pests such as soil dwelling grubs, root worms, borers, subterranean termites and also for the control of fleas, ticks on cattle, cockroaches etc. In agriculture, it was mainly used in crops such as grains, cotton, fruits, nuts, vegetable crops etc. (Hua *et al*., 2009). Among the insecticides, chlorpyrifos was the most widely used during 2019- 2020 in India. Its consumption has risen from 471 MT in 2014-15 to 1431 MT in 2019-20 (Nayak and Solanki, 2021).

2.2.1 Uses of chlorpyrifos

Pesticides are a boon for the farmers because it will increase the agricultural productivity. In order to make agriculture more productive and profitable, the intensity of crop protection should increase by 15-20 fold around the world.

Chlorpyrifos is an effective insecticide used for the control of wide range of pests including Coleoptera, Diptera, Homoptera and Lepidoptera in different crops. Complete control of the mustard aphid was effectively done by application of 0.03 per cent chlorpyrifos (Upadyay and Agarwal, 1993). Chlorpyrifos when applied at 0.04 per

cent on pea caused the complete control of pea leaf minor *Chromatomyla horticola* (Khajuria and Sherma, 1995). Red hairy caterpillar *Amsacta moorei* would be controlled under the laboratory condition by using chlorpyrifos concentration at 0.2 per cent (Tandi *et al.,* 1993)

Aerial application of chlorpyrifos was used for the control of surface feeding insects of rice, cotton, mustard and chickpea (Dhawan and Simwat, 1996). Soil application of chlorpyrifos was commonly used against root damaging insect larvae which attack the crops such as cole crops, ground nut, vegetables, cardamom, tobacco and onion (Rouchoud *et al.,* 1991). Soil dwelling termites can be controlled by the application of 1 to 2 per cent chlorpyrifos. Chlorpyrifos was used for controlling the termites in three different type of soils which showed that the concentration of chlorpyrifos were seven times lower in sandy soils compared with sandy loam and sandy clay loam (Forscher and Townsend, 1998).

2.2.2 Adverse effect of chlorpyrifos

Pesticides are quick, easy and inexpensive tool for controlling the insect pests (Kumar *et al.,* 2013). Use of organophosphate pesticides are higher due to their low persistence and less harmful to the environment than the organochlorines. The excessive use of chlorpyrifos in plants inhibits seedling emergence, abnormal cell division and fruit deformities (Galloway and Handy, 2003). According to Tu (1981), pesticide application to the soil reduces the microbial population and enzymatic activities, which affects soil fertility adversely. Unscientific use of chlorpyrifos contaminated the ecosystems (Blondell and Dobozy, 1997). The major degradation product of chlorpyrifos is TCP, it is highly toxic and has higher water solubility than parental compound which leads to contamination of soil and water systems.

Adverse effect of chlorpyrifos on non target organisms includes low birth weights, nervous system disorders, birth defects and endocrine disruption. Farmers are directly exposed to the toxic chemicals during the use of pesticides in the agricultural field without protective gears, which cause harmful effects on human beings. Anwar *et al.* (2009) reported that harmful effects of chlorpyrifos in humans include skin irritation, respiratory failures, twitching of muscles, convulsion and death. It has been reported

that the human body absorbs chlorpyrifos through oral route (70 %) and through skin (3 %) (Nolan *et al.,* 1984). The organophosphates do not accumulate in the human organs which gets readily bio transformed in the liver and they are usually excreted out within 24 h by urination and less likely in the faces (Griza *et al.,* 2008).

Gilani *et al.* (2016) reported that chlorpyrifos kills the fishes at very minute concentration in aquatic system. After effects of chlorpyrifos in birds includes nestlings, deformities and death (George *et al.,* 2014). Because of its toxic and persistent nature its need to be remediated.

2.2.3 Persistence of chlorpyrifos in soil

Chlorpyrifos is highly persistent insecticide and its degradation was slow in the environment. Long term availability of chlorpyrifos for the pest control directly related to the persistence of the pesticides in the environment.

Biological active period of chlorpyrifos ranges from 20 days to several months (Awasthi and Prakash, 1998). Half life of chlorpyrifos in soil ranges from 7 to 120 days. Persistence of chlorpyrifos was higher in organic soil than mineral soil (Gebremariam, 2012).

Residues of chlorpyrifos has higher affinity for aquatic sediments (Gebremariam, 2012). Various factors that govern the persistence of organophosphates in soil were clay content, organic matter and application rate (Awasthi and Prakash, 1997).

Tashiro and Kuhr (1978) found out that in sandy loam soil, chlorpyrifos has half life of 7-16 days, where as in clayey soil it was up to 120 days (Freed *et al.*, 1979). According to Getzin (1985), chlorpyrifos has degradation half life of 4 and 12 weeks in silt loam and clay loam respectively. Half-life of chlorpyrifos in water ranges from 35 to 78 days and it was moderately soluble in water (Racke *et al.,* 1996).

Chishti *et al.* (2013) confirmed that complete degradation or 100 per cent recovery of the active ingredient of chlorpyrifos was not possible during the dissipation. According to Nawaz *et al*. (2011), residue of chlorpyrifos found in the environment depends on its initial concentration as well as the biodegradation rate.

Halimah *et al*. (2010) stated that chlorpyrifos residue was detected in the soil at 20 cm depth after 5 to 7 days of its application.

Chlorpyrifos was relatively immobile vertically in soil and there was not proved the ground water contamination. But insecticide residues cause wide spread contamination of drinking water (Mukherjee *et al*., 1996).

Chlorpyrifos was applied on chilli crop at the concentration of 500 to 1000 g ai ha^{-1} and the residues recorded on 4.43 and 2.01 days were 0.59 and 2.02 mg kg^{-1} (Jyot *et al.,* 2013).

Suri and Joia (1996) found out that chlorpyrifos residues of 0.422 and 1.190 mg kg^{-1} were observed in the wheat cropped soil after the incorporation of chlorpyrifos at the rate of 1 and 2 kg ai ha^{-1} with the half life values of 23 and 23.2 days respectively.

Initial residues of chlorpyrifos recorded in the cabbage soil was 1.10 to 2.21 mg kg^{-1} with the application of chlorpyrifos methyl at 720 g m^{-2} (Hou *et al*., 2005).

Chlorpyrifos 20 EC was applied on processed black tea at the concentration of 750 and 1000 ml ha^{-1} in wet and dry seasons and the residues observed at the half life values of 1.62 and 1.68 days in wet season were 14.25 and 16.92 mg kg^{-1} and in dry season were 8.24 and 11.04 mg kg^{-1} (Manikandan *et al.,* 2001).

2.3 FATE OF CHLORPYRIFOS IN THE ENVIRONMENT

Pesticides undergo various changes in the environment depending on the physio- chemical properties of pesticides and the soil. Pesticides in the environment were influenced by many processes and these processes explains fate and persistence of pesticides (Mustapha *et al.,* 2018). When the pesticides were introduced in to the environment, it would undergo transfer and degradation processes.

2.3.1 Transfer process of chlorpyrifos

The transfer process includes adsorption, absorption, volatilisation, spray drift, run off and leaching. Adsorption of chlorpyrifos by soil or organic matter reduces the pesticides bioavailability and mobility in the soil. Air borne movement of pesticides called drift, would contaminates the crops which ready for harvest (Maybank *et al.,*

1978). Lower pH favours the uptake (Garcinuno *et al.*, 2003) whereas organic matter decreased the uptake of pesticides by plants (Yu *et al.*, 2009). Leaching and runoff of the pesticide causes surface water contamination. All these transfer process constantly raised the level of pesticides in the environment.

2.3.2 Degradation or breakdown process of chlorpyrifos

Degradation of the chlorpyrifos was advantageous but the process was harmful when it degraded even before the target pest has been controlled. During the degradation, chlorpyrifos molecule breakdown to form the intermediate products. Its further breakdown leads to the formation of carbon dioxide and water.

Organophosphorus compounds are esters of phosphoric acids which include the phenyl, aliphatic and heterocyclic derivatives. Due to its toxicity, its remediation from the environment is quite important. Residues of organophosphorus compounds were found in environment and they were also degradable in nature. Degradation of chlorpyrifos depend upon different factors such as physical and chemical properties of soil and the pesticide molecule (Li *et al.*, 2008).

Major degradation process were microbial degradation, photolysis, volatilization and chemical hydrolysis (Racke *et al.*, 1988). Degradation of pesticides were done by different methods among this, biological method was more efficient (Racke *et al.*, 1990).

Chemical methods used for the degradation were oxidation, Fenton oxidation, electro oxidation, electro coagulation and the photocatalytic degradation (Pangarkar *et al.*, 2014).

Under the physical methods photodegradation, electro dialysis, reverse osmosis, membrane distillation, adsorption and nano filtration were found to degrade chlorpyrifos effectively (Pangarkar *et al.*, 2014).

Bioremediation does not need disposal of chemical pesticides as compared to physio chemical degradation methods. Biodegradation is an in-situ remediation technique. Microorganisms present in the contaminated area has the ability to do the degradation due to the continuous exposure to the pesticides. Most of the times, external

nutrient supply was not required as the chemical serve as source of energy and nutrient for the degradation process (Ortiz-Hernandez *et al.*, 2013).

Each technique provides unique and different approaches and also entails certain advantages over others under particular situation.

2.3.3 Chlorpyrifos degradation pathway

Oxidative desulfuration of chlorpyrifos leads to the formation of unstable chlorpyrifos oxon which was highly toxic than parental compound and it was responsible for inhibition of acetyl choline esterases (Nolan *et al*., 1984). The unstable chlorpyrifos oxon was 400 times more active than chlorpyrifos and undergo hydrolysis leading to the formation of TCP (3,5,6- trichloro – 2 pyridinol) and diethylthiophosphate (DETP) (Racke *et al.*, 1994). The TCP is pridinol ring with 3 chlorine atoms attached to it. It was reported that TCP was used by *Pseudomonas* as a sole source of carbon and energy. The intermediate product TCP was highly toxic and more mobile than parental compound and has antimicrobial property but not any insecticidal property (Gilani *et al*., 2016). This TCP has half-life of 360 days which was higher than parental compound (Thengodkar and Sivakami, 2010). Soil microorganisms have the capability to degrade the TCP about 65-80 per cent within 14 days (Racke *et al.*, 1988). The DETP hydrolysed to produce phosphorothioic acid and ethanol molecules, which act as a source of carbon, sulphur and phosphorus to the microorganisms (Rokade and Mali, 2013). Intermediate products formed during breakdown of chlorpyrifos were TCP, DETP, dihydroxypyridine, chlorodihydro-2-pyridone, maleamide semialdehyde and tetrahydro-2-pyridone (Singh and Walker, 2006). Complete degradation of chlorpyrifos leads to the formation of CO_2 and water (Singh and Walker, 2006).

According to Sardar and Kole (2005), the intermediate product of chlorpyrifos TCP, transformed in to 3,5,6-trichloro-2-methoxy-pyridine (TMP). Reductive dechlorination of TMP results into the formation of 2,3 dihydroxy pyridine which yield 2,5,6 trihydroxy pyridine. Oxidation of metabolites results into the formation of carbon and inorganic phosphates and amines.

Reddy *et al.* (2013) reported the intermediate product of chlorpyrifos, 2,3-dihydroxy pyridine converted to maleamic acid and further reaction leads to the formation of carbon dioxide, water, ammonium carbonate and sodium chloride.

Chlorpyrifos degradation leads to the formation of DETP and TCP and the DETP converted into thiophosphate while the TCP converted into 2-pyridinol (Rokade and Mali, 2013).

2.4 MICROBIAL DEGRADATION

Microorganisms such as bacteria and fungi have high potential for growth on chlorpyrifos (Gilani *et al*., 2016). Biodegradation was more efficient and economic approach (Xu *et al.,* 2008) than chemical and physical process in which microbes convert toxic compound into less toxic compounds. Microorganisms degrade the pesticides in two ways. In first type organic compounds were completely degraded into their metabolites along with the release of energy and nutrients, which was utilized by microorganisms (Alexander, 1999). It is called as catabolism. In the second type organic compounds were not completely metabolized called co- metabolism and they do not provide any benefit to the microorganisms (Alexander, 1999).

Microorganisms can degrade the pesticides and used as a source of nutrients for their growth. Chlorpyrifos serve as a source of carbon, nitrogen and phosphorous to the degrading microorganisms (Gilani *et al*., 2016). Degradation of chlorpyrifos was described by first order model (Hua *et al.,* 2009). Microbial degradation of chlorpyrifos depends the different factors like metabolite available for the degradation, physiological status of microbes, capacity of microorganism to survive and proliferate in the contaminate site and the sustainable microbial population (Gilani *et al*., 2016). Factors such as low temperature, anaerobic condition and salinity lowered the microbial degradation (Bondarenko and Gan, 2004).

There are three types of biodegradations. They are bacterial, fungal, enzymatic degradation. Optimum environmental conditions such as pH, temperature, salinity, availability of water was necessary for the efficient degradation process.

2.4.1 Bacterial degradation

Bacteria has been used extensively for the degradation of pesticides. Bacterial strains isolated from the contaminated site widely used for the degradation of pesticides. The important groups of bacterial genera used for the degradation are *Pseudomonas, Bacillus, Klebsiella, Flavobacterium, Acinetobacter, Aerobacter, Alcaligenes, Micrococcus, Neisseria, Sphingomonas, Burkholderia, Micrococcus and Arthrobacter* (Mohammed and Bartakke, 2015).

An experiment was conducted by Ajaz *et al.* (2005) by screening 20 bacterial isolates three of them showed higher resistance to chlorpyrifos and used for the study. Bacterial isolates such as *Pseudomonas putida, Klebsiella* sp. and *Aeromonas* sp. offered the resistance of chlorpyrifos up to 2 mg ml^{-1}, 4 mg ml^{-1} and 8 mg ml^{-1} respectively.

According to Singh *et al.* (2009) *Pseudomonas aeruginosa* is a versatile microorganism widely used for the dissipation of xenobiotic compounds. More than 98% degradation of chlorpyrifos was done in the presence of biosurfactant 0.1 g L^{-1} where as in the absence of biosurfactant 84 per cent degradation within 120 hours of incubation.

El-Helow *et al.* (2013) reported that *Bacillus subtilis* Y242 grows on a medium containing chlorpyrifos concentration up to 150 mg L^{-1} and used the chlorpyrifos as a source of carbon and energy for their growth and reproduction during the degradation. Which degraded the chlorpyrifos up to 95.12 per cent within 48 hours of incubation.

An experiment was done by Anwar *et al*. (2009), biodegradation of chlorpyrifos using bacterial strain C2A1. Degradation would be enhanced with the addition of yeast extract and glucose and achieved the degradation efficiency of 90 per cent within 8 days of incubation. Microbial strain used the chlorpyrifos as a source of carbon and energy.

A study conducted by Zhu *et al*. (2010) using the strain of a bacteria *Bacillus licheniformis* isolated from soil for the degradation of chlorpyrifos contaminated soil having concentration up to 100 mg kg^{-1}. Optimum conditions like 35°C and pH 7.5

required for the rapid degradation by bacteria, results in higher degradation of chlorpyrifos up to 99 per cent within 14 days of incubation.

Degradation study by using two streptomyces strains AC5 and AC7 have capability to degrade chlorpyrifos. Both the strains of streptomyces degrade the chlorpyrifos concentration of 25 to 50 mg L^{-1} up to 90 per cent within 24 hours of incubation (Briceno *et al.,* 2012).

Silambarasan and Abraham, (2013) isolated the bacterial strain from the soil contaminated with chlorpyrifos. It has high potential to degrade the chlorpyrifos and TCP and identified the bacterial strain as *Alcaligenes* sp JAS1 which degraded the chlorpyrifos concentration of 300 mg L^{-1} within 12 hours of incubation.

2.4.1.1 Action of Pseudomonas sp in pesticide degradation

Pseudomonas was the dominant group of bacteria present in soil and it has vital role in organic matter degradation and they have the capability to degrade petroleum products, aromatic hydrocarbons and the other pesticides (Sarkar *et al*., 2009).

Pseudomonas aeruginosa and *Pseudomonas putida* have the highest degradation capacity among the *Pseudomonas* species (Vijayalakshmi and Usha, 2012). *Pseudomonas aeruginosa* is gram negative bacterium has the high potential for chlorpyrifos degradation (Xu *et al*., 2008). *Pseudomonas aeruginosa* reported 92 per cent chlorpyrifos degradation in soil at 37^0 C within 30 days (Lakshmi *et al.*, 2008). Chlorpyrifos up to 500 mg L^{-1} would be completely degraded by *Pseudomonas aeruginosa* within 7 days.

Walker *et al.* (1993) reported that *Pseudomonas* and *Bacillus* have different enzymes which enable them to degrade different group of pesticides.

Maya *et al*. (2011) evaluated the degradation potential of seven bacterial isolate for the degradation of chlorpyrifos and TCP. Results indicated that the degradation potential was highest for *Pseudomonas* followed by *Agrobacterium* and *Bacillus.* It has been also observed that these bacterial isolates were more efficient in degradation of TCP than chlorpyrifos.

An investigation was carried out by Awad *et al*. (2011) by using five bacterial strains of *Pseudomonas stutzeri* which was isolated from contaminated soils of Egypt. Strain of *Pseudomonas stutzeri* B-CP5 has capacity to degrade 300 mg L^{-1} of chlorpyrifos in the contaminated soils of Egypt within 7 days at pH 7 and 30^0 C.

Research work on chlorpyrifos degradation was conducted by using strain *Pseudomonas desmolyticum* NCIM 2112. Optimum conditions such as pH 7 and 30^0 C were required for the efficient degradation of chlorpyrifos. *Pseudomonas desmolyticum* NCIM 2112 degraded 98 per cent of chlorpyrifos within 6 days and converted this toxic compound into non toxic metabolites such as 2- pyidinol and thiophosphate (Rokade and Mali, 2013).

Sharma *et al.* (2017) observed that *Pseudomonas indoloxydans* ASK was able to grow on chlorpyrifos containing medium concentration up to 400 mg L^{-1}. This organism possessed high organophosphorus hydrolase activity at optimum conditions like 37^0 C and pH 8.0 after the 48 hours of incubation period. The strain showed the degradation of chlorpyrifos up to 82.72 per cent after 96 hours of incubation.

Rokade and Mali (2013) reported that degradation of chlorpyrifos by *Pseudomonas* increased with the addition of nutrients. In the presence of $NaNO_3$ degradation was found to be 98 per cent. The degradation of chlorpyrifos was 60 per cent and 50 per cent in the presence of peptone and malt extract respectively.

2.4.2 Fungal degradation

Fungal population introduces minor structural changes to the pesticide and converts into non toxic metabolites releasing them back to the soil and they were further degraded by bacterial species through process such as cometabolism and mineralization (Coelho *et al.,* 2015).

A promising approach for the detoxification of chlorpyrifos on vegetable would be successfully done by using cell free extract from the fungal strain of *Verticillium* which was isolated from soil contaminated with chlorpyrifos and the degradation enhanced with increase in the concentration of chlorpyrifos (Yu *et al.,* 2006).

According to Bumpus *et al.* (1993), *Phanerochaete chrysosporium* has the ability to degrade the chlorpyrifos up to 27.5 per cent during the 18^{th} day of incubation under nitrogen limited condition.

A degradation experiment was conducted by two fungi *Trichoderma viride* and *Aspergillus niger* and evaluated for the degradation of chlorpyrifos. *Trichoderma viride* showed the highest degradation potential of 95.7 per cent while the *Aspergillus niger* showed 72.3 per cent within 14 days of incubation (Mukherjee and Gopal, 1996).

Fungal strain of *Verticillium* sp. has the capability to reduce the half life of chlorpyrifos up to 37 per cent (Fang *et al.,* 2008). The fungal inoculated soils showed the highest degradation of chlorpyrifos and degradation rates reported were 3.61 times faster than sterilized soil under the laboratory condition.

Chen *et al*. (2012) found out that new fungal strain *Cladosporium cladosporoides* strain Hu-01 has the capability to completely degraded the chlorpyrifos into CO_2 and water under the optimum conditions such as 26.8^0 C and pH 6.5. It would tolerate the chlorpyrifos concentration up to 500 mg ml^{-1} and it utilized the chlorpyrifos as a source of carbon and energy during the degradation.

2.4.2.1 Action of Trichoderma sp in pesticide degradation

Alvarenga *et al.* (2015) reported that *Aspergillus sydowii* CBMAI 935 and *Trichoderma* sp. CBMAI 932 were choose as best chlorpyrifos dissipating strains from seven marine derived fungi. Both strains were capable of dissipating chlorpyrifos in high percentage and also lowering the concentration of TCP. *Trichoderma* sp. CBMAI 932 reported the 72 per cent degradation within 30 days where as in *Aspergillus sydowii* CBMAI 935 showed the 63 per cent degradation within the same period.

Jayaraman *et al.* (2012) conducted the co- culture experiment by using *T.viride*, *T. harzianum* and its consortium for the degradation of chlorpyrifos. Highest degradation of chlorpyrifos showed by *T.viride* (99.06%) followed by *T. harzianum* (97.57%) and its consortium (59.70%) within 10 days of incubation. Consortium of this fungi possess low rate of degradation compared to individual strains.

Jayaraman *et al.* (2012) confirmed that strains of *T. viride* and *T. harzianum* decrease their growth with increase in the concentration of chlorpyrifos and radial growth of the organisms increased with increase in the incubation days.

Growth of fungus increased with the addition of organophosphous insecticides which might be due to accumulation of organic and inorganic compounds by fungi (Omar, 1998).

2.4.3 Action of consortium on pesticide degradation

Mixed cultures of microorganism are more efficient in pesticide degradation than single cultures (Sariwati *et al*., 2017).

According to John *et al.* (2016), bacterial consortium consisted of *Staphylococcus warneri*, *Pseudomonas putida* and *Stenotrophomonas maltophilia* capable of giving 90 per cent degradation of chlorpyrifos (125 mg kg^{-1}) than single cultures under optimized conditions of pH 7 and temperature 30^0 C within the 8 days of incubation period.

Barathidasan *et al.* (2014) claimed that complete degradation of 50 mg L^{-1} of chlorpyrifos achieved by co-culture of *Cellulomonas fimi* and fungal strain *Phanerochaete chrysosporium* within 16 hours whereas fungus alone could degrade 50 mg L^{-1} of chlorpyrifos within 6 days. This study pointed out the advantage of consortia over single cultures and also potential to clean up contaminated environment by microbial cultures.

John *et al*. (2014) observed that microbial consortium consisting of *Klebsiella* sp. and two different strains of *Pseudomonas aeruginosa* enhanced the dissipation of chlorpyrifos when amended with NPK nutrients.

Flavobacterium sp. and *Escherichia coli* clone co-metabolically degrade the chlorpyrifos but these organisms do not use the chlorpyrifos as a source of carbon and energy (Mallick *et al.,* 1999; Richnis *et al.,* 1997).

Microbial consortium consisting of *Pseudomonas aeruginosa, Bacillus cereus, Klebsiella sp and Serratia marscecens* developed from pesticide-contaminated soil of Punjab degraded the chlorpyrifos effectively (Lakshmi *et al.*, 2009).

Mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* shows 72- 83 per cent chlorpyrifos dissipation within 96 hours of incubation (Shaker *et al.,* 1988).

2.5 CHEMICAL DEGRADATION

Pesticides undergo chemical degradation by hydrolysis and volatilization. Pesticides would be easily hydrolysed when the functional groups of the pesticides were attached with weak or labile bond. The process of breaking such labile bond is called hydrolysis and the hydrolytic enzymes catalyse this process (Armstrong and Konrad, 1974). Once pesticide was volatilized, it may diffuse into the atmosphere and either be destroyed or continued as an environmental risk (Armstrong and Konrad, 1974).

Hydrolysis, volatilization and oxidation were the main reactions involved in chemical degradation. Among the chemical treatments, oxidation process degrades the pesticides by utilizing the hydroxyl radicals. Alkaline hydrolysis used for removing certain pesticides but this process done at laboratory conditions and this will lead to the formation of secondary pollutants (Ortiz-Hernandez *et al.,* 2013). Hydrolytic degradation of chlorpyrifos would be low in pure water, half life of chlorpyrifos was 29- 74 days under conditions such as pH 7 and 25^0 C but the degradation may be enhanced under certain environmental conditions (Mukherjee *et al.,* 2006)

2.5.1 Degradation of pesticides by hydrogen peroxide

Lambert *et al.* (1996) conducted a degradation study to examine the effects of ozon and ozon in combination with hydrogen peroxide for the dissipation of five herbicides namely atrazine, benazoline, bentazone, imazapyr and triclopyr. Hydrogen peroxide applied with ozone was effective for removal of all the herbicides whereas the degradation by ozone alone was effective only for bentazone at the concentration of 2 µg L^{-1} and at the pH of 7.5.

According to Ho *et al.* (2020), removal of chlorpyrifos residues on the surface of apples would be effectively done by advanced oxidative process by combining H_2O_2 and UV-C light.

Using UV radiation with H_2O_2 was a more effective method than UV-TiO_2 for the degradation of organophosphorus compounds (Doong and Chang, 1997). Addition of H_2O_2 was more effective than TiO_2 in the photocatalytic oxidation of organophosphorus compounds.

Research work conducted by Chelme-Ayala *et al.* (2011) with ozone (O_3) and O_3 combined with hydrogen peroxide (H_2O_2) for the degradation of bromoxynil and trifluralin in natural waters. The results pointed out that higher degradation was observed by using O_3/H_2O_2 process.

Investigation was carried out to check the efficiency of hydrogen peroxide, ultrasonic waves or the combined H_2O_2 sonication for the detoxification of organochlorine pesticides. Kida *et al.* (2018) confirmed that degradation was more effective by using combined effects of H_2O_2 sonication process than H_2O_2 alone.

2.5.2 Degradation of pesticides by Fenton reagent

Electro Fenton process was successfully used for the degradation of organophosphorus compounds (Guivarch *et al.,* 2003). Electro-Fenton process was efficient method for the degradation of chlorpyrifos in the initial concentration of 30-240 mg L^{-1}.

Aziz *et al.* (2013) stated that complete degradation of chlorpyrifos was attained at initial concentration of 30 mg L^{-1} within 60 min. At initial concentration of chlorpyrifos 240 mg L^{-1} the removal was 72 per cent within 90 min. Optimum dose of Fe^{2+} for this process was found to be 20 mg L^{-1} and at the pH value of 3. Electro Fenton process follow pseudo- first order kinetics.

The extension of the Fenton process was mainly influenced by the H_2O_2 dosage, because it was the source of hydroxyl radicals during the light irradiation time (Zazo *et al.*, 2009). For the dissipation of organophosphorus pesticides addition of ferrous iron in combination with UV light was a more powerful method than UV/H_2O_2 treatment (Doong and Chang, 1998).

FeGAC/H_2O_2 process has been applied for the degradation of aqueous solution containing chlorpyrifos, cypermethrin and chlorothalonil. Complete degradation of

chlorpyrifos occurred in 1 minute by FeGAC (5 g L^{-1}) H_2O_2 process under operating conditions like pH 3 and 60 min reaction time (Affam *et al.,* 2016).

Alalm *et al.* (2017) conducted an experiment by using solar photo Fenton process for the dissipation of phenol. Study pointed out that complete dissipation of phenol was attained when the initial concentration of phenol at 100 mg L^{-1} after 45 min of irradiation. Degradation of phenol boosted with increase in the dosage of H_2O_2 and $FeSO_4.7H_2O$ and the optimum dosages required for the degradation were 44 mmol L^{-1} for H_2O_2 and 0.5 g L^{-1} of $FeSO_4.7H_2O$.

Li *et al*. (2009) reported that degradation efficiency of triazophos in wastewater was 96.3 per cent under the ideal conditions such as dosage of 2.5 g L^{-1} of $FeSO_4.7H_2O$ and 100 mL L^{-1} of 30% H_2O_2 solution, pH value of 4 and stirring time of 90 minutes.

For reducing the levels of pesticides in waste water, H_2O_2/Fe^{2+}/UV system would be applied and works as a new developing methodology (Tamimi *et al.,* 2008). According to Tamimi *et al.* (2008), photo-Fenton was more efficient than Fenton process alone for the methomyl degradation. Optimum conditions required for the degradation of methomyl for the Fenton and photo- Fenton process was observed at pH 3, with an initial Fe^{2+} concentration of 0.5 mmol L^{-1} and initial H_2O_2 concentration of 1 mmol L^{-1} with a pesticide concentration of 0.123 mmol L^{-1}.

2.6 PHYSICAL DEGRADATION

Photodecomposition is one of the common methods used for the degradation of chlorpyrifos. Sun light and the UV light were the main agents used for the of photodecomposition of chlorpyrifos. Solar radiation brings about many chemical changes in the environment. Sufficient energy of sunlight and UV light were necessary for the many chemical transformation of pesticides. Intermediate products formed during the degradation were similar to biological reactions, however unique structures were produced by photodecomposition (Sadegh-Zadeh *et al.,* 2017). Degradation of pesticides by UV light was restricted to the residues close to the surface, since penetration of UV light in to the solid matter was limited. For the photodecomposition by sunlight, it must be in contact with the pesticides. Factors which influence the photodecomposition of pesticides were duration of exposure, intensity, wavelength of

light and the presence of water and air. Decomposition of pesticides by light has considerable importance (Sadegh-Zadeh *et al*., 2017). Photodegradation reduced under the shade condition (Chai *et al.,* 2009). Therefore, the persistence of chlorpyrifos increased under the dark condition. The dissipation half life of chlorpyrifos in air due to volatilization was 72 hours (Lyman *et al.,* 1990) and due to photolysis was 6.34 hours (Howard *et al.,* 1991).

2.6.1 Degradation of pesticides by sunlight

Barcelo *et al.* (1993) observed that degradation products like 3,5,6 trichloro-2-pyridinol, fenamiphos sulfoxide and vamidothion sulfoxide were formed during the photodegradation of chlorpyrifos, fenamiphos and vamidothion respectively in water containing 2 to 4 per cent methanol and the photodegradation followed the order of fenamiphos > chlorpyrifos > vamidothion.

Lu *et al.* (2020) developed a promising technology to remove the organic pollutants from the environment by acid-activated Fe (VI)/simulated sunlight system. Rapid dissipation of dimethoate was attained by the use of acid-activated Fe (VI)/simulated sunlight system within 20 minutes.

Sivagami *et al.* (2014) reported that photodegradation of organophosphorus insecticides such as monocrotophos (MCP), endosulfan (ES) and chlorpyrifos would be done by using floating polymeric TiO_2 coated beads under solar radiation. Higher degradation of these pesticides obtained with solar radiation which indicates its potential for practical application.

Diazinon would be degraded by sunlight irradiation which was examined by using nanosized photocatalyst TiO_2. During the photodegradation, heteroatoms S, P and N were released as sulphate, phosphate and ammonium and nitrate ions respectively (Molla *et al.,* 2020).

Photodegradation study of organophosphorus insecticides such as ethyl-parathion, methyl-parathion, fenitrothion, fenthion in water and soil were carried out by Sakellarides *et al*. (2003). The half-lives of the organophosphorus insecticides vary from 0.4 to 35.4 days in natural waters and in soil it varies from 3.4 to 21.3 days.

Fenthion sulfone, O, O, O -triethyl phosphorothioate, O, O, O -triethyl phosphorothioate, fenthion sulfoxide, aminoparathion and paraoxon were the main intermediate products formed during the degradation.

2.6.2 Degradation of pesticides by UV light

Chlorpyrifos undergo three different photochemical reactions such as hydrolysis, dechlorination, and oxidation under the exposure of UV light. Chlorpyrifos undergo oxidation and dehalogenation and formed the intermediate products. The intermediate products further broken-down leads to the formation of chloropyridinols and O, O-diethyl phosphorothioic acid which were unstable and undergo hydrolysis without accumulation in soil (Walia *et al.*, 1988).

Zhang *et al.* (2011) revealed that chlorpyrifos ethyl would be dissipated by ultrasonic irradiation but the toxicity was raised.

According to John and Shaike (2015), degradation by UV light was less effective process would be accelerated by the addition of hydrogen peroxide or ozone.

Djelal *et al.* (2016) confirmed that the amount of chlorpyrifos ethyl in aqueous solution lowered more rapidly with UV/H_2O_2 treatment due to the increased production of hydroxyl radicals. Chlorpyrifos removal was decreased after 90 minutes of irradiation time.

The chlorpyrifos degradation by UV/H_2O_2 process occurs much more quickly than by using only UV or H_2O_2 (Djelal *et al.*, 2016) Therefore, in the whole UV/H_2O_2 process UV contribution cannot be disregarded.

Doong and Chang (1998) confirmed that photocatalytic degradation of organophosphorous pesticides (OPPs) such as methamidophos, malathion, diazion and phorate would be done by using UV radiation in combination with hydrogen peroxide. Degradation efficiency of organophosphorus compounds would be enhanced by the addition of Fe compounds. Complete degradation of pesticides can be obtained with the addition of iron compounds and illuminated with UV light. Degradation of organophosphorus compounds was more efficient in the UV/Fe/H_2O_2 system than in the UV/H_2O_2 system.

2.7 EFFECT OF CHLORPYRIFOS RESIDUES ON MICROBIAL POPULATION

Microbial community was important parameter which determines the soil health (Brussaard, 2007). Martinez-Toledo *et al.* (1992) reported that insecticides have deleterious effect on soil microorganisms. The number of microbes or population decreased by the action of pesticides which might be also affects the soil fertility and productivity (Wainwright, 1978). Jana *et al.* (1988) revealed that degradation products of insecticides were assimilated by the microorganisms. The effect of pesticide on microorganism was depends not only the chemical itself but also pesticide concentration, soil type and microbial composition.

Chlorpyrifos has inhibitory effect on bacterial population (Pandey and Singh, 2004). Sardar and Kole (2005) reported that chlorpyrifos has inhibitory effect on dinitrogen fixing bacteria thereby it affects the nitrogen fixation and N, P, and K mineralization. Major metabolite of chlorpyrifos degradation, TCP (3,5,6-trichloro-2-pyridinol) has inhibitory effect on available N, P, K and ammonification (Racke *et al.*, 1990).

Menon *et al.* (2005) reported the overall inhibition of chlorpyrifos on enzymatic activities such as alkaline phosphatase, dehydrogenase and urease activities.

Xiaoqiang *et al.* (2008) confirmed that chlorpyrifos has inhibitory effect on bacteria, fungus and actinomycetes population after the chlorpyrifos application. Bacterial population decreased by 44.1 per cent one day after the application of chlorpyrifos compared to the control. Bacterial population inhibited up to seventh day thereafter increases and return to initial level on 14^{th} day after the application of chlorpyrifos. The fungal population reduced by 61.1 per cent on one day after the application of chlorpyrifos and not recovered the fungal population to initial level until 14^{th} day. Actinomycetes population decreased up to 7^{th} day after the application there after the population is recovered to similar level that of control.

There are contradictory evidences on changes in microbial population due to chlorpyrifos application. Pozo *et al*. (1995) observed that chlorpyrifos has stimulatory effect on bacterial and fungal population. Non inhibitory effect of chlorpyrifos reported by several researchers on total viable count of any kind of bacteria (Sarnaik *et al.* 2006)

or fungal populations, nitrifying and denitrifying bacteria (Pozo *et al.*, 1995). Recommended level of chlorpyrifos application was not detrimental to soil microbial community but higher doses would cause inhibitory effect on microbial activity (Dutta *et al.*, 2010).

Materials and methods

3. MATERIAL AND METHODS

An investigation entitled “Approaches to assess chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)” was carried out at College of Agriculture (COA), Padannakkad during September 2019 to July 2021. The objective of the study was evaluation and impact assessment of chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11) by using physical, chemical and biological methods. The whole study was conducted in two steps.

I. Incubation experiment No. 1
II. Incubation experiment No. 2

3.1. DETAILS OF SOIL SAMPLE USED FOR THE STUDY

The soil type used for the experiment was laterite soil which was collected from nearby areas of Pilicode [(12^{o} 12′ 8.1″) N latitude and (75^{o} 09′ 51.8″) E longitude] and brought to the College of Agriculture, Padannakkad and used for conducting both the experiments, incubation experiment No.1 and incubation experiment No. 2. Portions of soil samples were air dried, sieved using a 2 mm sieve to separate coarser fragments and stored in air tight container for studying initial soil properties. Physical properties such as particle density, bulk density, textural analysis, moisture content, chemical parameters such as pH, EC, organic carbon, available nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B), chlorides, phosphates and exchangeable ions, biological properties such as enzymatic activity (dehydrogenase, phosphatase and urease) and microbial biomass carbon were determined as per standard procedure.

Table1: Analytical procedure of physical, chemical and biological parameters

Sl. No.	Parameter	Method	Reference
1	Bulk density (g cm^{-3})	Undisturbed core sampler	Blake (1965)
2	Particle density (g cm^{-3})	Pycnometer method	Vadyunina and Korchagina (1986)
3	Moisture content (%)	Gravimetric method	Hesse (1971)
4	Textural analysis	International pipette method	Robinson (1922)
5	pH	pH meter (1:2.5 soil water ratio)	Jackson (1973)
6	EC (dS m^{-1})	Conductivity meter (1:2.5 soil water ratio)	Jackson (1973)
7	Organic carbon (%)	Walkley and Black method	Walkley and Black (1934)
8	Available N (kg ha^{-1})	Alkaline permanganate method	Subbaiah and Asija (1956)
9	Available P (kg ha^{-1})	Extraction using Bray No.1 solution and estimation using spectrophotometer	Bray and Kurtz (1945)
10	Available K (kg ha^{-1})	Neutral normal ammonium acetate extraction and estimation using flame photometry	Jackson (1973)
11	Available Ca (mg kg^{-1})	Versanate titration method	Hesse (1971)
12	Available Mg (mg kg^{-1})	Versanate titration method	Hesse (1971)
13	Available S (mg kg^{-1})	$CaCl_2$ extraction and estimation using spectrophotometer	Massoumi and Cornfield (1963)
14	Available Fe (mg kg^{-1})	0.1 N HCl extraction and estimation using atomic absorption Spectroscopy	Sims and Johnson (1991)
15	Available Mn (mg kg^{-1})	0.1 N HCl extraction and estimation using atomic absorption Spectroscopy	Sims and Johnson (1991)

16	Available Zn (mg kg^{-1})	0.1 N HCl extraction and estimation using atomic absorption Spectroscopy	Emmel *et al.* (1977)
17	Available Cu (mg kg^{-1})	0.1 N HCl extraction and estimation using atomic absorption Spectroscopy	Emmel *et al.* (1977)
18	Available B (mg kg^{-1})	Hot water extraction and estimation using spectrophotometer	Bingham (1982)
19	Chloride (mg kg^{-1})	Mohr method	Richards (1954)
20	Phosphate (mg kg^{-1})	Extraction using Bray No.1 solution and estimation using spectrophotometer	Bray and Kurtz (1945)
21	Microbial biomass carbon (µg g^{-1})	Fumigation extraction method	Jenkinson and Poulson (1976)

3.1.1 Exchangeable ions

Exchangeable cations such as K, Na, Ca and Mg were extracted by neutral normal ammonium acetate in which K and Na determined by flame photometry (Jackson, 1973) whereas Ca and Mg determined by versanate titration method (Hesse, 1971). Exchangeable anions such as carbonates, bicarbonates, chlorides, sulphates, phosphates and nitrates were determined separately. Carbonate and bicarbonates were determined in soil saturation extract by titration with 0.1N H_2SO_4 (Richards, 1954). Chloride content in soil was determined by Mohr's method (Richards, 1954). Sulphate and phosphate ions were determined by photoelectric colorimetry**.** Ammoniacal and nitrate nitrogen were determined separately by Macro-Kjeldahl distillation and titrimetry (Keeney and Bremner, 1966).

3.1.2. Enzymatic activity

3.1.2.1 Dehydrogenase activity in soil (Casida et al., 1964)

The dehydrogenase activity in soil was estimated as per the procedure given by Casida *et al.* (1964). Five grams of soil sample was treated with one ml of 3 per cent solution of triphenyl tetrazolium chloride and 2.5 ml of distilled water and incubated at 30^0 C. After 24 hours incubation, added 10 ml methanol to each tube. Shaken the contents for one minute. Filtered the suspension and made up to 50 ml with methanol. Measured the absorbance of orange colour at 485 nm in UV spectrophotometer with methanol as blank. Calculated the amount of Triphenyl formazan and expressed dehydrogenase activity as µg TPF $g^{-1}24$ h^{-1}.

2.1.2.2. Phosphatase activity in soil (Tabatabai and Bremner, 1969)

The phosphatase activity was determined by the procedure as given by Tabatabai and Bremner, (1969). One gram of soil sample was treated with 0.2 ml of toluene, 4 ml of modified universal buffer and 1 ml of p-nitrophenol phosphatase solution and swirled for a few seconds. After one hour incubation, added 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. Filtered the suspension and measured the absorbance of yellow coloured filtrate by UV spectrophotometer at 420 nm. Calculated the p-nitrophenol content of filtrate and express phosphatase activity as µg PNP g^{-1} soil h^{-1}.

2.1.2.3. Urease activity in soil (Tabatabai and Bremner, 1972)

Urease activity in soil was estimated as per the procedure given by Tabatabai and Bremner, (1972). Five grams of soil sample was treated with 0.2 ml of toluene and 9 ml of Tris (Hydroxymethyl) amino methane buffer. Added 1 ml of 0.2 M urea solution and placed in an incubator at 37°C. After 2 hours added 35 ml of KCl-Ag_2SO_4 solution. Swirled the flasks for few seconds and made the contents to 50 ml by addition of KCl-Ag_2SO_4 solution and mixed the contents. Determined the ammoniacal-nitrogen in soil suspension by steam distillation method. For control, followed the same procedure as described for assay of urease activity except that added 1 ml of 0.2 M urea solution after the addition of 35 ml KCl-Ag_2SO_4 solution. Expressed urease activity as µg NH^{4+}-N g^{-1}soil h^{-1}.

3.2. INCUBATION EXPERIMENT NO.1

An incubation experiment No.1 was carried out to know the pattern and time required for degradation of chlorpyrifos in soil. Soils described in 3.1 were used for the study. Pot culture study was conducted in five pots filled with 10 kg soil and drenched with chlorpyrifos at the concentration of 2.5 ml L^{-1} (20 EC). (Drench load was 1L/10 kg soil and size of the pot was 2204 cubic inches [36 L]). Soil was analyzed at weekly intervals to determine the duration of chlorpyrifos degradation. Statistical analysis of incubation experiment No. 1 was done by using single sample t test by comparing with initial soil analysis.

3.2.1 Soil analysis of incubation experiment No.1

Analysis of parameters such as pH, chlorides (mg kg^{-1}), phosphates (mg kg^{-1}), microbial biomass (µg g^{-1}) and content of chlorpyrifos (mg kg^{-1}) were analyzed at weekly intervals. EC (dS m^{-1}), soil moisture (%) and exchangeable ions (meq 100 g^{-1} of soil) were carried out at 28 days interval.

3.2.2. Estimation of chlorpyrifos content in soil

In a centrifuge tube, a 10 g soil sample was taken and added 20 ml acetonitrile (CH_3CN) and shaken well for one minute. Then added 4 g anhydrous $MgSO_4$ and 1g NaCl to the tube and centrifuged at 3300 rpm for 4 minutes. Then 10 ml supernatant was transferred to a 15 ml centrifuge tube containing 0.25 g primary secondary amine (PSA) and 1.5 g anhydrous $MgSO_4$ and was mixed thoroughly by using a vortex mixer for 30 seconds. Then centrifuged at 4400 rpm for 10 minutes. Transferred 4 ml supernatant to the test tube and evaporated in nitrogen evaporator at 50^0 C and the volume was made up to 1 ml with hexane for gas chromatography analysis.

3.3 INCUBATION EXPERIMENT NO.2

Incubation experiment No.2 was carried out to assess the best method of degradation of chlorpyrifos in soil. Incubation experiment No.2 was conducted after the completion of incubation experiment No.1. From the results of incubation experiment No.1, the pattern and duration of chlorpyrifos degradation was understood and the duration of incubation experiment No.2 was decided for 60 days. Experiment

was laid out in completely randomized design with12 treatments replicated thrice. In each treatment about 10 kg soil was maintained in each pot. Soils were drenched with chlorpyrifos in all the treatments at the concentration of 2.5 ml L^{-1} (20 EC). (Drench load was 1L/10 kg soil). Physical, chemical and biological agents were applied and evaluated to study their effect on degradation of chlorpyrifos.

3.3.1. Design and layout

Experimental design – Completely Randomized Design

Replication - 3

Treatments - 12

Total no.of pots - 36

Pot size - 36 L

3.3.3. Treatments

T_1 - Control

T_2 - Chemical agent 1(Hydrogen peroxide-5%)

T_3 - Chemical agent 2 (Fenton reagent -0.5%)

T_4 - Chemical agent 1+ Chemical agent 2

T_5 - Biological agent 1 (*Pseudomonas fluorescens*)

T_6 - Biological agent 2 (*Trichoderma viride*)

T_7 - Biological agent 1+ Biological agent 2

T_8 - Physical treatment 1 (Sunlight – 6hrs)

T_9 - Physical treatment 2 (Ultra violet – 4hrs)

T_{10} - Physical treatment 1 + Physical treatment 2

T_{11}- Soil under saturated condition at 5 cm level of submergence

T_{12} - Soil under saturated condition at 5 cm level of submergence with azolla.

3.3.4 Details of physical, chemical and biological agents

Preparation of Fenton reagent

Fenton reagent was prepared in the laboratory of department of soil science and agricultural chemistry. Fenton reagent was prepared by using ferrous sulphate

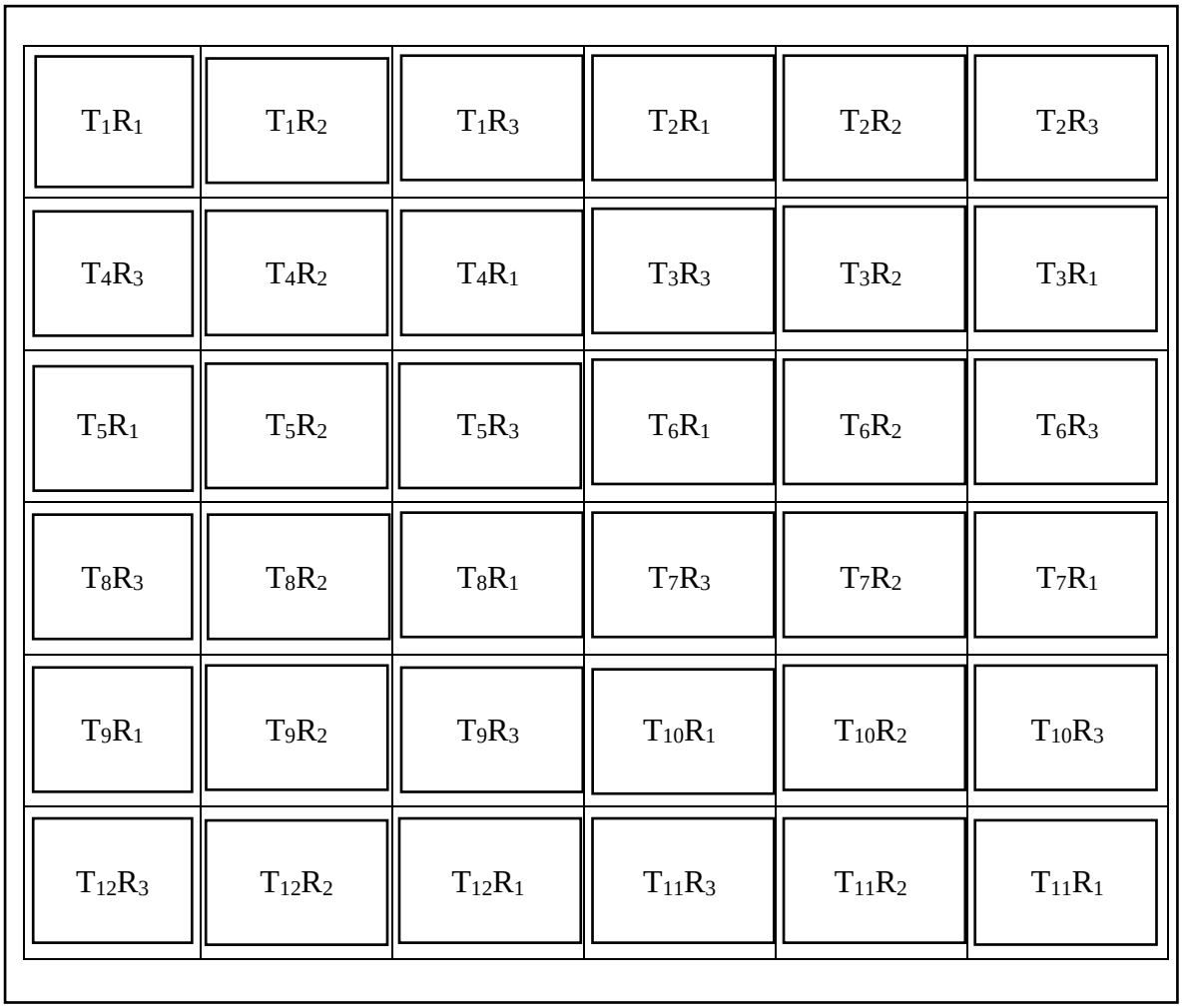


Plate: 1 - Layout of the incubation experiment No. 2

heptahydrate and hydrogen peroxide. Three different concentrations of Fenton reagent were prepared.

First concentration - $FeSO_4$. $7H_2O$ -1% and H_2O_2 – 5%

Second concentration - $FeSO_4$. $7H_2O$ -3% and H_2O_2 – 8%

Third concentration - $FeSO_4$. $7H_2O$ -5% and H_2O_2 – 10%

Fenton reagent was prepared by adding 10 ml ferrous sulphate heptahydrate to the 20 ml hydrogen peroxide in the ratio 1:2. Using this solution 0.5 per cent Fenton reagent was prepared. The different concentrations of Fenton reagents were titrated against strong reducing agent and found out that among these, 3% $FeSO_4$. $7H_2O$ and 8% H_2O_2 had best oxidation potential and it was used for the experiment. Chemical treatments were given on the next day after drenching of chlorpyrifos at the rate of 500 ml reagent per pot.

Biological agents used for the study were *Pseudomonas fluorescens* and *Trichoderma viride* which was obtained from department of Agricultural Microbiology, Kerala Agricultural University, Vellanikkara. Purification of mother culture was done in the laboratory of department of Agricultural microbiology, College of Agriculture, Padanakkad. Mass multiplication of *Pseudomonas fluorescens* and *Trichoderma viride* were done by using kings B medium and potato dextrose medium respectively. Microorganisms were mixed into the soil at the rate of 100 ml per pot on the next day after the application of chlorpyrifos.

Colony forming unit of microorganisms were

Pseudomonas fluorescens : 9.5×10^{-6}

Trichoderma viride : 5×10^{-4}

Physical agents included sunlight and UV light. Ultraviolet lamp 11 watt was used for the study. Pots were maintained in sunlight for three different durations such as 4hr, 6hr and 8hr. Preliminary study showed that 6hr was found to be performed well under sunlight. With respect to UV light, among the three durations (4hr, 6hr and 8hr),

4 hr duration was observed to be ideal for the study. Hence, pots were maintained under the sunlight for 6 hr and under the UV light for 4 hr.

Treatments T_{11} and T_{12} included soil under submerged condition with and without azolla. Azolla culture used for the study was *Azolla Pinnata*, was obtained from department of Soil Science and Agricultural Chemistry, College of Agriculture, Padannakkad. Each pot was filled with 10 kg soil and water level was maintained in the pot to 5 cm level from the surface of top soil. Three pots were maintained with azolla culture under saturated condition at 5 cm level of submergence and other three pots contained no azolla. A PVC pipe having perforations in the base portion and diameter of 5 cm was inserted into these pots to collect the leachate from the soil depth. The leachate in the pot entered in to the pipe through the holes in its bottom. Leachate were collected from the pots at biweekly intervals by using a syringe and water analysis was done.

3.3.5. Soil analysis of incubation experiment No.2

Analysis of parameters such as pH, chlorides (mg kg^{-1}), phosphates (mg kg^{-1}), enzymatic activity, microbial biomass carbon (µg g^{-1}) and content of chlorpyrifos (mg kg^{-1}) were analyzed at 14 days interval. EC (dS m^{-1}), soil moisture (%), exchangeable ions (meq 100 g^{-1} of soil) and organic matter (%) were estimated at 28 days interval. Duration of the experiment was decided from the results of incubation experiment No.1.

3.3.6 Water Analysis of incubation experiment No.2

Analysis of parameters such as pH, EC (dS m^{-1}), chlorides (mg L^{-1}), phosphates (mg L^{-1}) and content of chlorpyrifos (µg L^{-1}) were analysed in the water sample of treatment T_{11} and T_{12} at 14 days interval.

3.3.6.1 Estimation of chlorpyrifos in water sample

Leachate was collected from the treatments T_{11} and T_{12}. Taken 750 ml water sample in a 1L separating funnel. Added 150 g sodium chloride and 75 ml dichloromethane DCM (CH_2Cl_2), shaken for 5 minutes at 250 rpm. Organic layer was collected from the separating funnel by repeated partitioning with 40 ml dichloromethane followed by addition of 50 ml n-Hexane. The collected organic layer

was mixed together then added 5-10 g anhydrous sodium sulphate and concentrated to 5 ml. Added twice times 20 ml n- Hexane, concentrated near to dryness and made up to volume 1 ml by using n-Hexane. The sample was then subjected to GC-MS analysis.

3.4 STATISTICAL ANALYSIS

The data obtained from incubation experiment No.1 and experiment No.2 were statistically analyzed by using analysis of variance techniques (ANOVA) as applied to Completely Randomized Design described by Panse and Sukhatme, 1985.

Plate 2 A: Preparation of chlorpyrifos @ 2.5 ml/L Plate 2 B: Drenching of chlorpyrifos to soil

Plate 2: Incubation experiment No. 1

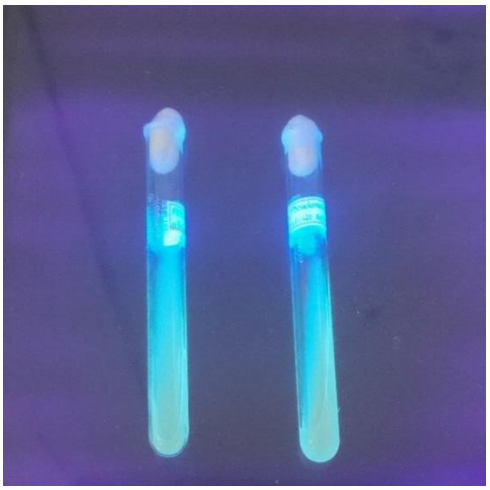
Plate 3 A: Fenton Reagent -(3% $FeSO_4$. $7H_2O$ and 8% H_2O_2)

Plate 3 B: Fenton Reagent – 0.5%

Plate 3: Chemical treatments for incubation experiment No. 2

Plate 4 A: *Pseudomonas fluorescens* slant

Plate 4 B: *Trichoderma viride* slant



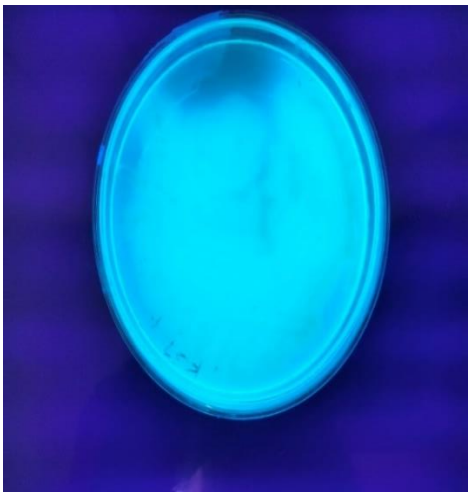


Plate 4 C: View of *Pseudomonas fluorescens* under UV light

Plate 4 D: *Trichoderma viride*

Plate 4 E: Broth of *Pseudomonas fluorescens* Plate 4 F: Broth of *Trichoderma viride*

Plate 4: Biological treatments for incubation experiment No. 2

Plate 5 A: Application of H_2O_2 to soil Plate 5 B: Application of Fenton reagent to soil

Plate 5 C: Application of *Pseudomonas fluorescens* Plate 5 D: Application of *Trichoderma viride*

Plate 5: Application of treatments to the soil

Plate 6 A: Pots under sunlight treatment

Plate 6 B: Pots under UV light treatment

Plate 6 C: Application of azolla

Plate 6 D: Soil under saturated condition at 5 cm level of submergence with azolla

Plate 6 E: Field view of incubation experiment No.2

Plate 6: Treatments in the incubation experiment No.2

Results

4. RESULTS

The investigation was conducted to evaluate the different approaches of chlorpyrifos degradation in northern laterite soils of Kasaragod. The data obtained from the experiment were subjected to statistical analysis and the results were given in this chapter.

4.1 SOIL CHARACTERISTICS OF EXPERIMENTAL SOIL

Laterite soils used for the experiment was collected from Pilicode. Initial properties of the experimental soil before the application of chlorpyrifos are furnished below. The initial properties of the experimental soil are presented in the Table 2. Soil belongs to the textural class of sandy loam and the moisture content of initial soil was found to be 7.78%. Bulk density and particle density of the soil were 1.41 g cm^{-3} and 2.47 g cm^{-3} respectively. It was acidic in reaction with a pH of 4.4 and low in electrical conductivity (0.189 dS m^{-1}). Organic carbon (0.360 %), nitrogen (250.88 kg ha^{-1}) and potassium (116.03 kg ha^{-1}) of the initial soil were rated as low whereas phosphorus (64.6 kg ha^{-1}) was rated as high. Calcium (118.60 mg kg^{-1}), magnesium (10.25 mg kg^{-1}) and sulphur (4.01 mg kg^{-1}) were rated as low in soil. Micronutrients such as Fe (46.4 mg kg^{-1}), Mn (17.64 mg kg^{-1}), Zn (1.83 mg kg^{-1}) and Cu (2.56 mg kg^{-1}) were rated as sufficient in the soil sample whereas boron was found to be deficient (0.283 mg kg^{-1}). Chloride and phosphate ions in soil were found to be 14.20 mg kg^{-1} and 29.36 mg kg^{-1} respectively. Exchangeable ions in soil were found to be 1.76 meq $100g^{-1}$ of soil. Chlorpyrifos residue in the soil was found to be nil. Biological properties of soil such as dehydrogenase activity, phosphatase activity and urease activity were rated as 11.78 µg TPF g^{-1} soil day^{-1}, 13.62 µg PNP g^{-1} soil h^{-1} and 33.25 µg NH_4^+-N g^{-1} soil h^{-1} respectively. Microbial biomass carbon of initial soil was found to be 91.73 µg g^{-1}.

Table 2: Properties of the experimental soil

Sl. No.	Parameter	Value
Physical parameters		
1	Moisture content (%)	7.78
2	Bulk density (g cm^{-3})	1.41
3	Particle density (g cm^{-3})	2.47
4	Textural composition	
	Sand (%)	77.8
	Silt (%)	19.7
	Clay (%)	3.32
	Textural class	Sandy loam
Chemical parameters		
5	pH	5.14
6	EC (dS m^{-1})	0.189
7	Organic carbon (%)	0.360
8	Available N (kg ha^{-1})	250.88
9	Available P (kg ha^{-1})	64.6
10	Available K (kg ha^{-1})	116.03
11	Available Ca (mg kg^{-1})	118.60
12	Available Mg (mg kg^{-1})	10.25
13	Available S (mg kg^{-1})	4.01
14	Available Fe (mg kg^{-1})	46.4
15	Available Mn (mg kg^{-1})	17.64
16	Available Zn (mg kg^{-1})	1.83
17	Available Cu (mg kg^{-1})	2.56
18	Available B (mg kg^{-1})	0.283
19	Chlorides (mg kg^{-1})	14.20
20	Phosphates (mg kg^{-1})	29.36
21	Exchangeable ions (meq $100g^{-1}$ of soil)	1.76
22	Chlorpyrifos residue (mg kg^{-1})	Nil
Biological parameters		
22	Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})	11.78
23	Phosphatase activity (µg PNP g^{-1} soil h^{-1})	13.62
24	Urease activity (µg NH_4^+-N g^{-1} soil h^{-1})	33.25
25	Microbial biomass carbon (µg g^{-1})	91.73

4.2 INCUBATION EXPERIMENT NO.1

An incubation experiment was carried out to know the pattern and time required for degradation of chlorpyrifos in laterite soil at College of Agriculture, Padannakkad during September 2019 to July 2021. The pots were filled with 10 kg soil and drenched with chlorpyrifos at the concentration of 2.5 ml L^{-1} (20 EC). Soil was analysed at weekly intervals to determine the duration and pattern of chlorpyrifos degradation. Results obtained from the study was statistically analysed by using single sample t test, compared with initial soil analysis and the important findings are presented below:

4.2.1 Soil properties

4.2.1.1 Soil *pH*

The soil pH was recorded periodically after the application of chlorpyrifos and presented in the Table 3. Data revealed that soil pH decreased significantly from 3rd week up to 8th week with respect to initial soil pH. The trend of decrease in the soil pH was observed throughout the degradation period.

4.2.1.2 Chlorides (mg kg^{-1})

Chloride ion in soil was recorded at weekly intervals after the application of chlorpyrifos and given in the Table 4. Effect of chlorpyrifos application on chloride ion in soil was found to be significant at 8th week in association with pretreatment chloride ion. Chloride ions in soil showed increasing trend throughout the incubation period.

Table 3: Effect of chlorpyrifos application on soil pH at weekly intervals in incubation experiment No.1

Variables	Soil pH							
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T_1R_1	5.10	5.07	5.07	5.05	5.07	5.05	5.03	5.01
T_1R_2	5.08	5.08	5.09	5.02	5.06	5.05	5.03	5.06
T_1R_3	5.12	5.09	5.06	5.05	5.03	5.04	5.05	5.01
T_1R_4	5.13	5.12	5.09	5.06	5.01	5.00	4.99	4.98
T_1R_5	5.12	5.09	5.07	5.03	5.05	5.03	5.01	5.05
Mean	5.11	5.09	5.07	5.04	5.04	5.03	5.02	5.02
Initial value	5.14							
t (0.01)	1.18	3.58	7.33*	10.61*	7.05*	9.27*	9.61*	6.69*
P value	0.326	0.023	0.001	0.0004	0.002	0.0007	0.0006	0.002

(*Significant)

Table 4: Effect of chlorpyrifos application on soil chloride content (mg kg^{-1}) at weekly intervals in incubation experiment No.1

Variables	Chloride content (mg kg^{-1})							
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T_1R_1	14.2	14.2	14.2	14.2	21.3	28.4	28.4	28.4
T_1R_2	14.2	21.3	21.3	21.3	21.3	21.3	21.3	21.3
T_1R_3	14.2	14.2	14.2	14.2	14.2	14.2	14.2	21.3
T_1R_4	14.2	14.2	14.2	14.2	21.3	21.3	28.4	21.3
T_1R_5	14.2	14.2	21.3	21.3	14.2	14.2	14.2	21.3
Mean	14.2	15.62	17.04	17.04	18.46	19.88	21.3	22.72
Initial value	14.2							
t (0.01)	-	1	1.63	1.63	2.44	2.13	2.23	6*
P value	-	0.373	0.177	0.177	0.070	0.099	0.089	0.003

(*Significant)

4.2.1.3 Phosphates (mg kg^{-1})

The data relevant to phosphate ions in soil as influenced by the application of chlorpyrifos at weekly intervals shown in the Table 5. Effect of chlorpyrifos application on phosphate ion in soil was found to be significant at 1^{st}, 2^{nd}, 3^{rd} ,4^{th} and 6^{th} weeks in relation with native soil phosphate ion. The decline in the phosphate ion was noticed after the chlorpyrifos application but it was restored to initial level by the eighth week.

4.2.1.4 Microbial biomass carbon in soil (µg g^{-1})

The effect of chlorpyrifos application on microbial biomass carbon in soil at weekly intervals are given in the Table 6. Application of chlorpyrifos showed significant effect on microbial biomass carbon in soil at biweekly intervals except at 1^{st} week. Microbial biomass carbon decreased up to 6^{th} week but it was increased at the end of incubation period.

4.2.1.5 Electrical conductivity (dS m^{-1})

The effect of chlorpyrifos application on electrical conductivity of the soil at monthly intervals are shown in the Table 7. There was no considerable effect on electrical conductivity of the soil after the application of chlorpyrifos at 4^{th} week and 8^{th} weeks with respect to initial soil property. There was increase in the electrical conductivity of soil at 8^{th} week.

4.2.1.6 Soil moisture (%)

The data relevant to soil moisture content was recorded periodically at 4^{th} week and 8^{th} weeks after chlorpyrifos application and presented in the Table 7. The data given in the table showed that there was significant difference on soil moisture content at 4^{th} and 8^{th} weeks in association with initial soil moisture content.

Table 5: Effect of chlorpyrifos application on soil phosphates content (mg kg^{-1}) at weekly intervals in incubation experiment No.1

Variables	Phosphates content (mg kg^{-1})							
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T_1R_1	25.58	25.45	25.43	25.85	25.85	27.72	25.58	25.85
T_1R_2	25.45	24.11	23.79	26.35	25.44	27.29	29.99	30.90
T_1R_3	25.87	26.93	22.68	27.20	27.20	27.38	30.42	31.75
T_1R_4	26.49	26.29	26.02	27.44	29.71	26.29	28.08	27.44
T_1R_5	26.46	26.27	26.06	26.81	25.67	26.27	28.73	31.35
Mean	25.97	25.81	24.8	26.73	26.78	26.99	28.56	29.46
Initial value	29.36							
t (0.01)	15.54*	7.30*	6.79*	9.17*	3.24	7.90*	0.928	0.087
P value	0.0001	0.0018	0.0024	0.0007	0.031	0.0013	0.405	0.935

(*Significant)

Table 6: Effect of chlorpyrifos application on soil microbial biomass carbon (µg g^{-1}) at weekly intervals in incubation experiment No.1

Variables	Soil microbial biomass carbon (µg g^{-1})							
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T_1R_1	92.43	90.32	88.38	82.39	83.98	83.98	84.98	84.39
T_1R_2	91.90	87.45	84.21	84.56	84.01	84.01	83.01	85.84
T_1R_3	90.38	89.45	86.47	86.27	81.26	81.26	83.26	83.21
T_1R_4	88.65	86.83	82.39	82.17	81.31	81.31	80.51	84.34
T_1R_5	87.38	85.39	82.58	81.47	77.21	77.21	83.21	83.23
Mean	90.14	87.88	84.80	83.37	83.15	82.99	84.20	84.38
Initial value	91.73							
t (0.01)	2.42	5.12*	6.62*	10.21*	8.76*	12.21*	15.58*	19.70*
P value	0.072	0.006	0.002	0.0005	0.0009	0.0003	0.0008	0.0002

(*Significant)

Table 7: Effect of chlorpyrifos application on EC (dS/m) and moisture content of soil at monthly intervals in incubation experiment No.1

Variables	EC (dS m^{-1})		Soil moisture (%)	
	4^{th} week	8^{th} week	4^{th} week	8^{th} week
T_1R_1	0.182	0.193	21.67	19.30
T_1R_2	0.158	0.184	18.34	20.70
T_1R_3	0.168	0.193	19.54	22.67
T_1R_4	0.195	0.178	20.56	18.93
T_1R_5	0.179	0.205	22.02	16.83
Mean	0.176	0.190	20.13	20.81
Initial value	0.189		7.78	
t (0.01)	2.001	0.349	22.02*	16.83*
P value	0.116	0.744	0	0.0001

(*Significant)

Table 8: Effect of chlorpyrifos application on exchangeable K and Na ions in soil at monthly intervals in incubation experiment No.1

	K (meq $100g^{-1}$ soil)		Na (meq $100g^{-1}$ soil)	
	4^{th} week	8^{th} week	4^{th} week	8^{th} week
T_1R_1	0.125	0.109	0.102	0.091
T_1R_2	0.117	0.101	0.105	0.099
T_1R_3	0.120	0.105	0.100	0.101
T_1R_4	0.107	0.101	0.098	0.096
T_1R_5	0.100	0.088	0.098	0.101
Mean	0.114	0.101	0.101	0.098
Initial value	0.132		0.103	
t (0.01)	3.867*	8.692*	1.976	2.972
P value	0.0180	0.0009	0.1193	0.0410

(*Significant)

4.2.1.7 Exchangeable ions (meq 100g soil^{-1})

The effect of chlorpyrifos application on exchangeable potassium and sodium ions at monthly intervals recorded in the Table 8. Values specified in the table showed that effect of chlorpyrifos application on potassium ion of the soil was found to be significant at 8th week whereas non significant at 4th week with respect to initial potassium ions. Effect of chlorpyrifos application on sodium ion was found to be non significant at 4th and 8th weeks in relation to initial soil property. The trend of decrease in the potassium and sodium ions of soil were observed at 8th week.

Data relevant to exchangeable calcium, magnesium and ammonium ions in soil at monthly intervals are shown in the Table 9. The data given in the table showed significant difference in calcium ions of soil at 4th and 8th weeks in relation to initial calcium ions. There was no significant difference after the chlorpyrifos application on magnesium ions of soil at 4th and 8th weeks with respect to native magnesium ions. Effect of chlorpyrifos application on ammonium ion of soil was found to be non significant at 4th and 8th weeks in association with initial soil property. Magnesium and ammonium ions decreased whereas calcium ions increased at the 8th week.

Data recorded on phosphate and chloride ions in soil at monthly intervals are presented in the Table 10. Data revealed that effect of chlorpyrifos application on phosphate ion in soil was found to be significant at 4th week whereas non significant at 8th week in relation to initial soil phosphate ions. Effect of chlorpyrifos application on chloride ion of the soil was found to be significant at 8th week while non significant at 4th week with respect to initial chloride ions. Phosphate and chloride ions increased in the incubation period.

The data on sulphate, nitrate and bicarbonate ions in soil at monthly intervals are recorded and shown in the Table 11. Data given in the table showed that effect of chlorpyrifos application on sulphate ion was found to be significant at 8th week while non significant at 4th week. There was no considerable difference noticed after chlorpyrifos application on nitrate and bicarbonate ions in soil at 4th and 8th week intervals in association with initial soil property. However, slight increase in the sulphate and nitrate ions observed at 8th week.

The data obtained on exchangeable cations, anions and total ions at monthly intervals are shown in the Table 12. Effect of chlorpyrifos application on exchangeable cations in soil were found to be non significant at 4^{th} week and 8^{th} weeks with respect to initial cations. Exchangeable anions in soil were found to be significant at 8^{th} week while non significant at 4^{th} week in association with initial soil values. Total exchangeable ions in soil were found to be non significant at 4^{th} and 8^{th} week intervals in relation to initial soil values. There was increase in the exchangeable ions observed at the 8^{th} week.

4.2.1.8 Chlorpyrifos content (mg kg^{-1})

The data relevant to chlorpyrifos content in soil is given in the Table 13. Chlorpyrifos residue in the pre-treatment soil sample was found to be zero. The data revealed that chlorpyrifos residue was reduced throughout the incubation period. Chlorpyrifos was degraded 34.76% within the 60 days of incubation period.

4.2.1.9 Percentage variation in the soil parameters

The data relevant to percentage variation in the soil parameters after the application of chlorpyrifos is presented in the Table 14. Results from the incubation experiment No.1 pointed out that soil pH decreased after the application of chlorpyrifos to the soil. Chloride ion was increased about 60 per cent at the 8^{th} week of incubation. There was a decline in the phosphate ion noticed during the initial weeks later increased to pre-treatment value. There was slight increase in the EC (0.846%) and the exchangeable ions (6.25%) of soil. Chlorpyrifos was degraded 34.76% within 60 days of incubation period. Based on the results and findings from the incubation experiment No.1, duration of incubation experiment No.2 was decided to be 60 days.

Table 9: Effect of chlorpyrifos application on exchangeable Ca, Mg and ammonium ions in soil at monthly intervals in incubation experiment No.1

	Ca (meq $100g^{-1}$ soil)		Mg (meq $100g^{-1}$ soil)		NH_4^+ (meq $100g^{-1}$ soil)	
	4^{th} week	8^{th} week	4^{th} week	8^{th} week	4^{th} week	8^{th} week
T_1R_1	0.534	0.522	0.089	0.090	0.311	0.311
T_1R_2	0.528	0.544	0.091	0.079	0.311	0.311
T_1R_3	0.533	0.491	0.088	0.085	0.466	0.311
T_1R_4	0.514	0.554	0.084	0.075	0.466	0.466
T_1R_5	0.501	0.541	0.081	0.081	0.466	0.466
Mean	0.522	0.530	0.087	0.082	0.404	0.373
Initial value	0.593		0.085		0.311	
t (0.01)	11.25*	5.572*	0.922	1.104	1.633	2.45
P value	0.00035	0.00508	0.4087	0.3315	0.1777	0.0704

(*Significant)

Table 10: Effect of chlorpyrifos application on phosphate and chloride ions in soil at monthly intervals in incubation experiment No.1

	Phosphates (meq $100g^{-1}$ soil)		Chlorides (meq $100g^{-1}$ soil)	
	4^{th} week	8^{th} week	4^{th} week	8^{th} week
T_1R_1	0.246	0.246	0.2	0.4
T_1R_2	0.251	0.294	0.3	0.3
T_1R_3	0.259	0.302	0.2	0.3
T_1R_4	0.261	0.261	0.2	0.3
T_1R_5	0.255	0.298	0.3	0.3
Mean	0.254	0.280	0.24	0.32
Initial value	0.279		0.2	
t (0.01)	9.16*	0.088	1.633	6*
P value	0.00078	0.933	0.1778	0.0038

(*Significant)

Table 11: Effect of chlorpyrifos application on sulphate, nitrate and bicarbonate ions in soil at monthly intervals in incubation experiment No.1

	Sulphates (meq $100g^{-1}$ soil)		**Nitrates (meq $100g^{-1}$ soil)**		**Bicarbonates (meq $100g^{-1}$ soil)**	
	4^{th} week	8^{th} week	4^{th} week	8^{th} week	4^{th} week	8^{th} week
T_1R_1	0.017	0.027	0.043	0.043	0.001	0.001
T_1R_2	0.018	0.031	0.043	0.087	0.001	0.001
T_1R_3	0.013	0.033	0.043	0.043	0.001	0.001
T_1R_4	0.018	0.030	0.087	0.087	0.001	0.001
T_1R_5	0.018	0.040	0.043	0.043	0.001	0.003
Mean	0.017	0.032	0.052	0.061	0.001	0.001
Initial value	0.016		0.043		0.001	
t (0.01)	0.676	7.516*	1.638	1.006	-	1.001
P value	0.5359	0.00167	0.1768	0.3714	-	0.37345

(*Significant)

Table 12: Effect of chlorpyrifos application on exchangeable cations, anions and total ions in soil at monthly intervals in incubation experiment No.1

	Cations (meq $100g^{-1}$ soil)		**Anions (meq $100g^{-1}$ soil)**		**Total ions (meq $100g^{-1}$ soil)**	
	4^{th} week	8^{th} week	4^{th} week	8^{th} week	4^{th} week	8^{th} week
T_1R_1	1.16	1.12	0.509	0.719	1.67	1.84
T_1R_2	1.15	1.13	0.614	0.715	1.76	1.85
T_1R_3	1.30	1.09	0.517	0.681	1.82	1.77
T_1R_4	1.27	1.29	0.569	0.680	1.84	1.97
T_1R_5	1.24	1.27	0.619	0.685	1.86	1.96
Mean	1.22	1.18	0.566	0.696	1.79	1.88
Initial value	1.22		0.541		1.76	
t (0.01)	1.053	0.221	1.05	18.13*	1.02	3.21
P value	0.3517	0.8358	0.352	0.0001	0.366	0.032

(*Significant)

Table 13: Degradation of chlorpyrifos in soil (mg kg^{-1}) at weekly intervals in incubation experiment No.1

	Chlorpyrifos content in soil (mg kg^{-1})							
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
T_1R_1	73.45	69.25	66.75	62.34	55.76	52.12	50.97	47.87
T_1R_2	78.84	75.56	69.54	66.43	60.45	54.98	55.67	51.06
T_1R_3	79.07	76.78	71.68	67.12	61.32	56.56	53.47	51.23
T_1R_4	75.47	72.97	68.75	64.89	58.43	53.98	50.68	50.34
T_1R_5	77.89	73.78	67.98	64.12	58.64	52.98	51.07	50.45
Mean	76.94	73.66	68.94	64.98	58.92	54.12	52.37	50.19
% Decrease from the first week		4.2%	10.39 %	15.54 %	23.42 %	29.65 %	31.93 %	34.76 %

Table 14: Percentage variation in the soil parameters after the application of chlorpyrifos to the soil

Parameters	Percentage variation (%)
pH	2.29% (Decrease)
Chlorides (mg kg^{-1})	60% (Increase)
Phosphates (mg kg^{-1})	0.30% (Increase)
Electrical conductivity (dsm^{-1})	0.846% (Increase)
Exchangeable ions (meq $100g^{-1}$ soil)	6.25% (Increase)
Microbial biomass (µg g^{-1})	8.2 % (Decrease)
Chlorpyrifos content (mg kg^{-1})	34.76% (Decrease)

4.3 INCUBATION EXPERIMENT NO.2

Incubation experiment No.2 was carried out to assess the best method of degradation of chlorpyrifos in soil. Physical, chemical and biological agents were applied and evaluated for their effect on degradation of chlorpyrifos. Soil was analysed at biweekly intervals. Based on the results and findings from the incubation experiment No.1, duration of incubation experiment No.2 was decided to be 60 days. Results obtained from the study was statistically analysed and important findings are presented below.

4.3.1 Chemical properties

4.3.1.1 pH

The data summarized in Table 15 express the significant effect of various treatments on soil pH. At 2nd week, pot treated with combination of physical agents (T_{10}) recorded higher soil pH (5.12) which was on par with T_7 (5.12), T_5 (5.11), T_6 (5.11), T_8 (5.10) and T_9 (5.09) whereas lowest pH showed at T_4 (4.91). Treatments T_7 where combination of *Pseudomonas fluorescens* + *Trichoderma viride* was applied recorded the highest soil pH (5.11), though on par with treatments such as T_{10} (5.11), T_5 (5.10), T_8 (5.09), T_6 (5.09) and T_9 (5.08) while the treatment T_4 (4.87) showed the lowest pH at 4th week. At 6th week, T_{10} (5.08) was found to be on par with T_5 (5.07), T_7 (5.07), T_8 (5.06) and T_6 (5.06) and significantly superior to all other treatments while the minimum pH recorded at T_4 (4.89). At 8th week, T_{10} (Sunlight +UV light) recorded the highest pH of 5.08 which was on par with T_6 (5.07), T_5 (5.06) and T_7 (5.06) whereas the least pH was recorded at T_4 (4.93).

4.3.1.2 Chloride (mg kg^{-1})

Chloride content of the soil analysed at biweekly intervals are presented in Table 16.

The data revealed that effect of treatment application on chloride content of soil was statistically non significant at the biweekly intervals. However, increase in the chloride ion observed during the degradation of chlorpyrifos. At 8th week, T_7 (*Pseudomonas fluorescens + Trichoderma viride)* showed the highest value (30.76) whereas treatment T_1 (Control) had the lowest value (21.30) of chloride ions in soil.

Table 15: Effect of treatments on pH of soil at biweekly intervals in the incubation experiment No.2

Treatment	pH			
	2nd week	4th week	6th week	8th week
T_1	5.08^{b}	5.04^{b}	5.02^{b}	5.00^{d}
T_2	4.98^{c}	4.94^{c}	4.95^{c}	4.97^{e}
T_3	4.94^{d}	4.89^{d}	4.92^{cd}	4.96^{e}
T_4	4.91^{d}	4.87^{d}	4.89^{d}	4.93^{f}
T_5	5.11^{ab}	5.10^{a}	5.07^{a}	5.07^{a}
T_6	5.11^{ab}	5.09^{a}	5.06^{ab}	5.08^{a}
T_7	5.12^{a}	5.11^{a}	5.07^{a}	5.06^{ab}
T_8	5.10^{ab}	5.09^{a}	5.06^{ab}	5.02^{cd}
T_9	5.09^{ab}	5.08^{a}	5.05^{ab}	5.03^{bcd}
T_{10}	5.12^{a}	5.11^{a}	5.08^{a}	5.09^{abc}
SE(m)	0.011	0.009	0.015	0.012
CD (0.01)	0.045	0.036	0.059	0.048

Table 16: Effect of treatments on chloride content of the soil at biweekly intervals in the incubation experiment No.2

Treatment	Chloride content (mg kg^{-1})			
	2nd week	4th week	6th week	8th week
T_1	14.20	16.56	18.93	21.30
T_2	16.56	18.93	21.30	23.66
T_3	21.30	23.66	23.66	26.03
T_4	18.93	21.30	23.66	26.03
T_5	21.30	23.66	26.03	28.40
T_6	18.93	21.30	28.40	28.40
T_7	18.93	26.03	28.40	30.76
T_8	16.56	18.93	18.93	23.66
T_9	14.20	16.56	18.93	23.66
T_{10}	18.93	21.30	23.66	26.03
SE(m)	1.83	2.36	1.98	1.98
CD (0.01)	NS	NS	NS	NS

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.1.3 Phosphates (mg kg^{-1})

Phosphate ions of the soil are recorded at biweekly intervals and shown in Table 17.

With respect to phosphate ions in soil treatments showed significant superior results at the biweekly intervals. At 2nd week, pot received with *Trichoderma viride* (T_6) recorded the highest phosphate ions (29.25) in soil which was on par with T_3 (28.97), T_7 (28.76) and T_4 (28.42) while the minimum value recorded at T_9 (26.07). Treatment received the Fenton reagent (T_3) recorded the highest phosphate ions (27.59) in soil, though on par with T_7 (27.30), T_6 (27.20), T_4 (27.24) and T_5 (26.42) while the lowest amount recorded at T_1 (25.36) at 4th week. At 6th week, T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) recorded the highest phosphate content of 30.00 mg kg^{-1} which was on par with T_6 (29.69), T_3 (29.43), T_5 (29.43) and T_4 (28.90) while the minimum value recorded at T_1 (27.60). At 8th week, T_5 (31.80) recorded the highest phosphate ions in soil which was on par with T_6 (31.76) and T_7 (31.30) and significantly superior to all other treatments whereas lowest value of 28.86 mg kg^{-1} recorded at treatment T_1 (control).

4.3.1.4 Chlorpyrifos content in soil (mg kg^{-1})

Data recorded on chlorpyrifos residue in soil at biweekly intervals are recorded in the Table 18.

Imposition of different treatments had a significant effect on the chlorpyrifos residue of soil over control at the biweekly intervals. Among the treatments T_3 (61.55) showed highest removal of chlorpyrifos residue which was significantly superior to all other treatments whereas T_1 (73.63) recorded the highest chlorpyrifos residue in soil at 2nd week. At 4th week, highest dissipation of chlorpyrifos recorded at T_6 (41.66) which was on par with T_7 (43.31) while lowest degradation registered at T_1 (65.56). Treatment T_7 (26.78) was on par with T_5 (28.66) and significantly superior to all other treatments whereas the T_1 (53.45) had the lowest degradation at 6th week. At 8th week, T_7 (18.41) showed the highest removal of chlorpyrifos followed by T_5 (22.13) which was significantly superior to all other treatments while the lowest residue recorded at T_1 (48.72).

Table 17: Effect of treatments on phosphates content of the soil at biweekly intervals in the incubation experiment No.2

Treatment	Phosphates content (mg kg^{-1})			
	2nd week	4th week	6th week	8th week
T_1	26.53^{ef}	25.36^{d}	27.60^{e}	28.86^{d}
T_2	27.59^{cde}	26.11^{cd}	27.92^{de}	30.36^{bc}
T_3	28.97^{ab}	27.59^{a}	29.43^{abc}	30.67^{b}
T_4	28.42^{abc}	27.24^{ab}	28.90^{abcd}	29.60^{cd}
T_5	27.93^{bcd}	26.42^{bc}	29.43^{abc}	31.80^{a}
T_6	29.25^{a}	27.20^{ab}	29.69^{ab}	31.76^{a}
T_7	28.76^{ab}	27.30^{a}	30.00^{a}	31.30^{ab}
T_8	27.06^{def}	25.81^{cd}	28.75^{bcde}	29.44^{cd}
T_9	26.07^{f}	25.68^{cd}	27.73^{de}	29.10^{d}
T_{10}	27.49^{cde}	25.93^{cd}	28.50^{cde}	29.66^{cd}
SE(m)	0.394	0.296	0.396	0.336
CD (0.01)	1.58	1.19	1.12	1.35

Table 18: Effect of treatments on degradation of chlorpyrifos in soil at biweekly intervals in the incubation experiment No.2

Treatment	Chlorpyrifos content in soil (mg kg^{-1})				Percentage decrease from control
	2nd week	4th week	6th week	8th week	
T_1	73.63^{a}	65.56^{a}	53.45^{a}	48.72^{a}	33.83 %
T_2	66.74^{ef}	53.70^{d}	46.53^{bc}	38.43^{d}	47.80 %
T_3	61.55^{g}	52.49^{de}	38.55^{d}	30.70^{f}	58.30 %
T_4	63.74^{f}	50.46^{e}	40.53^{d}	32.56^{e}	55.77 %
T_5	66.59^{cd}	44.96^{f}	28.66^{f}	22.13^{h}	69.94 %
T_6	66.27^{de}	41.66^{g}	31.74^{e}	24.77^{g}	66.35 %
T_7	65.39^{ef}	43.31^{fg}	26.78^{f}	18.41^{i}	74.99 %
T_8	70.41^{b}	59.55^{c}	48.51^{b}	41.59^{c}	43.51 %
T_9	71.69^{b}	62.49^{b}	51.67^{a}	43.36^{b}	41.11 %
T_{10}	68.19^{c}	57.62^{c}	45.36^{c}	38.53^{d}	47.67 %
SE(m)	0.578	0.712	0.689	0.553	
CD (0.01)	2.32	2.86	2.77	2.22	

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.1.5 Electrical conductivity of soil (dSm^{-1})

Electrical conductivity of soil analysed at monthly intervals and presented in the Table 19.

At 4th week and 8th weeks, treatments had no significant influence on the electrical conductivity of soil. After the chlorpyrifos application electrical conductivity of the soil increases though there was no significant variation among treatments.

4.3.1.6 Organic matter content of soil (%)

Organic matter content of soil was recorded at monthly intervals and shown in the Table 19.

Treatments did not show any significant effect on the soil organic matter content at 4th week but it showed significant effect at 8th week. Treatments T_7 (0.638), T_6 (0.621), T_5 (0.603) and T_{10} (0.569) were found to be on par with highest value recorded at T_7 (0.638) followed by T_6 (0.621), T_5 (0.603) and T_{10} (0.569) while the treatments T_3 (0.483) and T_4 (0.483) registered the lowest organic matter content at 8th week.

Table 19: Effect of treatments on EC and organic matter content in soil at monthly intervals in the incubation experiment No.2

Treatment	EC (dSm^{-1})		Organic matter (%)	
	1st month	2nd month	1st month	2nd month
T_1	0.181	0.185	0.552	0.534^{cde}
T_2	0.183	0.186	0.534	0.517^{de}
T_3	0.194	0.195	0.517	0.483^{e}
T_4	0.191	0.191	0.500	0.483^{e}
T_5	0.191	0.196	0.586	0.603^{abc}
T_6	0.188	0.194	0.603	0.621^{ab}
T_7	0.189	0.193	0.603	0.638^{a}
T_8	0.187	0.188	0.569	0.552^{bcde}
T_9	0.182	0.183	0.552	0.534^{cde}
T_{10}	0.194	0.194	0.569	0.569^{abcd}
SE(m)	0.004	0.003	0.033	0.027
CD (0.01)	NS	NS	NS	0.110

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.1.7 Potassium (meq 100g^{-1} soil)

The data relevant to exchangeable potassium ions at 4th and 8th weeks are given in the Table 20. Imposition of different treatments had a significant effect on potassium ions of the soil at 4th and 8th weeks. At 4th week, treatments T_2 (0.146), T_3 (0.141), T_4 (0.142), T_{10} (0.140), T_5 (0.139) T_6 (0.138) and T_7 (0.136) were found to be on par while the lowest value recorded at T_8 (0.115). Treatments T_2 (0.138), T_5 (0.136), T_3 (0.136), T_6 (0.135), T_7 (0.134) and T_4 (0.131) were found to be on par while treatment T_{10} (0.103) showed the lowest value of potassium ions in soil at the 8th week.

4.3.1.8 Sodium (meq 100g^{-1} soil)

Sodium ions in the soil at 4th and 8th weeks were recorded and shown in the Table 20. Data revealed that there was no significant difference between various treatments at 4th and 8th weeks. However, there was decrease in the sodium ions observed during the degradation.

4.3.1.9 Calcium (meq 100 g^{-1} soil)

The data summarized in Table 21 express the significant effect of various treatments on exchangeable calcium ions. At 4th week, T_5 (0.647) recorded the highest calcium ions which was on par with T_7 (0.627), T_6 (0.618), T_8 (0.613), T_9 (0.599) and T_2 (0.591) whereas the lowest value recorded at T_1 (0.512). At 8th week, T_2 (0.635) recorded the maximum calcium ions which was on par with T_9 (0.626), T_8 (0.622) and T_5 (0.614) while the lowest calcium observed at T_1 (0.527).

4.3.1.10 Magnesium (meq 100g^{-1} soil)

The data recorded on magnesium ions in soil at 4th and 8th weeks are presented in the Table 21. Treatments significantly influenced the exchangeable magnesium ions in soil at 4th and 8th weeks. At the 4th week, treatments T_4 (0.090), T_1 (0.089), T_7 (0.084), T_8 (0.084), T_3 (0.084), T_5 (0.083) and T_9 (0.083) were found to be on par and significantly superior to all other treatments while the treatment T_{10} (0.076) recorded the lowest value. Treatments T_7 (0.091) recorded the highest magnesium ions in soil which was on par with T_5 (0.091), T_6 (0.090), T_4 (0.090), T_2 (0.090) and T_8 (0.089) while the T_1 (0.082) with lowest value of magnesium ions in soil at 8th week.

Table 20: Effect of treatments on exchangeable potassium and sodium ions in soil at monthly intervals in the incubation experiment No.2

Treatment	Potassium (meq 100g^{-1} soil)		Sodium (meq 100g^{-1} soil)	
	1st month	2nd month	1st month	2nd month
T_1	0.119^{b}	0.111^{b}	0.105	0.101
T_2	0.146^{a}	0.138^{a}	0.118	0.103
T_3	0.141^{a}	0.136^{a}	0.119	0.109
T_4	0.142^{a}	0.131^{a}	0.115	0.106
T_5	0.139^{a}	0.136^{a}	0.112	0.103
T_6	0.138^{a}	0.135^{a}	0.109	0.106
T_7	0.136^{a}	0.134^{a}	0.113	0.108
T_8	0.115^{b}	0.114^{b}	0.107	0.111
T_9	0.118^{b}	0.105^{b}	0.106	0.100
T_{10}	0.140^{a}	0.103^{b}	0.109	0.099
SE(m)	0.004	0.004	0.008	0.007
CD (0.01)	0.017	0.017	NS	NS

Table 21: Effect of treatments on exchangeable calcium, magnesium and ammonium ions in soil at monthly intervals in the incubation experiment No.2

Treatment	Calcium (meq 100g^{-1} soil)		Magnesium (meq 100g^{-1} soil)		Ammonium (meq 100g^{-1} soil)	
	1st month	2nd month	1st month	2nd month	1st month	2nd month
T_1	0.512^{d}	0.527^{c}	0.089^{ab}	0.082^{d}	0.415	0.363
T_2	0.591^{abc}	0.635^{a}	0.082^{bcd}	0.090^{ab}	0.311	0.259
T_3	0.533^{cd}	0.554^{bc}	0.084^{abc}	0.086^{bc}	0.363	0.311
T_4	0.583^{abc}	0.606^{ab}	0.090^{a}	0.090^{ab}	0.363	0.311
T_5	0.647^{a}	0.614^{a}	0.083^{abc}	0.091^{a}	0.311	0.259
T_6	0.618^{ab}	0.603^{ab}	0.079^{cd}	0.090^{ab}	0.311	0.259
T_7	0.627^{ab}	0.604^{ab}	0.084^{abc}	0.091^{a}	0.311	0.259
T_8	0.613^{ab}	0.622^{a}	0.084^{abc}	0.089^{abc}	0.311	0.311
T_9	0.599^{abc}	0.626^{a}	0.083^{abcd}	0.084^{cd}	0.311	0.311
T_{10}	0.573^{bcd}	0.587^{ab}	0.076^{d}	0.085^{cd}	0.363	0.311
SE(m)	0.024	0.020	0.003	0.002	0.052	0.073
CD (0.01)	0.093	0.079	0.011	0.005	NS	NS

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.1.11 Ammonium (meq 100g^{-1} soil)

The data on ammonium ions in soil at 4th and 8th weeks are shown in the Table 21. Treatments did not show any significant effect on ammonium ions in soil at 4th and 8th weeks. However, ammonium ion in soil was decreased at the 8th week during the degradation of chlorpyrifos.

4.3.1.12 Phosphates (meq 100g^{-1} soil)

Effect of treatments on phosphate ions in soil at 4th and 8th weeks are depicted in the Table 22. Treatments imposed had significant effect on the phosphate ions in soil at monthly intervals. At 4th week, significantly higher phosphate ion was observed for the treatment T_3 (0.263) which was on par with T_7 (0.260), T_4 (0.259) and T_6 (0.259) whereas T_1 (0.242) had the lowest value. At 8th week, T_5 (0.303) registered the highest phosphate ions in soil which was on par with T_6 (0.303) and T_7 (0.298) while the T_1 recorded the lowest value.

4.3.1.13 Chlorides (meq 100g^{-1} soil)

The data pertaining to the effect of treatments on chloride ions in soil at 4th and 8th weeks are presented in the Table 22. Treatments showed the significant superior results on the chloride ions in soil over control at 8th week whereas in 4th week treatments found to be non significant. Treatment T_7 (0. 467) recorded the highest chloride ions in soil which was on par with T_5 (0.400) and T_6 (0.400) whereas the lowest value recorded at T_1 (0.300) at 8th week.

4.3.1.14 Sulphates (meq 100g^{-1} soil)

Sulphate ions of the soil at 4th and 8th weeks were statistically analysed and depicted in the Table 23. Effect of chlorpyrifos on sulphate ion was found to be significant at 4th week whereas non significant at 8th week. At 4th week, T_5 (0.024) recorded highest sulphate ions in soil which was on par with T_7 (0.023), T_4 (0.023), T_2 (0.022) and T_6 (0.022) while the lowest value recorded at T_1 (0.020), T_9 (0.020) and T_{10} (0.020).

4.3.1.15 Nitrates (meq 100g^{-1} soil)

Effect of treatments on nitrates ions of soil at 4th and 8th weeks were analysed and depicted in the Table 23. Data revealed that there was no significant difference between various treatments. However, nitrate ion in soil was found to be increased at the 8th week.

4.3.1.16 Bicarbonates (meq 100g^{-1} soil)

The data recorded on bicarbonates at 4th and 8th weeks are given in the Table 23. Treatments were found to be statistically significant at 8th week whereas non significant at 4th week. At 8th week, treatments T_{10} (0.003), T_2 (0.003), T_3 (0.003), T_5 (0.003) and T_8 (0.003) were found to be on par while the treatments T_6 (0.002), T_7 (0.002), T_4 (0.002), T_1 (0.002) and T_9 (0.002) reported the minimum bicarbonates value of 0.002.

4.3.1.17 Carbonates (meq 100g^{-1} soil)

Carbonate content of the soil was analysed at biweekly intervals and found out that carbonates are absent in the soil.

4.3.1.18 Exchangeable cations (meq 100g^{-1} soil)

Effect of treatments on exchangeable cations of soil at 4th and 8th weeks were statistically analysed and given in the Table 24. Treatments did not show any significant effect on exchangeable cations of the soil at 4th and 8th week intervals. However, exchangeable cation in soil was found to be decreased.

Table 22: Effect of treatments on phosphate and chloride ions in soil at monthly intervals in the incubation experiment No.2

Treatment	Phosphates (meq 100g^{-1} soil)		Chlorides (meq 100g^{-1} soil)	
	1st month	2nd month	1st month	2nd month
T_1	0.242^{d}	0.275^{d}	0.233	0.300^{c}
T_2	0.249^{cd}	0.289^{bc}	0.267	0.333^{bc}
T_3	0.263^{a}	0.292^{b}	0.333	0.367^{bc}
T_4	0.259^{ab}	0.282^{cd}	0.300	0.367^{bc}
T_5	0.252^{bc}	0.303^{a}	0.333	0.400^{ab}
T_6	0.259^{ab}	0.303^{a}	0.300	0.400^{ab}
T_7	0.260^{a}	0.298^{ab}	0.367	0.467^{a}
T_8	0.246^{cd}	0.280^{cd}	0.267	0.333^{bc}
T_9	0.245^{cd}	0.277^{d}	0.233	0.333^{bc}
T_{10}	0.247^{cd}	0.282^{cd}	0.300	0.367^{bc}
SE(m)	0.003	0.003	0.033	0.028
CD (0.01)	0.011	0.014	NS	0.110

Table 23: Effect of treatments on sulphate, nitrate and bicarbonate ions in soil at monthly intervals in the incubation experiment No.2

Treatment	Sulphates (meq 100g^{-1} soil)		Nitrates (meq 100g^{-1} soil)		Bicarbonates (meq 100g^{-1} soil)	
	1st month	2nd month	1st month	2nd month	1st month	2nd month
T_1	0.020^{cd}	0.035	0.044	0.058	0.002	0.002^{c}
T_2	0.022^{abc}	0.038	0.073	0.073	0.002	0.003^{a}
T_3	0.020^{cd}	0.033	0.044	0.073	0.002	0.003^{a}
T_4	0.023^{ab}	0.038	0.058	0.058	0.002	0.002^{c}
T_5	0.024^{a}	0.039	0.073	0.073	0.002	0.003^{ab}
T_6	0.022^{abc}	0.042	0.058	0.088	0.002	0.002^{bc}
T_7	0.023^{ab}	0.046	0.058	0.058	0.002	0.002^{bc}
T_8	0.021^{bcd}	0.037	0.088	0.088	0.002	0.003^{ab}
T_9	0.020^{cd}	0.034	0.044	0.058	0.002	0.002^{c}
T_{10}	0.020^{d}	0.039	0.058	0.073	0.002	0.003^{a}
SE(m)	0.001	0.003	0.011	0.013	0.000	0.000
CD (0.01)	0.002	NS	NS	NS	NS	0.001

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.1.19 Exchangeable anions (meq 100g^{-1} soil)

The data recorded on exchangeable anions of soil at 4th and 8th weeks were statistically analysed and depicted in the Table 24. Data revealed that treatments showed significantly higher exchangeable anions in the soil over the control at the 8th week while non significant at 4th week. Treatments T_7 (0.871), T_6 (0.835) and T_5 (0.818) were found to be on par with highest value recorded at T_7 (0.871) followed by T_6 (0.835) and T_5 (0.818) while T_1 (0.670) recorded the lowest value of exchangeable anions at 8th week.

4.3.1.20 Exchangeable ions (meq 100g^{-1} soil)

Effect of treatments on exchangeable ions of soil at 4th and 8th weeks were analysed and presented in the Table 24. Treatments found to be statistically non significant in exchangeable ions of soil at 4th and 8th week intervals. However, increase in the exchangeable ions observed at the 8th week.

4.3.2 Physical properties

4.3.2.1 Soil moisture (%)

The data summarized in Table 25 express the significant effect of various treatments on soil moisture at 4th and 8th weeks. The statistically analysed data revealed that treatments were found to be having significant effect on soil moisture content at 4th and 8th weeks. At 4th week, T_6 (21.43) recorded the highest moisture content which was on par with T_1 (20.90), T_4 (20.76), T_5 (20.56), T_9 (20.53), T_7 (20.40), T_2 (20.23) and T_3 (19.33) while the treatment received with sunlight registered the lowest moisture content. At 8th week, treatments T_9 (22.63) (21.79), T_2 (21.53), T_5 (21.33), T_3 (21.09), T_6 (20.68) and T_1 (20.63) were found to be on par while the soil moisture significantly lower (13.88) in pot treated with sunlight (T_8).

Table 24: Effect of treatments on exchangeable cations, anions and total ions in soil at monthly intervals in the incubation experiment No.2

Treatment	Exchangeable cations (meq $100g^{-1}$ soil)		Exchangeable anions (meq $100g^{-1}$ soil)		Exchangeable ions (meq $100g^{-1}$ soil)	
	1^{st} month	2^{nd} month	1^{st} month	2^{nd} month	1^{st} month	2^{nd} month
T_1	1.23	1.18	0.540	0.670^{c}	1.78	1.85
T_2	1.24	1.22	0.612	0.736^{bc}	1.86	1.96
T_3	1.24	1.19	0.662	0.768^{bc}	1.90	1.96
T_4	1.29	1.24	0.642	0.746^{bc}	1.93	1.99
T_5	1.29	1.20	0.684	0.818^{ab}	1.97	2.02
T_6	1.25	1.19	0.641	0.835^{ab}	1.89	2.02
T_7	1.27	1.19	0.710	0.871^{a}	1.98	2.06
T_8	1.23	1.24	0.623	0.741^{bc}	1.85	1.98
T_9	1.21	1.22	0.544	0.704^{c}	1.76	1.93
T_{10}	1.26	1.18	0.627	0.764^{bc}	1.88	1.95
SE(m)	0.071	0.070	0.037	0.035	0.07	0.081
CD (0.01)	NS	NS	NS	0.139	NS	NS

Table 25: Effect of treatments on soil moisture content in soil at monthly intervals in the incubation experiment No.2

Treatment	Soil moisture (%)	
	1^{st} month	2^{nd} month
T_1	20.90^{a}	20.63^{ab}
T_2	20.23^{ab}	21.53^{ab}
T_3	19.33^{ab}	21.09^{ab}
T_4	20.76^{a}	19.96^{b}
T_5	20.56^{a}	21.33^{ab}
T_6	21.43^{a}	20.68^{ab}
T_7	20.40^{a}	21.79^{ab}
T_8	15.26^{c}	13.88^{c}
T_9	20.53^{a}	22.63^{a}
T_{10}	17.50^{bc}	15.68^{c}
SE(m)	0.948	0.860
CD (0.01)	3.81	3.44

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.3 Biological properties

4.3.3.1 Dehydrogenase activity in soil (µg TPFg^{-1} soil day^{-1})

The data relevant to dehydrogenase activity in soil is shown in the Table. 26.

Imposition of treatments had significant effect on dehydrogenase activity of the soil at biweekly intervals. The combination of *Pseudomonas fluorescens* + *Trichoderma viride* (T_7) treated pot recorded highest dehydrogenase activity (11.57) in the soil which was statistically on par with T_5 (11.19) and T_6 (10.92) while the lowest value recorded at T_8 (9.44) at 2nd week. At 4th week, treatment T_7 (10.55) was on par with T_5 (10.40), T_{10} (10.23), T_6 (10.19) and T_2 (10.15) and significantly superior to all other treatments while the minimum value recorded at T_4 (9.04). Treatment T_5 (11.39) where *Pseudomonas fluorescens* applied, recorded the highest dehydrogenase activity which was on par with T_6 (11.24) and T_7 (10.84) while the lowest value recorded at T_1 (8.46) at 6th week. At 8th week, Treatment T_5 (11.74) recorded the highest dehydrogenase activity in the soil which was on par with T_6 (11.43) and T_7 (11.37) and significantly superior to all other treatments while the lowest activity was found in the treatment T_1 (7.83).

4.3.3.2 Phosphatase activity in soil (µg PNP g^{-1} soil h^{-1})

The data on effect of treatment application on the phosphatase activity in soil is depicted in the Table 27.

Results on phosphatase activity of the soil revealed that treatments were found to be significant at the biweekly intervals. The maximum phosphatase activity at 2nd week was found in the treatment T_6 (13.70) which was on par with T_5 (13.29) and lowest activity recorded at T_4 (10.65). At 4th week, treatment T_5 (15.59) recorded the highest activity which was on par with T_7 (14.53) and T_6 (14.28) and significantly superior to all other treatments while the minimum activity showed at T_9 (10.05). At 6th week, pot treated with combination of *Pseudomonas fluorescens* + *Trichoderma viride* (T_7) showed the highest activity (17.53) which was on par with T_5 (16.63) whereas the minimum activity was recorded at T_1 (11.11). At 8th week, maximum phosphatase activity was recorded at T_6 (17.06) followed by T_7 (16.53) and significantly superior to all other treatments while the T_1 (12.04) registered the lowest activity.

Table 26: Effect of treatments on soil dehydrogenase activity (µg TPFg^{-1} soil day^{-1}) in soil at biweekly intervals in the incubation experiment No.2

Treatment	Dehydrogenase enzymatic activity (µg TPF g^{-1} soil day^{-1})			
	2nd week	4th week	6th week	8th week
T_1	9.51^{de}	9.16^{cd}	8.46^{e}	7.83^{c}
T_2	10.46^{bc}	10.15^{ab}	9.73^{bc}	10.21^{b}
T_3	10.25^{cd}	9.83^{bc}	9.27^{cd}	10.33^{b}
T_4	9.67^{de}	9.04^{d}	8.64^{e}	8.59^{c}
T_5	11.19^{ab}	10.40^{ab}	11.39^{a}	11.74^{a}
T_6	10.92^{abc}	10.19^{ab}	11.24^{a}	11.43^{a}
T_7	11.57^{a}	10.55^{a}	10.84^{a}	11.37^{a}
T_8	9.44^{e}	9.10^{d}	8.75^{de}	8.37^{c}
T_9	9.61^{de}	9.45^{cd}	9.03^{de}	8.25^{c}
T_{10}	10.70^{bc}	10.23^{ab}	10.09^{b}	10.26^{b}
SE(m)	0.263	0.232	0.199	0.289
CD (0.01)	1.05	0.933	0.799	1.16

Table 27: Effect of treatments on soil phosphatase activity (µg PNP g^{-1} soil h^{-1}) in soil at biweekly intervals in the incubation experiment No.2

Treatment	Phosphatase activity (µg PNP g^{-1} soil h^{-1})			
	2nd week	4th week	6th week	8th week
T_1	11.37^{ef}	10.91^{ef}	11.11^{f}	12.04^{g}
T_2	11.88^{de}	11.58^{de}	12.08^{ef}	12.16^{g}
T_3	12.47^{bcd}	13.10^{bcd}	13.55^{d}	14.48^{de}
T_4	10.65^{f}	10.17^{ef}	11.20^{f}	12.49^{fg}
T_5	13.29^{ab}	15.59^{a}	16.63^{ab}	15.79^{bc}
T_6	13.70^{a}	14.28^{ab}	15.24^{bc}	17.06^{a}
T_7	12.84^{b}	14.53^{ab}	17.53^{a}	16.53^{ab}
T_8	12.73^{bc}	13.62^{bc}	14.14^{cd}	14.70^{cd}
T_9	10.86^{f}	10.05^{f}	11.84^{ef}	12.28^{g}
T_{10}	11.96^{cde}	12.47^{cd}	13.00^{de}	13.54^{ef}
SE(m)	0.282	0.514	0.477	0.392
CD (0.01)	1.13	2.07	1.92	1.57

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.3.3 Urease activity in soil (µg NH_4^+-N g^{-1} soil h^{-1})

The data on urease activity in soil at biweekly intervals are presented in the Table 28.

Treatments showed non significant effect on urease activity of the soil at 2nd and 4th weeks. At 6th and 8th weeks, significant difference was noticed among the treatments. The maximum urease activity in soil at 2nd week was recorded in the treatment T_6 (35.58) and lowest activity noticed at T_9 (31.50) and T_1 (31.5). At 4th week, maximum activity recorded at T_5 (34.41) and the minimum activity observed at T_9 (30.33). At 6th week, treatment T_7 (35.58) recorded the highest value which was on par with T_5 (35.00) and T_6 (34.41) while T_3 (28.58) showed the minimum activity. At 8th week, treatments T_7 (36.16), T_5 (35.58) and T_6 (35.00) were found to be on par and significantly superior to all other treatments while the lowest activity reported at T_1 (28.58) and T_3 (28.58).

4.3.3.4 Microbial biomass carbon in soil (µg g^{-1})

The data recorded on microbial biomass carbon in soil at biweekly intervals are depicted in the Table 29.

The effect of treatment application on microbial biomass carbon in was found to be non significant at 2nd week whereas significant at 4th, 6th and 8th weeks. Treatments T_7 (94.40), registered the highest microbial biomass carbon which was on par with T_5 (92.56) and T_6 (91.33) and significantly superior to all other treatments while T_4 (77.46) showed the lowest value of microbial biomass carbon at 4th week. At 6th week, T_7 (96.26) recorded the maximum activity which was on par with T_5 (95.33) and T_6 (93.56) while the treatment T_4 (79.56) showed the minimum value. At 8th week, treatments T_7 (99.15), T_5 (97.33) and T_6 (95.46) were found to be on par and highest value recorded at T_7 (96.82) followed by T_5 (96.33) and T_6 (95.46) whereas the treatment T_4 (78.23) showed the minimum value of microbial biomass carbon.

Table 28: Effect of treatments on soil urease activity (µg NH_4^+-N g^{-1} soil h^{-1}) in soil at biweekly intervals in the incubation experiment No.2

Treatment	Urease activity (µg NH_4^+-N g^{-1} soil h^{-1})			
	2nd week	4th week	6th week	8th week
T_1	31.50	30.91	29.75^{cd}	28.58^{d}
T_2	33.25	31.50	30.91^{cd}	30.91^{bc}
T_3	32.08	31.50	28.58^{d}	28.58^{d}
T_4	32.66	32.08	30.91^{cd}	29.16^{d}
T_5	35.00	34.41	35.00^{a}	35.58^{a}
T_6	35.58	33.83	34.41^{ab}	35.00^{a}
T_7	34.41	33.25	35.58^{a}	36.16^{a}
T_8	33.83	32.66	30.33^{cd}	31.50^{b}
T_9	31.50	30.33	29.75^{cd}	29.75^{cd}
T_{10}	33.25	32.08	32.08^{bc}	31.50^{b}
SE(m)	0.825	0.804	0.804	0.452
CD (0.01)	NS	NS	3.21	1.81

Table 29: Effect of treatments on microbial biomass carbon in soil at biweekly intervals in the incubation experiment No.2

Treatment	Soil microbial biomass carbon (µg g^{-1})			
	2nd week	4th week	6th week	8th week
T_1	88.23	84.53^{bc}	83.10^{cd}	83.76^{c}
T_2	87.30	84.30^{bc}	82.96^{cde}	84.74^{bc}
T_3	87.20	83.20^{bc}	81.30^{de}	79.63^{d}
T_4	86.56	82.46^{c}	79.56^{e}	78.23^{d}
T_5	91.93	92.56^{a}	95.33^{a}	97.33^{a}
T_6	90.46	91.33^{a}	93.56^{a}	95.46^{a}
T_7	92.40	94.40^{a}	96.26^{a}	99.15^{a}
T_8	88.43	84.40^{bc}	85.63^{bc}	85.81^{b}
T_9	89.20	85.56^{bc}	81.53^{de}	83.36^{c}
T_{10}	89.53	86.40^{b}	87.30^{b}	85.60^{bc}
SE(m)	1.23	1.11	1.17	1.08
CD (0.01)	NS	4.49	4.72	4.35

T_1: Control; T_2: Hydrogen peroxide-5%; T_3: Fenton reagent -0.5%; T_4: Hydrogen peroxide-5% + Fenton reagent -0.5%; T_5: *Pseudomonas fluorescens;* T_6: *Trichoderma viride*; T_7: *Pseudomonas fluorescens* + *Trichoderma viride*; T_8: Sunlight – 6 hr; T_9: Ultra violet – 4 hr; T_{10}: Sunlight – 6 hr + Ultra violet – 4 hr.

4.3.4 Properties of water (leachate) sample

Pots were filled with 10 kg soil and water level maintained in the pot was 5 cm level from the surface of top soil. Treatment includes soil under saturated condition at 5 cm level of submergence (T_{11}) and soil under saturated condition at 5 cm level of submergence with azolla (T_{12}). Leachate from the pots collected at biweekly intervals through perforated pipes by using a syringe and water analysis was done and the results obtained are presented below:

4.3.4.1 pH and electrical conductivity (dS m^{-1})

The data on pH of water was recorded at biweekly intervals and shown in the Table 30. Treatments did not show any significant effect on water pH at biweekly intervals. However, pH of water increased to neutral range.

The data relevant to electrical conductivity of water was recorded at biweekly intervals and depicted in the Table 29. Effect of treatments on electrical conductivity of water was found to be statistically non significant at biweekly intervals. However, electrical conductivity of water increased under the saturated condition.

4.3.4.2 Chlorides (mg L^{-1}) and phosphates

The data pertaining to the effect of treatments on chloride and phosphate ions in water at biweekly intervals were recorded and given in the Table 31. Effect of treatments on chlorides and phosphate ions of water were found to be non significant in both the treatments at biweekly intervals. However, increase in the chloride and phosphate ions were observed during the degradation period.

4.3.4.3 Chlorpyrifos in water sample (µg L^{-1})

Data relevant to chlorpyrifos residue in water sample were recorded and presented in the Table 32.

Treatments were found to be having non significant effect on chlorpyrifos residue of water at 2^{nd} week and 4^{th} weeks while significant at 6^{th} and 8^{th} weeks. The data indicated that at 6^{th} week, highest chlorpyrifos degradation was found in the treatment T_{11} (4401.10) followed by T_{12} (4939.69). At 8^{th} week, highest removal of

chlorpyrifos recorded at T_{11} (2598.62) and the lowest removal of chlorpyrifos was observed at T_{12} (3318.07). Trend of decrease in the chlorpyrifos residue was observed throughout the degradation period.

Table 30: Effect of treatments on pH and EC of water (leachate) under submerged condition at biweekly intervals in the incubation experiment No.2

Treatment	pH				EC (dS m^{-1})			
	2nd week	4th week	6th week	8th week	2nd week	4th week	6th week	8th week
T_{11}	5.88	6.31	6.68	7.05	0.19	0.21	0.24	0.24
T_{12}	5.96	6.65	6.96	7.26	0.20	0.22	0.21	0.19
SE(m)	0.104	0.079	0.060	0.064	0.005	0.007	0.009	0.009
CD (0.01)	NS	NS	NS	NS	NS	NS	NS	NS

Table 31: Effect of treatments on chloride and phosphate ions in water (leachate) under submerged condition at biweekly intervals in the incubation experiment No.2

Treatment	Chlorides (mg L^{-1})				Phosphates (mg L^{-1})			
	2nd week	4th week	6th week	8th week	2nd week	4th week	6th week	8th week
T_{11}	23.66	28.40	30.76	33.13	34.70	41.26	49.10	56.96
T_{12}	23.66	26.03	30.76	30.76	35.16	40.56	46.86	52.90
SE(m)	2.36	3.34	2.36	2.36	0.694	1.66	0.657	0.971
CD (0.01)	NS	NS	NS	NS	NS	NS	NS	NS

Table 32: Effect of submerged condition on degradation of chlorpyrifos at biweekly intervals in the incubation experiment No.2

Treatment	Concentration of chlorpyrifos in water sample (µg L^{-1})			
	2nd week	4th week	6th week	8th week
T_{11}	11030.33	7257.75	4401.10[a]	2598.62[a]
T_{12}	11648.33	7855.34	4939.69[b]	3318.07[b]
SE(m)	553.58	130.44	59.38	105.78
CD (0.01)	NS	NS	386.65	688.75

T_{11}: Soil under saturated condition at 5 cm level of submergence; T_{12} - Soil under saturated condition at 5 cm level of submergence with azolla.

Plate 7: Decaying of azolla under submerged condition

Discussion

5. DISCUSSION

The result obtained from the study entitled "Approaches of chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)" are briefly discussed in this chapter. The investigation was conducted in two steps which included incubation experiment No.1 and incubation experiment No.2.

5.1 INCUBATION EXPERIMENT NO.1

The experiment was carried out to know the duration and pattern of chlorpyrifos degradation in laterite soils. For this physical, chemical and biological properties of chlorpyrifos treated soil were studied at periodic intervals.

5.1.1 Effect of chlorpyrifos application on soil properties

5.1.1.1. pH, chlorides and phosphates at weekly intervals

Results revealed that significant decrease in the soil pH (2.29%) was observed after the application of chlorpyrifos at weekly intervals except at 1st and 2nd weeks. This could be due to release of H^+ and chloride ions during the degradation leading the formation of acidic compounds. Martin (1966) reported that pesticides containing Cl, Br, N and S would undergo reaction to produce acidic compounds which further causes decrease in the soil pH.

Significant increase in the chloride ion was observed at 8th week of incubation period which showed release of chloride ions from the chlorpyrifos compound. Chloride ion in the soil was gradually increased throughout the incubation period after the application of chlorpyrifos. Chloride values observed in the 1st week of incubation was similar to the initial chloride ions in the soil which proves that there was no release of chloride ions at the 1st week. The chloride ions showed gradual increase from the 2nd week of incubation period. At the 8th week, chloride ions in soil showed significant increase. This might be due to increased rate of chlorpyrifos degradation. Bose *et al.* (2021) reported that during the degradation of chlorpyrifos, the intermediate product, TCP (3,5,6- trichloro – 2 pyridinol) undergoes dechlorination leading to increased level of chloride ions in the soil.

Effect of chlorpyrifos application on phosphate ion in soil was found to be significant from 1st to 4th weeks. There was slight decline during 1st, 2nd and 3rd weeks

of chlorpyrifos degradation and increase in the phosphate ion was noticed afterwards till the 8th week of incubation. Lowering of soil pH might be responsible for the fixation of phosphorus in soil up to 3rd week leading to decrease in the soil phosphate ions. Later phosphate ions increased up to 8th week of incubation which might be due to release of phosphate ions from chlorpyrifos compound. The findings were in accordance with the results obtained by Sardar and Kole (2005). They reported that phosphate ions in the chlorpyrifos treated soil decreases immediately after the application of chlorpyrifos later it showed increasing trend. Yu *et al.* (2011) reported that organophosphorus compounds such as methamidophos and glyphosate treated soil released the phosphate ions into the soil and also observed that soil available phosphorus increased significantly during the degradation period. Shifu and Gengyu (2005) reported that during the photocatalytic degradation of organophosphorus compounds, phosphate ions were released to the soil.

5.1.1.2 Microbial biomass carbon at weekly intervals

Effect of chlorpyrifos application on microbial biomass carbon of soil was found to be significant in all the weeks except at 1st week. Decrease in the microbial biomass carbon was observed immediately after the application of chlorpyrifos and later increased at the end of incubation period. This decrease in the microbial biomass carbon might be due to the lowering of microbial population immediately after application of chlorpyrifos (Bacteria and fungus). Shan *et al.* (2006) reported that chlorpyrifos treated soil showed significant decrease in the soil bacterial, fungal and the actinomycetes population at the chlorpyrifos concentration of 10 mg kg^{-1}. Xiaoqiang *et al.* (2008) reported that chlorpyrifos has inhibitory effect on microbial population on 7th day of incubation. Fungal population reduced to 61.1 per cent after the application of chlorpyrifos and did not return to initial level whereas the bacterial population returned to initial pre-treatment population (Xiaoqiang *et al.*, 2008).

5.1.1.3 Electrical conductivity and Soil moisture content at monthly intervals

Effect of chlorpyrifos application on electrical conductivity of the soil was found to be non significant at 4th and 8th weeks after the application of chlorpyrifos. However, slight increase in the electrical conductivity of the soil was observed at 8th week, which might be due to increased soluble ions in the soil solution. Exchangeable ions were released to the soil during the degradation process. Lipman *et al.* (1926) reported that EC of soil solution is controlled by sum of concentration of cations and anions.

Soil moisture showed significant difference with respect to treatment application at 4th week and 8th weeks. Earlier study conducted by Getzin (1981), reported that chlorpyrifos degradation did not have any influence on soil moisture content. This was in contrast to the findings of the present study.

5.1.1.4 Exchangeable ions at monthly intervals

Effect of chlorpyrifos application on exchangeable ions was studied at monthly intervals. The application of chlorpyrifos has significant effect on exchangeable potassium ions of soil at 8th week whereas effect was non significant at 4th week. Decrease in the potassium ion was observed throughout the degradation period. These findings were in accordance with Sardar and Kole (2005) reported that potassium ions in soil did not show significant difference after the chlorpyrifos application but there was slight decrease in the potassium ions during the degradation. Effect of chlorpyrifos application on calcium ion in soil was found to be significantly increased at 4th and 8th weeks of incubation. Sodium, magnesium and ammonium ions in soil were found to be non significant with respect to chlorpyrifos application. However, a trend of decrease in the sodium, magnesium and ammonium ions were observed at the 8th week of incubation.

Phosphate ion in soil was found to be significant only at 4th week with respect to chlorpyrifos application. Increase in the phosphate ion was noticed at the 8th week of incubation period and it was not found to be significant. Increase in the phosphate ions could be due to breakage of phospho- ester bond in the chlorpyrifos. Tang *et al.* (2011) confirmed that hydrolysis and oxidation of chlorpyrifos leads to release of phosphate

ions to the soil. Chloride and sulphate ions were found to be significant at 8th week whereas non significant at 4th week. This could be due to release of chloride and sulphate ions during the degradation of chlorpyrifos. During the process of degradation, homolytic cleavage of chlorine atoms in the chlorpyrifos compound was responsible for the release of chloride ions to the soil (Feng *et al.*, 1997). Pengphol *et al.* (2012) stated that sulphate ion was released during the degradation of chlorpyrifos. Effect of chlorpyrifos on nitrate and bicarbonate ions of soil were found to be non significant at 4th week and 8th weeks of incubation study. However, increase in the nitrate ion was observed at the 8th week.

Exchangeable cation in soil was found to be non significant at 4th and 8th weeks. However, there was decrease in the exchangeable cations observed at 8th week. Exchangeable anions and total exchangeable ions (cations and anions) in soil were found to be significantly increased at the 8th week. This could be due to release of ions from the chlorpyrifos compound during the process of degradation.

Results from the incubation experiment No.1 showed, degradation of chlorpyrifos decreased the soil pH by 2.29% within 60 days of incubation period. Chloride ions in soil showed significant increase at the 8th week of incubation. Phosphate ion was released from the chlorpyrifos compound as a result of breakage of phosphor ester bond indicating the degradation of chlorpyrifos. Chlorpyrifos residue in soil was reduced to 34.76 % within 60 days of incubation period.

5.2 INCUBATION EXPERIMENT NO.2

Based on the results and findings of the incubation experiment No.1, duration of incubation experiment No.2 was chosen for 60 days. Chemical and biological properties of the soil were studied and results from the experiment are briefly discussed below.

5.2.1 Effect of chlorpyrifos application on soil chemical properties

5.2.1.1. Soil pH

Data given in the figure 1 showed that there was a gradual decrease in the soil pH in all the treatments during the degradation of chlorpyrifos. At 8th week, treatment

received with combination of physical agents (Sunlight + Ultra violet), showed the highest pH which was on par with T_6 (*Trichoderma viride*), T_5 (*Pseudomonas fluorescens*), and T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) and least pH was recorded at T_4 (Hydrogen peroxide + Fenton reagent). Gradual decrease in the soil pH was observed up to 8^{th} week in all the treatments except chemical treatments. This might be due to the reason that application of chlorpyrifos will affect the pH of soil. Bisht *et al.* (2019) reported that pH of soil was reduced significantly during the degradation of pesticides such as endosulfan and chlorpyrifos and lowering of the soil pH might be due to dehalogenation during the degradation which leads to the formation of acids for both the pesticides. There was a decline in the soil pH in chemical treatments up to 4^{th} week and thereafter increase in the soil pH was observed. The decrease in soil pH in the chemical treatments might be due to application of H_2O_2 and Fe_2SO_4 leading to the formation of acids. This acidic condition causes hydroxyl free radicles to be formed from the Fenton reagent and leads to increase in the H^+ ions in the soil solution. Akhtar *et al.* (2004) reported that change in soil pH during the degradation had no effect on degradation of active ingredient of chlorpyrifos and fenpropathrin and he also observed that persistence of these pesticide was not influenced by the pH of soil. According to Gilani *et al.* (2010), chlorpyrifos degradation do not correlate with change in soil pH during the degradation study.

5.2.1.2. Chlorides

The results obtained from the study revealed that effect of treatments on chloride ion in soil was non significant at biweekly intervals. However, treatments showed gradual increase in the chloride ions at biweekly intervals. Increased chloride ions in soil might be due to enhanced rate of chlorpyrifos degradation as the treatments with faster degradation releases more chloride ions to the soil. T_7 (*Pseudomonas fluorescens* + *Trichoderma viride)* with the highest amount of chloride ions while the lowest chloride ions observed in control. During the degradation of chlorpyrifos, dehalogenation takes place which leads to release of chloride ions but among the treatments, difference in chloride ion was not significant.

According to Feng *et al.* (1997), release of chloride ion was stoichiometrically and concurrently related to removal of chlorpyrifos. Feng *et al.* (1997) reported that complete degradation of 40 mg TCP/L could releases 21.6 mg L^{-1} chloride ions. According to Wang *et al.* (2019), during the chlorpyrifos degradation concentration of chloride ion was increased from 19.9 mg L^{-1} in the first 2 hours to 39.9 mg L^{-1} within the 16 hours of incubation. Feng *et al.* (1997) reported that exposure of chlorpyrifos to UV light for about 6 hours causes the photocatalytic degradation and releases 36.5 mg L^{-1} chloride ions into the reacting solution. During the degradation, chlorpyrifos compound undergoes homolytic cleavage of chlorine atom and photo nucleophilic substitution responsible for the release of chloride ions to the soil (Feng *et al.*, 1997).

5.2.1.3. Phosphates

Effect of treatments on phosphates content of soil was found to be significant at biweekly intervals. At 2nd week, pot treated with *Trichoderma viride* recorded the higher amount of phosphate ions in soil while pot treated with ultra violet light showed the lower value of phosphate ions. Treatment received with Fenton reagent showed the higher amount of phosphate ions in soil at 4th week while T_1 (control) showed the lowest value of phosphate ions. Combination of *Pseudomonas fluorescens* + *Trichoderma viride* recorded the maximum value of phosphates in soil at 6th week while T_1 (control) recorded the lowest value. Pot treated with *Pseudomonas fluorescens* recorded the maximum value of phosphate ions and T_1 (control) recorded the lowest value of phosphate ion in soil at the 8th week.

Significant increase in the phosphate ion was observed during the incubation period. Phosphate ion in soil was found to decreased in the initial weeks. Fixation of phosphorus might be due to lowering of soil pH. Later, phosphate ion was increased which might be due to the release of phosphate ions from the chlorpyrifos compound during the degradation. These findings are accordance with the results reported by Sardar and Kole (2005). Phosphate ions in the chlorpyrifos treated soil decreases immediately after the application of chlorpyrifos and later showed increasing trend as the treatment with higher degradation showed more release of phosphate ions. Biological treatments showed the higher amount of phosphate ions in soil. Adelowo *et al*. (2015) reported utilization of organophosphates by microorganism as a source of

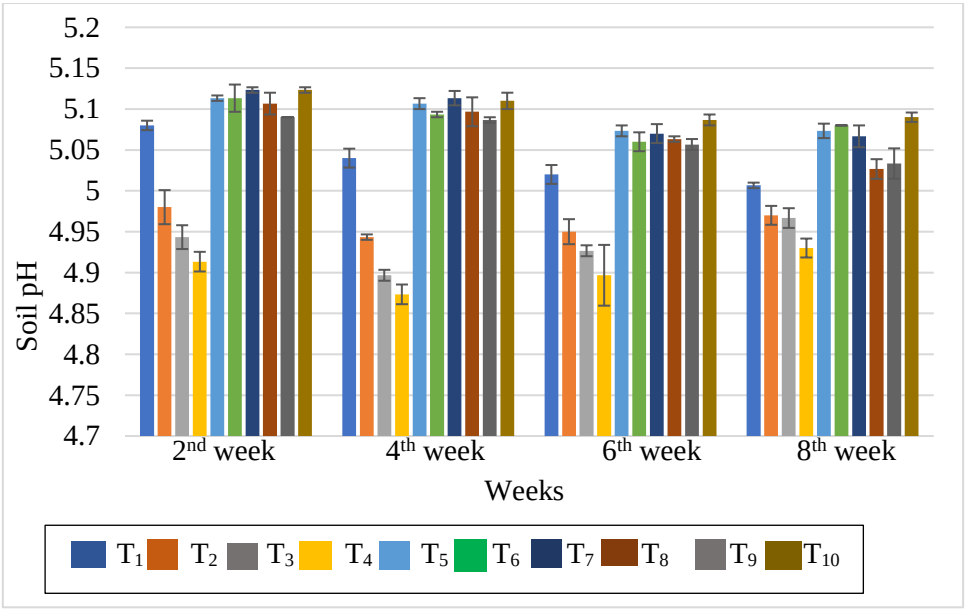


Fig.1 Effect of treatments on pH of soil

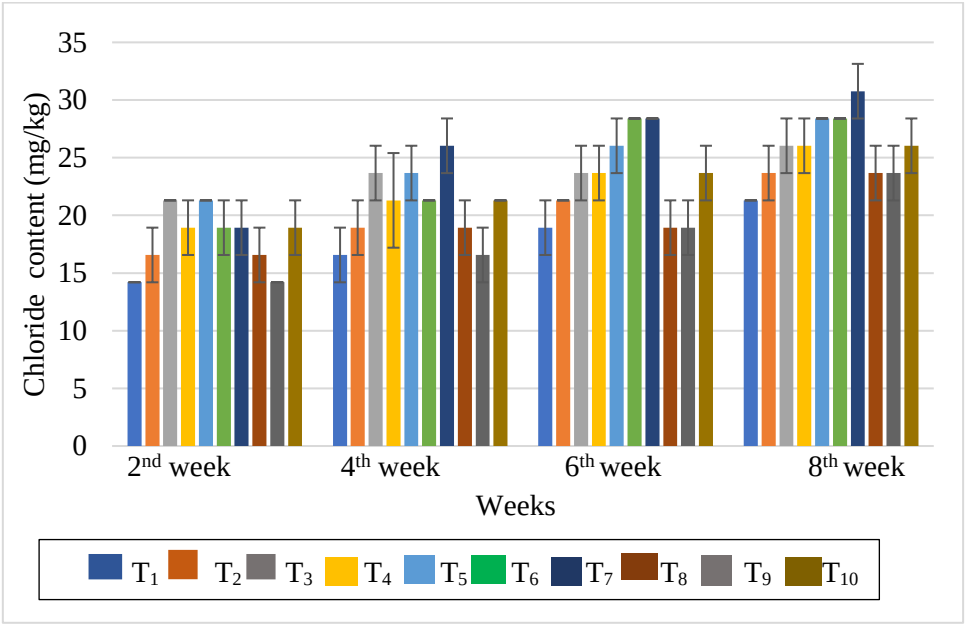


Fig.2 Effect of treatments on chloride content of soil

phosphate ions for their growth and reproduction. Release of phosphate ion during the dissipation of chlorpyrifos was due to the cleavage of carbon – phosphorus linkage. Tang *et al.* (2011) confirmed that hydrolysis and oxidation of chlorpyrifos leads to the release of phosphate ions to the soil.

5.2.1.4 Chlorpyrifos residue in soil

Treatments showed significant decrease in the chlorpyrifos residue of soil. Treatments showed the superior results over the control at biweekly intervals. At 2nd week, Fenton reagent (61.55 mg/kg) recorded the highest chlorpyrifos removal followed by T_4 (Hydrogen peroxide- + Fenton reagent) and T_5 (*Pseudomonas fluorescens*). Marican and Duran-Lara (2018) reported that combination of hydrogen peroxide and iron salts in the Fenton reagent act as strong oxidizing agent and leads to the formation of hydroxyl radicals which can oxidize the organics. At 4th week, highest removal of chlorpyrifos was recorded in the treatment received with *Trichoderma viride*. Pot treated with combination of biological agents (*Pseudomonas fluorescens* + *Trichoderma viride*) recorded the highest dissipation of chlorpyrifos (18.41 mg/kg) at 6th and 8th weeks followed by T_5 (*Pseudomonas fluorescens*). This might be due to combination of *Pseudomonas fluorescens* and *Trichoderma viride* acting as best agent for the degradation of chlorpyrifos. Sariwati *et al*. (2017) reported that mixed cultures of microorganism were more efficient in pesticide degradation than single cultures. Treatment T_1 (control) recorded the highest chlorpyrifos residue at the biweekly intervals which could be due to lower rate of degradation.

At 2nd week, chemical treatments recorded the highest removal of chlorpyrifos than biological treatments but from 4th week onwards biological treatments showed the highest dissipation of chlorpyrifos. Degradation of chlorpyrifos was slow in biological treatments at 2nd week due to lower microbial population and later the multiplication of microbes leads to the higher rate of degradation. Chlorpyrifos residue in soil decreased linearly throughout the incubation period in all the treatments. Treatment T_7 (*Pseudomonas fluorescens + Trichoderma viride)* showed the highest rate of degradation followed by T_5 (*Pseudomonas fluorescens*) and T_6 (*Trichoderma viride*). This might be due to the reason that combination of these two organisms were resistant to the chlorpyrifos and compatible to each other in the chlorpyrifos treated medium and

these would be acts as good degradation agent than the single cultures. Sasikala *et al.* (2012) reported that higher rate of degradation of chlorpyrifos showed by consortium might be due to synergistic effect of microbes. According to John *et al.* (2014), novel consortium of three species *Staphylococcus warneri*, *Pseudomonas putida* and *Stenotrophomonas maltophilia* would be efficiently used for the degradation of chlorpyrifos than single cultures and was capable of giving 90 per cent degradation of chlorpyrifos. Similar results reported by Sasikala *et al.* (2012) showed that consortium of *Pseudomonas aeruginosa*, *Bacillus cereu*s, *Klebsiella* sp and *Serratia marscecens* would be successfully used for the degradation of chlorpyrifos and observed that consortium could degrade the 65.87 per cent within 21 days of incubation.

Highest degradation of chlorpyrifos was shown by T_7 (*Pseudomonas fluorescens + Trichoderma viride)* followed by T_5 (*Pseudomonas fluorescens*) and T_6 (*Trichoderma viride*). The individual microorganisms also act as a good degradation agent for the chlorpyrifos dissipation. Lakshmi *et al*. (2008) found out that *Pseudomonas fluorescens* has the ability to degrade the chlorpyrifos up to 75-87 per cent. Jayaraman *et al.* (2012) reported that strains of *Trichoderma viride* and *Trichoderma harzianum* was efficiently used for the degradation of chlorpyrifos. Lower rate of degradation was observed in the treatment T_1 (control). This could be due to low rate of removal of chlorpyrifos residues from the control.

5.2.1.5 Electrical conductivity, Organic matter

There was no appreciable change in electrical conductivity of soil with respect to treatment application. However electrical conductivity of the soil increased at the 8^{th} week. This could be due to sustainable release of exchangeable ions to the soil. Lipman *et al.* (1926) reported that the EC of soil solution is controlled by sum of concentration of cations and anions.

Effect of treatments on organic matter content of soil was found to be significant at 8^{th} week, wherein treatments T_7 (*Pseudomonas fluorescens + Trichoderma viride)*, T_6 (*Trichoderma viride*), T_5 (*Pseudomonas fluorescens*) and T_{10} (Sunlight + Ultra violet) were found to be on par with highest organic matter content recorded at T_7 (0.638) and the lowest value was on T_3 (0.483) and T_4 (0.483). Change in the organic

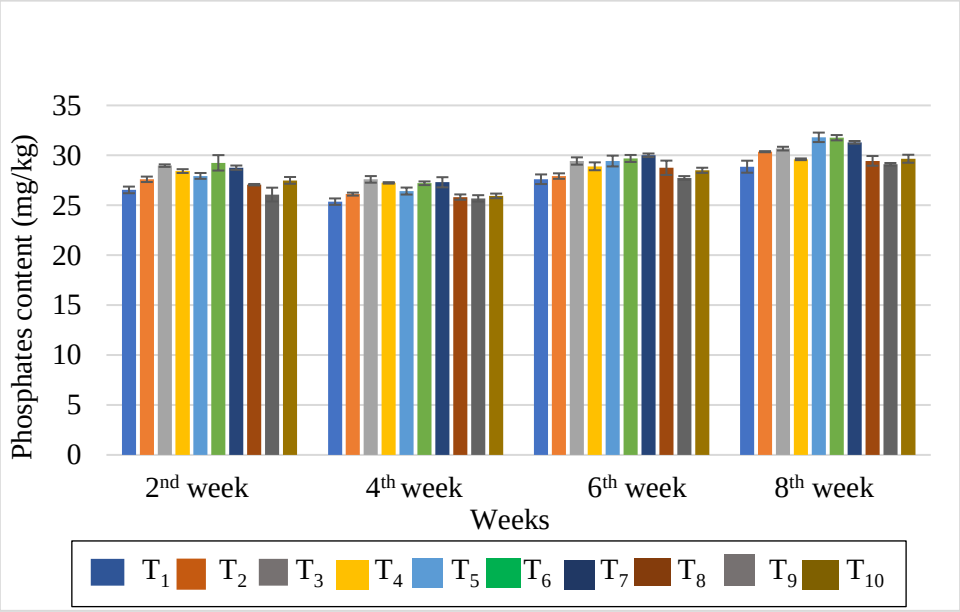


Fig.3 Effect of treatments on phosphate content of soil

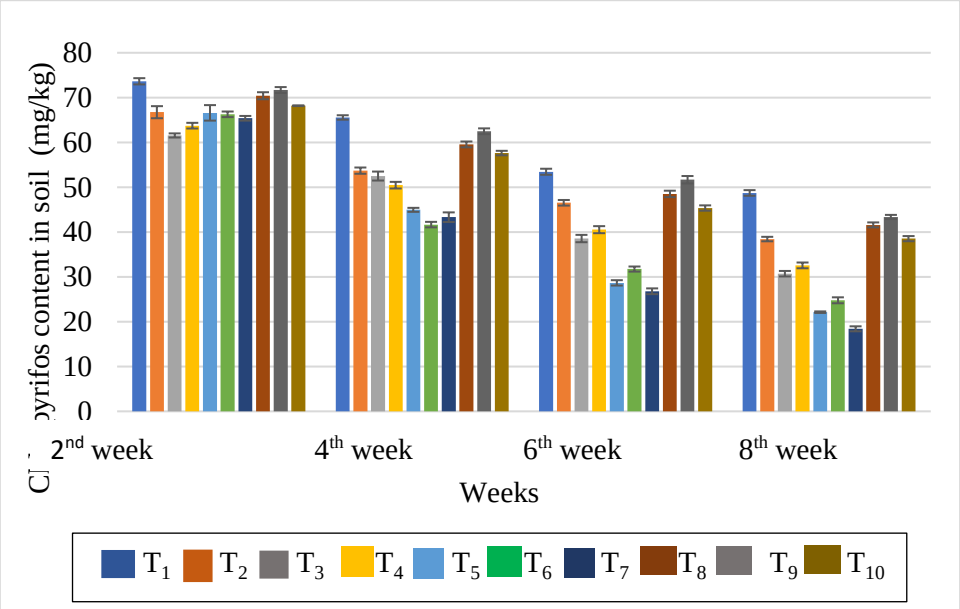


Fig.4 Effect of treatments on chlorpyrifos residue in soil

matter content varies with treatments. Increase in the organic matter content of soil was observed in biological treatments. The remaining treatments such as physical, chemical and control showed the decrease in organic matter content at 8th week. Mall *et al.* (2013) reported that chlorpyrifos treated soil showed highest organic carbon content on 14th day and thereafter gradually declined with the time of incubation.

5.2.1.6 Exchangeable cations

Effect of treatments on potassium ion in soil was found to be significant at 4th and 8th week intervals. At 4th week, highest potassium content was observed at T_2 (Hydrogen peroxide) and the lowest value was at T_8 (Sunlight). At 8th week, treatment received with hydrogen peroxide showed highest potassium ions in soil while the lowest value at T_{10} (Sunlight+ Ultra violet). The trend of decrease in the potassium ion was observed in all the treatments including the control at 8th week. Sahrawat (1976) reported that insecticide treated soil showed slight increase in available K during the second month. Later it decreased and followed the values similar to those in untreated soils. Racke *et al.* (1996) reported that low level of potassium was associated with accelerated level of chlorpyrifos degradation. In the present study the trend of decrease of potassium ion was observed in the 8th week which is in concordance with the reports of Racke *et al.* (1996).

Sodium ions in soil did not show significant differences with respect to treatment application. However, sodium ions in soil decreased at 8th week of incubation.

Exchangeable calcium in soil was significantly influenced by the treatment application. Significant increase in the calcium ions observed at 4th and 8th weeks. At 4th week, pot treated with *Pseudomonas fluorescens* showed higher calcium ions in soil followed by T_7 (*Pseudomonas fluorescens* + *Trichoderma viride)* and the lowest value was at T_1 (control). At 8th week, highest calcium ions recorded at T_4 (Hydrogen peroxide + Fenton reagent) followed by T_9 (Ultra violet) and the lowest calcium recorded at T_1 (control). At the 8th week, calcium ions increased in all the treatments except biological treatments. Biological treatments showed trend of decrease in the calcium ions at 8th week. This might be due to utilization of calcium ions for cell development of microbes.

Effect of treatments on exchangeable magnesium ion of soil was found to be significant at 4th and 8th week intervals. At the 4th week, T_4 (Hydrogen peroxide + Fenton reagent) recorded the highest magnesium ions in soil while the treatment received with combination of sunlight and ultra violet registered the lowest value. Treatments T_5 (*Pseudomonas fluorescens)* and T_7 (*Pseudomonas fluorescens* + *Trichoderma viride)* showed the highest and T_1 (control) with the lowest value of magnesium ions in soil recorded at 8th week. Increase in the magnesium ion was observed at all the treatments except control. Bulu *et al.* (2019) reported that exchangeable calcium and magnesium were higher in soils treated with pesticides compared to control. Martin (1966) reported that pesticide treated soil showed increase in the soluble Ca, Mg, P, S, Zn and Mn.

Effect of treatments on ammonium ions in soil did not show significant difference at 4th and 8th weeks. The trend of decrease in the ammoniacal nitrogen noticed at 8th week. This might be due to conversion of ammoniacal nitrogen to nitrate nitrogen. Affam and Chaudhuri (2013) reported that during the degradation of chlorpyrifos, there was decrease in ammoniacal nitrogen observed, which gets converted in to nitrate nitrogen.

There was no appreciable change in exchangeable cations with respect to treatment application at 4th and 8th week intervals. The effect of chlorpyrifos application on exchangeable cations in different treatments were studied and found out that potassium, sodium and ammonium ions in soil decreased whereas calcium and magnesium ions increased at the 8th week. At the 8th week, exchangeable cations in soil were found to be decreased.

5.2.1.7 Exchangeable anions

Phosphate ion in soil was found to be significant at 4th and 8th weeks. At 4th week, highest phosphate ion was recorded in treatment received with Fenton reagent while the lowest value observed at T_1 (control). At 8th week, maximum phosphate ions observed at pot treated with *Pseudomonas fluorescens* which was on par with T_6 (*Trichoderma viride*) and T_7 (*Pseudomonas fluorescens* + *Trichoderma viride)* whereas the lowest value recorded at T_1 (control). Tang *et al.* (2011) confirmed that hydrolysis

and oxidation of chlorpyrifos leads to release of phosphate ions to the soil. Higher phosphate ions in soil indicate the higher rate of degradation.

Effect of treatments on chloride ion in soil was found to be significantly increased at the 8th week. Biological treatments such as T_7 (*Pseudomonas fluorescens + Trichoderma viride)*, T_5 (*Pseudomonas fluorescens*) and T_6 (*Trichoderma viride*) were found to be superior to all other treatments while the lowest value recorded at T_1 (control) at 8th week. At the 8th week chloride ions increased in all the treatments due to increased rate of chlorpyrifos degradation. Pengphol (2012) reported that during the degradation of chlorpyrifos chloride ions were released to the soil.

Effect of treatments on sulphate ion in soil was found to be significant at 4th week. At 4th week, maximum amount of sulphate ion was found in the treatment T_5 (*Pseudomonas fluorescens*) followed by T_7 (*Pseudomonas fluorescens + Trichoderma viride)* and the minimum value recorded at T_1, T_3, T_9 and T_{10}. However, an increase in the sulphate ion was observed at the 8th week. Martin (1966) reported that pesticide containing sulphur compound releases the sulphur ions in to the soil. Giri *et al.* (2011) reported that 2,4 - D and Dithane M- 45 treated soil showed significant increase in the available sulphur.

Treatments not have any significant influence on nitrate ions in soil at 4th and 8th week. However, nitrate ions increased at the 8th week. According to Affam and Chaudhuri (2013), during the photocatalytic degradation of chlorpyrifos ammoniacal nitrogen decreased and nitrate nitrogen increased indicating mineralization of compound.

Effect of treatments on bicarbonates of soil was found to be significant at 8th week. Treatment T_{10} (Sunlight+ Ultra violet) was found to be on par with T_3, T_2, T_8 and T_5 whereas the treatments T_6, T_7, T_4, T_1 and T_9 recorded the minimum bicarbonates value of 0.002.

Effect of treatments on exchangeable anions of the soil were found to be significant at 8th week whereas non significant at 4th week. At 8th week, treatments T_7 (*Pseudomonas fluorescens + Trichoderma viride)*, T_6 (*Trichoderma viride*) and T_5 (*Pseudomonas fluorescens*) were found to be on par while lowest value of exchangeable

anions recorded at T_1 (0.670). Increase in the exchangeable anions was recorded at 8th week due to release of anions from the chlorpyrifos compound during the degradation.

5.2.1.8 Exchangeable ions

Exchangeable ion (exchangeable cations + exchangeable anions) in soil was not influenced significantly by treatment application at 4th and 8th week intervals. However exchangeable ions in soil increased at the 8th week. This might be due to release of ions into the soil during the degradation. If the pesticide is adsorbed as a cation or anion, it could replace the ions from the exchange sites on the soil colloids and releases the nutrients to the soil. Sahrawat (1976) confirmed that recommended level of pesticide to soil has little or no effect on soil available nutrients. Some insecticide has stimulatory effect on soil nutrients such as nitrogen, phosphorus and potassium.

5.2.2 Effect of chlorpyrifos application on soil physical property

5.2.2.1 Soil moisture content

Soil moisture exhibited significant differences at 4th and 8th weeks with respect to treatment application. T_6 (*Trichoderma viride*) recorded the highest moisture content which was on par with T_1, T_4, T_5, T_9, T_7, T_2 and T_3 at 4th week. At 8th week T_9 (Ultra violet) recorded the highest moisture content which was on par with T_7, T_2, T_5, T_3, T_6 and T_1. Treatment T_8 (Sunlight) recorded the lowest moisture content in soil at 4th and 8th week followed by T_{10} (Sunlight+ Ultra violet). This might be due to exposure of treatments under the sunlight for 6hr which has lead to great reduction in soil moisture. Cink and Coats (1993) reported that chlorpyrifos degradation is optimum under nearly field capacity. Lower degradation rate was observed at saturated condition as well as at low moisture content. At lower moisture content, the degradation rate was found to be lower.

5.2.3 Effect of chlorpyrifos application on soil biological properties

5.2.3.1 Dehydrogenase activity in soil

Dehydrogenase activity in the soil was found to be significant with respect to treatments at the biweekly intervals. Decrease in the dehydrogenase activity followed by gradual increase observed in the treatments. Pot treated with combination of

Pseudomonas fluorescens and *Trichoderma viride* recorded the highest dehydrogenase activity at 2nd and 4th weeks. At 6th and 8th weeks, maximum activity was observed at T_5 (*Trichoderma viride*). Treatment T_1 (control) registered the lowest activity of dehydrogenase at 6th and 8th weeks. Dehydrogenase activity of the soil was decreased up to 4th week. From the sixth week onwards, dehydrogenase activity of the soil increases in biological treatments. Treatments T_2 (Hydrogen peroxide), T_3 (Fenton reagent) and T_{10} (Sunlight+ Ultra violet) inhibit the dehydrogenase activity up to 6th week. Later its activity increased. Treatments such as T_1 (control), T_4 (Hydrogen peroxide+ Fenton reagent), T_8 (Sunlight) and T_9 (Ultra violet) showed the trend of gradual decrease in the dehydrogenase activity up to 8th week. John *et al.* (2018) reported that chlorpyrifos treated soil showed sequential decrease in the dehydrogenase activity of soil on 7th, 14th and 21st day of incubation. According to Singh *et al.* (2002), soil treated with combination of chlorothalonil and chlorpyrifos suppressed the dehydrogenase activity up to 50 per cent. Mayanglambam *et al.* (2005) reported that quinalphos (organophosphorus) treated soil showed 30 per cent reduction of dehydrogenase activity within 15 days of incubation and the enzymatic activity restored after 90 days of incubation due to adaptation of microorganisms to chemical stress. According to Jastrzębska (2011), dehydrogenase enzymes were very sensitive to chlorpyrifos and its activity was decreased at various concentration of chlorpyrifos but its activity was higher on 50th day compared to 10th day of incubation.

5.2.3.2 Phosphatase activity in soil

Phosphatase activity in the soil was found to be significantly increased at biweekly intervals with respect to treatment application. At 8th week maximum activity was recorded in treatment received with *Trichoderma viride* while the lowest activity was recoded at T_1 (control). The change in phosphatase activity of the chlorpyrifos treated soil in each treatment was different. Phosphatase activity of the soil in the biological treatments showed gradual increase up to 8th week except T_5 (*Pseudomonas fluorescens*) and T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) whereas decrease in phosphatase activity was observed at 8th week. Phosphatase activity of physical and chemical treatments decreases at first and then increased. Decline in the phosphatase activity could be due to inhibitory effect of chlorpyrifos or its metabolites directly on

phosphatase enzyme (Das and Mukherjee, 1999). Treatments, T_1 (control), T_2 (Hydrogen peroxide), T_4 (Hydrogen peroxide-5% + Fenton reagent -0.5%) and T_9 (Ultra violet) inhibited phosphatase activity up to 4^{th} week and there after its activity increased. Treatments T_3 (Fenton reagent), T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) and T_{10} (Sunlight+ Ultra violet) showed the gradual increase in the phosphatase activity up to 8^{th} week. This is in contrast to the work of Sikora *et al.* (1990), which suggested that an accelerated degradation of organophosphorus insecticides, including chlorpyrifos, was correlated with increased soil phosphatase activity. Study carried out by other researchers reported variable results on effect of pesticides on phosphatase activity (Kadyan and Chawla, 2020).

According to Aziz *et al.* (2021), amended and unamended chlorpyrifos contaminated treatments showed decreased phosphatase activity up to 15^{th} day and thereafter it stabilized and slowly increased at the end of incubation. Significant recovery of enzymatic activities might be due to removal of chlorpyrifos through degradation (Valle, 2006). Accelerating effect of biological treatments on phosphatase activities might be due to introduction of microbial communities. This will enhance the production of extracellular enzymes which was capable of degrading the chlorpyrifos (Tejada *et al.,* 2009). Hydrolysis of variety of organic phosphomonoesters is catalysed by the phosphatase enzyme and has been widely used for the degradation of organophosphorus pesticides (Kanekar *et al.,* 2004). Das and Mukherjee (1999) reported that phosphatase activity in the soil was increased by the application pesticides. Decline in the phosphatase activity in soil could be due to the inhibitory effect of chlorpyrifos or its metabolite on phosphate solubilizing soil microorganism or directly on phosphatase enzyme (Das and Mukherjee, 1999).

5.2.3.3 Urease activity in soil

Effect of treatments on urease activity in soil was found to be significant at 6^{th} and 8^{th} weeks. Urease activity in soil decreased at biweekly intervals. Treatment received with combination of *Pseudomonas fluorescens* and *Trichoderma viride* recorded the maximum activity of urease at 6^{th} and 8^{th} weeks. Urease activity lower in pot treated with Fenton reagent at 6^{th} week. T_1 (control) and T_3 (Fenton reagent) recorded the lowest urease activity at 8^{th} week. In biological treatments, urease activity

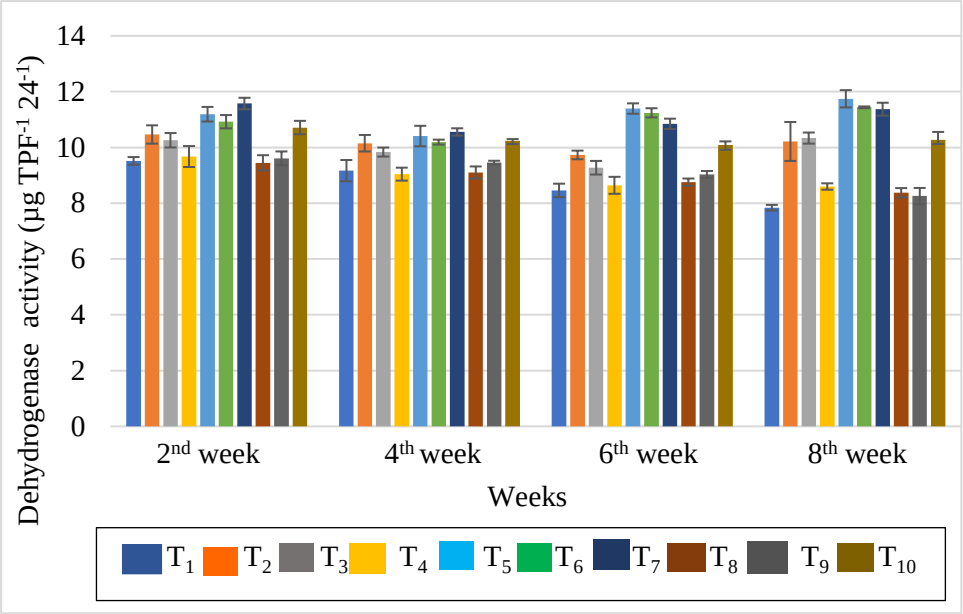


Fig.5 Effect of treatments on dehydrogenase activity of soil

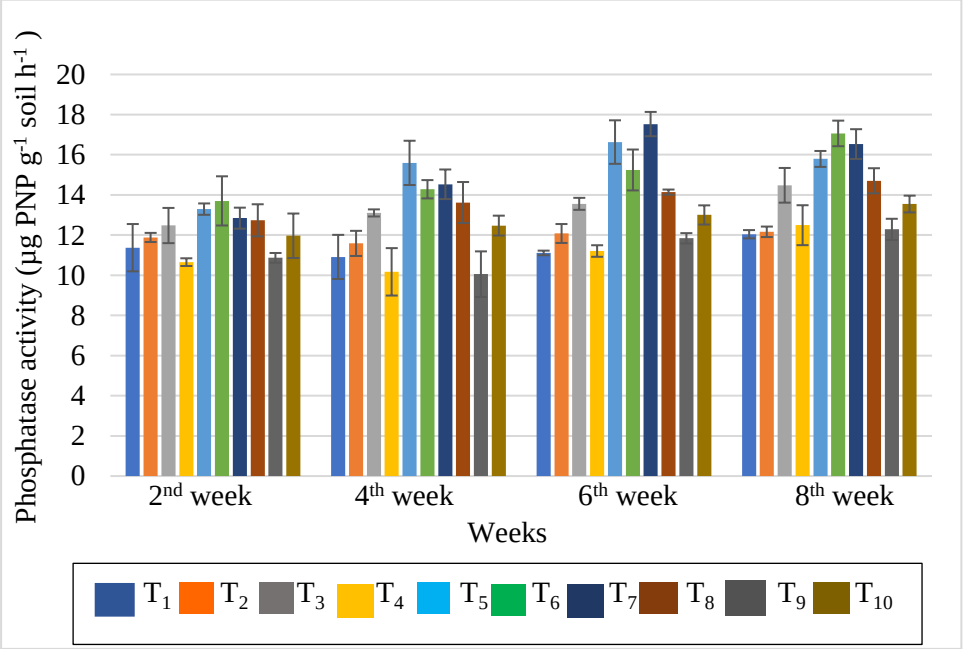


Fig.6 Effect of treatments on phosphatase activity of soil

of soil decreased up to 4th week and then its activity increases up to eighth week. Treatments such as T_8 (Sunlight) and T_{10} (Sunlight+ Ultra violet) showed the decreased activity of urease up to 6th week there after increase in the activity noticed at the 8th week. Inhibitory effect of chlorpyrifos on urease activity was noticed by Wang *et al.* (2010). Change in urease activity was due to inhibitory effects of chlorpyrifos oxon formed during the degradation. Application of pesticides into the soil reduces the urease activity by lowering the urea hydrolysis which was usually beneficial because it helps to maintain the nitrogen availability to plants (Antonious, 2003). Aziz *et al.* (2021) reported that chlorpyrifos has negative effect on urease activity throughout the incubation period. Hridya *et al.* (2014) reported that the application of *Azospirillum* and *Trichoderma* along with 50% recommended NPK increased urease enzymatic activity.

5.2.3.4 Microbial biomass carbon in soil

Microbial biomass carbon in soil decreased significantly in all the treatments except biological treatments. Treatment received with combination of biological agents (*Pseudomonas fluorescens* + *Trichoderma viride*) recorded the highest microbial biomass carbon (99.15 µg g^{-1}) whereas T_4 with lowest value (75.36 µg g^{-1}) of microbial biomass carbon in soil at all the biweekly intervals. Biological treatments showed gradual increase in the microbial biomass carbon at the biweekly intervals. Pots treated with control, chemical and physical treatments showed the trend of decrease in the microbial biomass carbon up to 6th week and later increased. The reduction in the microbial biomass carbon was due to the copper residues of chlorpyrifos resulted stress to the microbes. Chemical treatments showed the lowest microbial biomass carbon than that of control due to adverse effect of hydrogen peroxide and ferrous sulphate on the microbes. According to Singh *et al.* (2002), application of chlorpyrifos to the soil reduces the microbial biomass carbon. Chlorpyrifos application in groundnut field cause the short-term inhibitory effect on bacterial population which recovered to initial population after the 60 days of chlorpyrifos application whereas the fungal population stimulated after the soil treatment (Pandey and Singh, 2004). According to Tu (1970), initial reduction of microbial population followed by return to initial pre-treatment population due to the resistance acquired by microbes to pesticides.

5.2.4 Effect of chlorpyrifos application under submerged condition

5.2.4.1 pH, EC, chlorides and phosphates

pH of water (leachate) did not show significant difference with respect to treatment application. However, pH of leachate increased to near neutral pH at 8th week during the degradation of chlorpyrifos in both the treatments. Fadaei and Kargar, (2013) reported that pH of water increased from 5 to 9 during the photodegradation of chlorpyrifos under the UV/TiO_2 which also increases the degradation efficiency of chlorpyrifos. These findings were accordance with Femia (2013) reported that photodegradation of chlorpyrifos in natural water by UV/H_2O_2 showed increase in the pH of water during the progress of research.

Effect of treatments on electrical conductivity of the leachate was found to be non significant at biweekly intervals. Electrical conductivity of leachate increased during the degradation period. Muhamad (2010) reported that electrical conductivity of the solution was increased during the chlorpyrifos degradation. Increase in the electrical conductivity was observed due to conversion of organic chlorpyrifos in to inorganic species. Schwack and Flober-Muller (1990) reported that neutral organic molecules changed to conductive ionic species during the degradation of pesticides.

Chloride ion of the leachate was not influenced significantly by the treatment application at the biweekly intervals. Chloride ion was increased throughout the degradation period in both the treatments. During the degradation of chlorpyrifos, chloride ion is released due to the dechlorination of the compound (Schwack and Flober-Muller, 1990).

Treatments were found to be non significant on phosphate ions of leachate at weekly intervals. The trend of increase in the phosphate ion was observed throughout the incubation period. This could be due to release of phosphate ions during the degradation of chlorpyrifos.

5.2.4.2 Chlorpyrifos in water (leachate) sample

Treatments were found to be significant at 6th and 8th weeks whereas non significant at 2nd and 4th weeks. However highest removal of chlorpyrifos recorded at

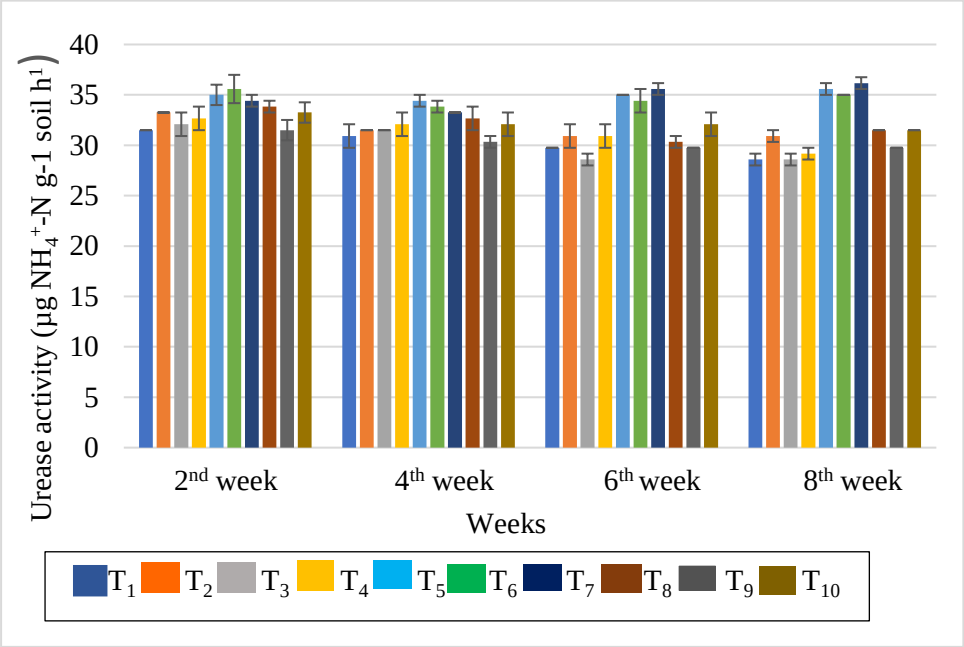


Fig.7 Effect of treatments on urease activity of soil

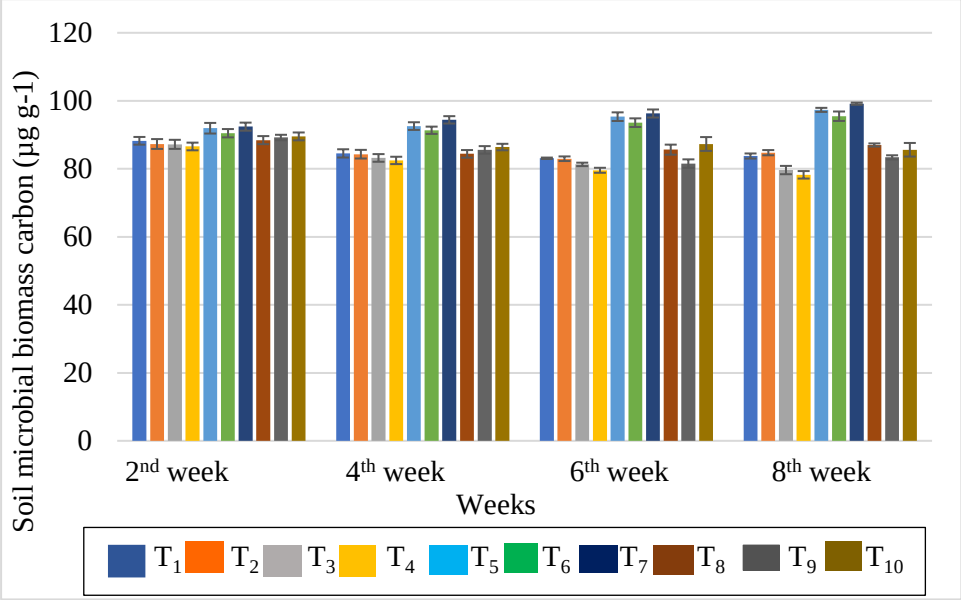


Fig.8 Effect of treatments on soil microbial biomass carbon of soil

T_{11} (soil under saturated condition at 5 cm level of submergence) followed by T_{12} (soil under saturated condition at 5 cm level of submergence with azolla) at the biweekly intervals. Chlorpyrifos residue was continuously decreased throughout the incubation period. Growth of azolla was normal under the submerged condition in the initial period of incubation study later stage, decaying of azolla was noticed. This could be due to adverse effect of chlorpyrifos on the growth of azolla. These findings were accordance with Prasad (2015) reported that growth of azolla was decreased to 30 per cent under the 5ppm concentration of chlorpyrifos whereas in control, growth of *Azolla pinnata* increased by 72-76 per cent.

The findings showed that application of biological agents in combination (*Pseudomonas fluorescens* + *Trichoderma viride*), degraded chlorpyrifos to 74.99 per cent and showed the highest rate of degradation throughout the incubation period followed by *Pseudomonas fluorescens* (69.94 %) and *Trichoderma viride* (66.35 %). Lowest rate of degradation was shown by control (33.83 %). Biological agents are more efficient than physical and chemical agents. Rate of degradation in the order biological > chemical > physical > control. Study related to the chemical parameters showed that significant decrease in the soil pH and significant increase in the phosphate ions were observed during the incubation period. Chloride ions showed non significant difference at biweekly intervals. However, increase in the chloride ion was observed during the incubation period which indicated the release of degradation products with advancement of degradation period. Biological properties of the soil were not much affected with treatments having biological agents whereas in chemical and physical treatments decrease in the biological properties observed. On account of these findings, we can recommend the use of combinations of biological agents or biological agents alone, as an ideal approach for degradation of chlorpyrifos in northern laterite soils. The treatments received with biological agents are best degraders and helps in maintaining the soil fertility or soil health.

Summary

6. SUMMARY

The investigation was conducted to evaluate the different approaches of chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU11)" are summarized in this chapter.

Investigation was carried out at College of Agriculture, Padannakkad during September 2019 to July 2021, with the objective of evaluation and impact assessment of chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11) by using physical, chemical and biological methods. The whole study consisted of two parts – incubation experiment No.1 and incubation experiment No. 2

The incubation experiment No. 1 was carried out to know the pattern and time required for degradation of chlorpyrifos in soil. Northern laterite soils (AEU 11) collected from the Pilicode were selected for the study. Pot culture study was conducted in five pots filled with 10 kg soil and drenched with chlorpyrifos at the concentration of 2.5 ml L^{-1} (20 EC). Soil was analyzed at weekly intervals and the results from the study were statistically analysed using single sample t test by comparing with initial soil sample properties. The results from the incubation study showed that chlorpyrifos residue was reduced at weekly intervals. Chlorpyrifos content was reduced 34.76 per cent within the 60 days of incubation period. Significant decrease on soil pH (2.29%) was noticed after application of chlorpyrifos. Chloride ions in soil significantly increased at the 8^{th} week. Slight decline in the phosphate ions was noticed during the initial weeks followed by increase in later stages of degradation period. Increase in the chloride and phosphate ions might be due to these ions were released during the degradation. During the degradation process, microbial biomass carbon (8.2%) in soil was reduced significantly. Effect of chlorpyrifos on electrical conductivity of the soil was found to be non significant. Exchangeable cations in soil were found to be non significant at monthly intervals whereas exchangeable anions were significant at 8^{th} week. Effect of chlorpyrifos on exchangeable ions were found to be non significant at 4^{th} and 8^{th} week intervals. However, there was slight increase in the exchangeable ions (6.25%) noticed during the degradation. Based on the results and findings from the incubation experiment No.1, duration of incubation experiment No.2 was decided for 60 days.

Incubation experiment No.2 was carried out to assess the best method of degradation of chlorpyrifos in soil. Experiment was laid out in completely randomized block design with 12 treatments replicated thrice. In each treatment, 10 kg soil was maintained and drenched with chlorpyrifos in all the treatments at the rate of 2.5 ml L^{-1} (20 EC). Physical, chemical and biological agents were applied and evaluated to study their effect on degradation of chlorpyrifos. The treatment combinations were control (T_1), hydrogen peroxide-5% (T_2), Fenton reagent -0.5% (T_3), hydrogen peroxide-5% + Fenton reagent -0.5% (T_4), *Pseudomonas fluorescens* (T_5), *Trichoderma viride* (T_6), *Pseudomonas fluorescens* + *Trichoderma viride* (T_7), sunlight – 6hrs (T_8), ultra violet – 4hrs (T_9), sunlight – 6hrs + ultra violet – 4hrs (T_{10}), soil under saturated condition at 5 cm level of submergence (T_{11}), soil under saturated condition at 5 cm level of submergence with azolla (T_{12}).

The chlorpyrifos degradation under the influence of different treatments showed significant reduction in chlorpyrifos residues. Application of combination of *Pseudomonas fluorescens* + *Trichoderma viride* (18.41 mg kg^{-1}) showed the higher rate of degradation throughout the incubation period followed by *Pseudomonas fluorescens* (22.13 mg kg^{-1}) and *Trichoderma viride* (24.77 mg kg^{-1}) within 60 days of incubation period. Lowest degradation was showed by control (48.72 mg kg^{-1}) throughout the incubation period. Biological agents showed the highest degradation followed by chemical and physical treatments. Among the chemical treatments Fenton reagent -0.5% showed the higher degradation (30.70 mg kg^{-1}) followed by combination of hydrogen peroxide-5% + Fenton reagent -0.5% (32.56 mg kg^{-1}) and hydrogen peroxide-5% (38.43 mg kg^{-1}). Physical agents showed lower degradation of chlorpyrifos as compared to chemical and biological treatments.

Results indicated that soil pH was found to be significantly decreased at the biweekly intervals. The soil pH in the treatments receiving chemical agents decreased at first then showed increasing trend. Treatment T_{10} (sunlight – 6hrs + ultra violet – 4hrs) recorded the highest pH (5.09) whereas T_4 (hydrogen peroxide-5% + Fenton reagent -0.5%) recorded the lowest pH (4.93) at the 8^{th} week. Chloride ion in soil was found to be non significant with respect to treatment application at biweekly intervals. However, chloride ion in soil was continuously increased throughout the incubation

period. Phosphate ions in soil decreased at first followed by gradual increase in the phosphate ions. At the 8th week, T_5 (*Pseudomonas fluorescens)* recorded the highest phosphate ions (31.80 mg/kg) in soil while the T_1 (control) recorded the lowest phosphate ions (28.86 mg/kg) in soil. Effect of chlorpyrifos with respect to soil EC was found to be non significant at monthly intervals. Effect of chlorpyrifos application on soil organic matter content was found to be significant at 8th week. Treatment T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) recorded the highest organic matter content whereas T_3 (Fenton reagent - 0.5%) and T_4 (hydrogen peroxide-5% + Fenton reagent -0.5%) showed the lowest value of organic matter. Soil moisture was found to be significant at 4th and 8th weeks. Effect of chlorpyrifos application on soil exchangeable anions were found to be significant at 8th week. Exchangeable cations and total exchangeable ions were found to be non significant at 4th and 8th week intervals.

Effect of treatments on biological properties of soil were analysed at weekly intervals. The treatment application has significant influence on soil dehydrogenase activity. Chlorpyrifos application has inhibitory effect on soil dehydrogenase activity but activity was recovered in the pots receiving biological treatments after first month of chlorpyrifos application. Chemical treatments alone (T_2, T_3) and combination of physical treatments (T_{10}) inhibited the dehydrogenase activity up to 6th week there after its activity increased. Physical treatments alone (Treatments T_8, T_9), combination of chemical treatments (T_4) and control showed the trend of gradual decrease in the dehydrogenase activity up to 8th week. Treatment T_5 (*Pseudomonas fluorescens)* recorded the highest dehydrogenase activity (11.74 µg TPF g^{-1} soil day^{-1}) while T_1 (control) showed the lowest dehydrogenase activity (7.83 µg TPF g^{-1} soil day^{-1}) at the 8th week. Study revealed that dehydrogenase enzyme was sensitive to chlorpyrifos later it recovered the activity at the end of incubation period. Effect of treatment application on soil phosphatase activity of chlorpyrifos treated soil was found to be significantly increased. Phosphatase activity of soil in the biological treatments showed gradual increase up to 8th week except T_5 and T_7 they showed decrease in phosphatase activity at 8th week. Control, chemical and physical treatments showed decreasing trend in the phosphatase activity during the initial period followed by increased the activity at 8th

week. Treatment T_6 (*Trichoderma viride*) recorded the highest (17.06 µg PNP g^{-1} soil h^{-1}) while the T_1 (Control) recorded the lowest activity (12.04 µg PNP g^{-1} soil h^{-1}) of phosphatase enzyme at the 8th week. Decline in the phosphatase activity in soil could be due to the inhibitory effect of chlorpyrifos or its metabolite on phosphate solubilizing soil microorganism or directly on phosphatase enzyme. Effect of treatments on soil urease activity was found to be significant at 6th and 8th weeks. Biological treatments showed slight decrease in the urease activity in the initial weeks thereafter retained the activity. The rest of treatments showed slight decrease in the urease activity up to 8th week. Treatment T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) showed the highest urease activity (36.16 µg NH_4^+-N g^{-1} soil h^{-1}) while T_1 (28.58 µg NH_4^+-N g^{-1} soil h^{-1}) and T_3 (28.58 µg NH_4^+-N g^{-1} soil h^{-1}) recorded the lowest urease activity in soil. Change in urease activity was due to inhibitory effects of chlorpyrifos oxon formed during the degradation. Microbial biomass carbon in soil was reduced significantly in all the treatments after the application of chlorpyrifos. There was trend of decrease at initial weeks followed by increase in the microbial biomass carbon noticed at the end of incubation. Treatment T_7 (99.15 µg/g) showed highest and T_4 (78.23µg/g) showed the lowest value of microbial biomass carbon at the 8th week.

Chlorpyrifos treated soil incubated under the submerged condition of 5 cm level with and without azolla. The treatments in the saturated conditions were T_{11} (soil under saturated condition at 5 cm level of submergence) and T_{12} (soil under saturated condition at 5 cm level of submergence with azolla). Chlorpyrifos residue in water (leachate) was found to be significant at 6th and 8th weeks. However, treatments T_{11} and T_{12} showed the trend of reduction of chlorpyrifos residue in water (leachate) at biweekly intervals. Treatment T_{11} (2598.62 µg L^{-1}) showed the highest removal of chlorpyrifos while T_{12} (3318.07 µg L^{-1}) with lowest removal of chlorpyrifos at the 8th week. Growth of azolla was normal up to 2nd week after the application of chlorpyrifos. The growth of azolla was arrested and declining of azolla was noticed during the later stage. The leachate of chlorpyrifos contaminated soil showed hazardous effect on azolla. pH, electrical conductivity, chlorides and phosphates of water (leachate) were found to be non significant at the biweekly intervals.

Results from the investigation revealed that combination of *Pseudomonas fluorescens* + *Trichoderma viride* (74.99 %) showed the highest rate of chlorpyrifos degradation followed by *Pseudomonas fluorescens* (69.94 %) and *Trichoderma viride* (66.35 %) within the 60 days of incubation study. Highest degradation rate was showed by biological agents followed by chemical and physical agents least was shown by control. During the degradation of chlorpyrifos, chloride and phosphate ions were released to the soil which was highest in T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*). Also, the biological properties of the soil were not much affected in the treatments such as T_5 (*Pseudomonas fluorescens*), T_6 (*Trichoderma viride*) and T_7 (*Pseudomonas fluorescens* + *Trichoderma viride*) after the application of chlorpyrifos. Chemical and physical treatments showed decline in biological activities and microbial biomass carbon. Therefore, from this study we can recommend the biological methods as best for the degradation of chlorpyrifos which was more efficient and effective method than chemical and physical methods and not harmful to the environment. It will maintain the soil health.

Future line of work

- There is a need for further investigation to understand the mechanisms of microbial degradation and the enzymes involved in degradation process.
- Use of plants with phytoremediation capabilities for removing chlorpyrifos from the soil and aquatic systems.
- Isolation of microorganisms from the pesticides contaminated sites used for the degradation of pesticides at field level.
- Evaluate the effect of lime application on degradation of chlorpyrifos in soil.

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“APPROACHES TO ASSESS CHLORPYRIFOS DEGRADATION IN NORTHERN LATERITE SOILS OF KASARAGOD (AEU 11)”

by

ARYA P. R.

(2019 - 11 - 173)

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

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

COLLEGE OF AGRICULTURE

PADANNAKKAD, KASARAGOD – 671314

KERALA, INDIA

2022

ABSTRACT

The investigation on "Approaches to assess chlorpyrifos degradation in northern laterite soils of Kasaragod (AEU 11)" was undertaken with the objective to evaluate the impact of physical, chemical and biological methods on degradation of chlorpyrifos in laterite soils. The study was carried out during 2019 – 2021 at College of Agriculture, Padannakkad in two incubation experiments.

The incubation experiment no. 1 was carried out to know the pattern and time required for degradation of chlorpyrifos in soil. Northern laterite soils (AEU 11) collected from Pilicode were selected for the study. Pot culture study was conducted in five pots filled with 10 kg soil and drenched with chlorpyrifos (20 EC) at the concentration of 2.5 ml/L. Soil was analyzed at weekly intervals and results from the incubation study showed that chlorpyrifos content was reduced to 34.76% within 60 days. Significant decrease on soil pH (2.29%) was noticed after the application of chlorpyrifos. Chloride and phosphate ions were increased during the incubation period due to release of these ions from the chlorpyrifos compound during the degradation process. Microbial biomass carbon (8.2%) in soil was reduced significantly. Based on the results and findings from the incubation experiment no.1, duration of incubation experiment no.2 was decided for 60 days.

The incubation experiment no. 2 was carried out to assess the best method of degradation of chlorpyrifos in laterite soil. The experiment was laid out in CRD with 12 treatments and three replications. Physical, chemical and biological agents were applied and evaluated to study their effect on degradation of chlorpyrifos. The treatment combinations were control (T_1), hydrogen peroxide-5% (T_2), Fenton reagent -0.5% (T_3), hydrogen peroxide-5% + Fenton reagent -0.5% (T_4), *Pseudomonas fluorescens* (T_5), *Trichoderma viride* (T_6), *Pseudomonas fluorescens* + *Trichoderma viride* (T_7), sunlight – 6hrs (T_8), ultra violet – 4hrs (T_9), sunlight – 6hrs + ultra violet – 4hrs (T_{10}), soil under saturated condition at 5 cm level of submergence (T_{11}) and soil under saturated condition at 5 cm level of submergence with azolla (T_{12})

Results from the incubation study revealed that combination of *Pseudomonas fluorescens* + *Trichoderma viride* showed the highest rate (74.99%) of chlorpyrifos degradation followed by *Pseudomonas fluorescens* (69.94 %) and *Trichoderma viride* (66.35 %) within 60 days. Effect of chlorpyrifos application on chemical properties of soil was studied at biweekly intervals. Soil pH was found to be significantly decreased throughout the incubation period. Highest pH (5.09) was recorded in treatment T_{10} (sunlight + UV light) whereas lowest in T_4 (4.93). Effect of treatments on chloride ions in soil were found to be non significant, however it was continuously increased throughout the incubation period. Phosphate ions in soil decreased significantly in the initial period followed by gradual increase in the phosphate ions in soil. In the 8^{th} week,

T_5 recorded the highest phosphate ions (31.80 mg kg^{-1}) while T_1 recorded the lowest phosphate ions (28.86 mg kg^{-1}) in soil.

The effect of treatment application on soil biological properties were studied and showed that chlorpyrifos has inhibitory effect on microbial biomass carbon, dehydrogenase, phosphatase and urease activities of the soil immediately after chlorpyrifos application but later restored the activities. The treatments that received biological agents were not much affected with respect to the biological properties of the soil. Biological treatments such as T_5 recorded the highest dehydrogenase activity (11.74 µg TPF g^{-1} soil day^{-1}) while the T_6 recorded the highest phosphatase activity (17.06 µg PNP g^{-1} soil hr^{-1}) of the soil. Treatment T_7 recorded the highest microbial biomass carbon (99.15 µg g^{-1}) and the urease activity (36.16 µg NH_4^+-N g^{-1} soil hr^{-1}) in soil.

There was a significant effect with respect to the two treatments maintained under submergence. The leachate from the chlorpyrifos treated soils were analysed at biweekly intervals. Treatments showed significant effect on leachate of chlorpyrifos residue at sixth and eighth week intervals. Treatment T_{11} (2598.62 µg L^{-1}) showed the highest degradation followed by T_{12} (3318.07 µg L^{-1}). The growth of azolla was normal during the initial period, later decaying of azolla was noticed. Growth of azolla was inhibited under the chlorpyrifos treatment because it could not tolerate the residual effect of chlorpyrifos.

The results from the investigation revealed that chlorpyrifos degradation using combination of *Pseudomonas fluorescens* + *Trichoderma viride* had the best potential to remove the residues of chlorpyrifos insecticide present in treated soils. Biological treatments are recorded as the prominent agents in chlorpyrifos degradation and also maintains the soil health. On account of these findings, we can recommend the use of biological agents in combination or alone, as an ideal approach for degradation of chlorpyrifos in laterite soils.

സംക്ഷിപ്തം

കാസർഗോഡിലെ വടക്കൻ ചെങ്കല്ല് മണ്ണിലെ ക്ലോർപൈറിഫോസ് നശീകരണം വിലയിരുത്തുന്നതിനുള്ള സമീപനങ്ങൾ (കാർഷിക പരിസ്ഥിതി യൂണിറ്റ് 11)

കാസർഗോഡിലെ വടക്കൻ ചെങ്കല്ല് മണ്ണിൽ (കാർഷിക പരിസ്ഥിതി യൂണിറ്റ് 11) ക്ലോർപൈറിഫോസ് നശീകരണത്തിന്റെ മൂല്യ നിർണയവും ആഘാതവും ഭൌതികവും രാസപരവും ജൈവശാസ്ത്രപരവുമായ രീതികൾ ഉപയോഗിച്ച് വിലയിരുത്തുക എന്ന ലക്ഷ്യത്തോടെയാണ് “കാസർഗോഡിലെ വടക്കൻ ചെങ്കല്ല് മണ്ണിൽ ക്ലോർപൈറിഫോസ് നശീകരണം വിലയിരുത്തുന്നതിനുള്ള സമീപനങ്ങൾ” എന്ന വിഷയത്തിൽ പഠനം നടപ്പിലാക്കിയത്. പടന്നക്കാട് കാർഷിക കോളേജിൽ 2019-2021 കാലയളവിൽ രണ്ട് ഭാഗങ്ങളായാണ് അന്വേക്ഷണം നടത്തിയത്.

മണ്ണിൽ ക്ലോർപൈറിഫോസ് നശീകരണത്തിന്റെ മാത്യകയും സമയവും അറിയുവാൻ വേണ്ടി ഇൻകുബേഷൻ പരീക്ഷണം നമ്പർ 1 നടത്തി. പീലിക്കോട് നിന്നുള്ള വടക്കൻ ചെങ്കല്ല് മണ്ണ് (കാർഷിക പരിസ്ഥിതി യൂണിറ്റ് 11) പഠനത്തിനായി തിരെഞ്ഞെടുത്തു. 10 കിലോഗ്രാം മണ്ണ് അഞ്ച് ചട്ടികളിൽ നിറച്ച് ക്ലോർപൈറിഫോസ് 2.5 മില്ലി/ലി (20 ഇസി) എന്ന തോതിൽ നനച്ചു. ക്ലോർപൈറിഫോസ് നശീകരണത്തിന്റെ ദൈർഘ്യവും മാത്യകയും നിർണയിക്കാൻ ആഴ്ചതോറുമുള്ള ഇടവേളകളിൽ മണ്ണ് വിശകലനം ചെയ്തു. ഇൻകുബേഷൻ കാലയളവിന്റെ രണ്ട് മാസത്തിനുള്ളിൽ ക്ലോർപൈറിഫോസിന്റെ അളവ് 34.76 ശതമാനമായി കുറഞ്ഞുവെന്ന് ഇൻകുബേഷൻ പഠനത്തിന്റെ ഫലം കാണിക്കുന്നു. ക്ലോർപൈറിഫോസ് പ്രയോഗത്തിനുശേഷം മണ്ണിന്റെ അമ്ലത്യം ഗണ്യമായി കുറഞ്ഞു (2.29%). നശീകരണ കാലഘട്ടത്തിൽ മണ്ണിലെ ക്ളോറൈഡ് അയോണുകളും ഫോസ്ഫേറ്റ് അയോണുകളും വർദ്ധിച്ചു ഇതിന് കാരണം ക്ലോർപൈറിഫോസ് സംയുക്തത്തിൽ നിന്നുമുള്ള ഈ അയോണുകളുടെ പ്രകാശനമാണ്. ക്ലോർപൈറിഫോസ് പ്രയോഗത്തിനുശേഷം മണ്ണിലെ മൈക്രോബിയൽ ബയോമാസ് കാർബൺ ഗണ്യമായി കുറയുകയും ചെയ്തു (8.2%). ഇൻകുബേഷൻ പരീക്ഷണം നമ്പർ 1 ൽ നിന്നുള്ള ഫലങ്ങളുടെയും കണ്ടെത്തലുകളുടെയും അടിസ്ഥാനത്തിൽ ഇൻകുബേഷൻ പരീക്ഷണം നമ്പർ 2 ന്റെ ദൈർഘ്യം 60 ദിവസത്തേക്ക് തീരുമാനിച്ചു.

മണ്ണിലെ ക്ലോർപൈറിഫോസിന്റെ ഏറ്റവും മികച്ച നശീകരണ രീതി വിലയിരുത്തുന്നതിനായി ഇൻകുബേഷൻ പരീക്ഷണം നമ്പർ 2 വടക്കൻ ചെങ്കല്ല് മണ്ണിൽ (കാർഷിക പരിസ്ഥിതി യൂണിറ്റ് 11) നടത്തി.

കംബ്ലീറ്റിലി റാൻഡമൈസഡ് ഡിസൈനിൽ 12 ട്രീറ്റ്മെന്റുകൾ 3 ആവർത്തനങ്ങളോടു കൂടിയാണ് പരീക്ഷണം നടത്തിയത്. ക്ലോർപൈറിഫോസിന്റെ നശീകരണത്തിനുവേണ്ടി ഭൌതികവും രാസപരവും ജൈവശാസ്ത്രപരവുമായ രീതികൾ പ്രയോഗിക്കുകയും അവയുടെ സ്വാധീനം വിലയിരുത്തുകയും ചെയ്തു. കണ്ട്രോൾ (T_1), ഹൈഡ്രജൻ പെറോക്സൈഡ്-5% (T_2), ഫെൻന്റോൺ റീയേജന്റെ-0.5% (T_3), ഹൈഡ്രജൻ പെറോക്സൈഡ്-5% + ഫെൻന്റോൺ റീയേജന്റെ-0.5% (T_4), സൂഡോമോണാസ് ഫ്ലൂറസെൻസ് (T_5), ട്രൈകോഡെർമ വിറിഡേ (T_6), സൂഡോമോണാസ് ഫ്ലൂറസെൻസ് + ട്രൈകോഡെർമ വിറിഡേ (T_7), സൂര്യപ്രകാശം - 6 മണിക്കൂർ (T_8), അൾട്രാ വയലറ്റ് - 4 മണിക്കൂർ (T_9), സൂര്യപ്രകാശം - 6 മണിക്കൂർ + അൾട്രാ വയലറ്റ് - 4 മണിക്കൂർ (T_{10}), 5 സെന്റീ മീറ്റർ വെള്ളത്തിനടിയിൽ പൂരിത അവസ്ഥയിലുള്ള മണ്ണ് (T_{11}), അസോള ഉപയോഗിച്ച് 5 സെന്റീ മീറ്റർ വെള്ളത്തിനടിയിൽ പൂരിത അവസ്ഥയിലുള്ള മണ്ണ് (T_{12}) എന്നിവയായിരുന്നു പഠനത്തിനായി ഉപയോഗിച്ച പരിചരണ മുറകൾ.

ഇൻകുബേഷൻ പഠനത്തിന്റെ ഫലങ്ങൾ വെളുപ്പെടുത്തിയത്, ഇൻകുബേഷൻ കാലയളവായ 60 ദിവസത്തിനുള്ളിൽ സൂഡോമോണാസ് ഫ്ലൂറസെൻസിന്റെയും ട്രൈകോഡെർമ വിറിഡേയുടെയും മിശ്രിതം ഉയർന്ന തോതിലുള്ള (74.99%) നശീകരണ നിരക്ക് കാണിച്ചു, തുടർന്ന് സൂഡോമോണാസ് ഫ്ലൂറസെൻസും (69.94%) ട്രൈകോഡെർമ വിറിഡേയും (66.35 %) ഉയർന്ന നിരക്ക് കാണിച്ചു. മണ്ണിന്റെ രാസഗുണങ്ങളിൽ ക്ലോർപൈറിഫോസ് പ്രയോഗത്തിനുശേഷം ട്രീറ്റ്മെൻറ്റിന്റെ സ്വാധീനം രണ്ടാഴ്ച ഇടവേളകളിൽ പഠിച്ചു. ഇൻകുബേഷൻ കാലയളവിൽ ഉടനീളം മണ്ണിന്റെ അമ്ലത്യം ഗണ്യമായി കുറഞ്ഞതായി കണ്ടെത്തി. ഏറ്റവും കൂടിയ അമ്ലത്യം (5.09), T_{10} (സൂര്യപ്രകാശം - 6 മണിക്കൂർ + അൾട്രാ വയലറ്റ് - 4 മണിക്കൂർ) രേഖപ്പെടുത്തിയപ്പോൾ ഏറ്റവും കുറവ് (4.93) T_4 (ഹൈഡ്രജൻ പെറോക്സൈഡ്-5% + ഫെൻന്റോൺ റീയേജന്റെ-0.5%) ലായിരുന്നു. മണ്ണിലെ ക്ളോറൈഡ് അയോണുകളിൽ ട്രീറ്റ്മെൻറ്റിന്റെ ഫലം പ്രധാന്യമില്ലാത്തതായി കണ്ടെത്തി. എന്നിരുന്നാലും ക്ളോറൈഡ് അയോണുകൾ ഇൻകുബേഷൻ കാലയളവിൽ തുടർച്ചയായി വർദ്ധിച്ചിരുന്നു. ആദ്യ ആഴ്ചകളിൽ ഫോസ്ഫേറ്റ് അയോണുകൾ ഗണ്യമായി കുറയുകയും പിന്നീട് ക്രമാനുഗതമായ വർദ്ധനവ് രേഖപ്പെടുത്തുകയും ചെയ്തു. എട്ടാമത്തെ ആഴ്ചയിൽ T_5 ഏറ്റവും കൂടുതൽ (31.80 മില്ലി ഗ്രാം /കിലോ ഗ്രാം) ഫോസ്ഫേറ്റ് അയോണുകളും T_1 ഏറ്റവും കുറവ് (28.86 മില്ലി ഗ്രാം /കിലോ ഗ്രാം) ഫോസ്ഫേറ്റ് അയോണുകളും രേഖപ്പെടുത്തി.

മണ്ണിന്റെ ജൈവിക ഗുണങ്ങളിൽ ക്ലോർപൈറിഫോസ് പ്രയോഗത്തിന്റെ പ്രഭാവം പഠിച്ചു. ക്ലോർപൈറിഫോസ് പ്രയോഗിച്ചതിനുശേഷം മണ്ണിന്റെ മൈക്രോബിയൽ ബയോമാസ് കാർബൺ, ഡീഹൈഡ്രോജിനേസ്, ഫോസ്ഫറ്റേസ്, യൂറിയേസ് എന്നിവയുടെ പ്രവർത്തനങ്ങൾ തടസ്സപ്പെടുന്നതായി കാണിച്ചു. എന്നാൽ പിന്നീട് പ്രവർത്തനം പുനസ്ഥാപിച്ചു. ജൈവശാസ്ത്രപരമായ രീതികൾ സ്വീകരിച്ച ട്രീറ്റ്മെൻറ്റികളിൽ ക്ലോർപൈറിഫോസിന്റെ ഉപയോഗം മണ്ണിന്റെ ജൈവിക ഗുണങ്ങളെ അധികം ബാധിച്ചിട്ടില്ല. ജൈവശാസ്ത്രപരമായ രീതികൾ സ്വീകരിച്ച ട്രീറ്റ്മെന്റ്കളായ T_5 ഏറ്റവും ഉയർന്ന ഡീഹൈഡ്രോജിനേസ് പ്രവർത്തനവും (11.74 µg TPF g^{-1} soil day^{-1}) T_6 ഉയർന്ന ഫോസ്ഫറ്റേസ് പ്രവർത്തനവും (17.06 µg PNP g^{-1} soil hr^{-1}) രേഖപ്പെടുത്തി. ട്രീറ്റ്മെന്റ് T_7 ഏറ്റവും ഉയർന്ന മൈക്രോബിയൽ ബയോമാസ് കാർബണും (99.15 µg g^{-1}) ഉയർന്ന യൂറിയേസ് പ്രവർത്തനവും (36.16 µg NH_4^+-N g^{-1} soil hr^{-1}) രേഖപ്പെടുത്തി.

ക്ലോർപൈറിഫോസിന്റെ നശീകരണത്തിൽ പൂരിത അവസ്ഥയിലുള്ള രണ്ട് ട്രീറ്റ്മെന്റ്കളിലും കാര്യമായ ഫലമുണ്ടായി. ക്ലോർപൈറിഫോസ് സംസ്കരിച്ച മണ്ണിൽ നിന്നുള്ള ലീച്ചേറ്റ് രണ്ടാഴ്ച ഇടവേളകളിൽ വിശകലനം ചെയ്തു. ആറാമത്തെയും എട്ടാമത്തെയും ആഴ്ചയിലെ ക്ലോർപൈറിഫോസ് അവശിഷ്ടങ്ങളുടെ ലീച്ചേറ്റിൽ ട്രീറ്റ്മെന്റ്കൾ കാര്യമായ സ്വാധീനം ചെലുത്തി. ഏറ്റവും കൂടുതൽ ക്ലോർപൈറിഫോസ് നശീകരണം രേഖപ്പെടുത്തിയത് ട്രീറ്റ്മെന്റ് T_{11} ആണ്, തുടർന്ന് ട്രീറ്റ്മെന്റ് T_{12} ഉം രേഖപ്പെടുത്തി. ആദ്യ ആഴ്ചകളിൽ അസോളയുടെ വളർച്ച സാധാരണ നിലയിൽ ആയിരുന്നു പിന്നീട് അസോളയുടെ അഴുകുൽ ശ്രദ്ധയിൽപ്പെട്ടു. ഇതിനു കാരണം അസോളയുടെ വളർച്ചയെ ക്ലോർപൈറിഫോസ് പ്രതികൂലമായി ബാധിക്കുന്നതാണ് .

സൂഡോമോണാസ് ഫ്ലൂറസെൻസിന്റെയും ട്രൈകോഡെർമ വിറിഡേയുടെയും മിശ്രിതത്തിന് ക്ലോർപൈറിഫോസ് അവശിഷ്ടങ്ങൾ മണ്ണിൽ നിന്ന് നീക്കം ചെയ്യാനുള്ള നല്ല കഴിവുണ്ടെന്ന് അന്വേക്ഷണത്തിലെ ഫലങ്ങളിൽ നിന്നും കണ്ടെത്തി. ജൈവശാസ്ത്രപരമായ രീതികൾ, ക്ലോർപൈറിഫോസിന്റെ നശീകരണത്തിൽ പ്രധാന പങ്ക് വഹിക്കുകയും മണ്ണിന്റെ ആരോഗ്യം നിലനിർത്തുകയും ചെയ്തു. ഈ കണ്ടെത്തലുകളുടെ അടിസ്ഥാനത്തിൽ, ചെങ്കല്ല് മണ്ണിലെ ക്ലോർപൈറിഫോസിന്റെ നശീകരണത്തിന് അനുയോജ്യമായ സമീപനം എന്ന നിലയിൽ, സംയുക്തമായോ ഒറ്റക്കോ ജൈവശാസ്ത്രപരമായ രീതികൾ ഉപയോഗിക്കാൻ നമുക്ക് ശുപാർശ ചെയ്യാം.

APPENDIX I

Carbonate in soil was found to be nil.

APPENDIX II

Date	Temperature		Relative humidity		Rainfall (mm)
	Max	Min	I	II	
07-12-2020	34	24.8	94	73	0
08-12-2020	34	23.5	91	64	1.1
09-12-2020	33	24	92	58	0
10-12-2020	33	23.5	96	61	0.2
11-12-2020	33	24	92	61	0
12-12-2020	34	24.5	92	72	0
13-12-2020	34	22	96	67	92
14-12-2020	34	23.5	88	67	0
15-12-2020	34	22	96	67	0
16-12-2020	34	22	91	67	0
17-12-2020	34	23.5	96	73	0
18-12-2020	34	23.5	96	61	0
19-12-2020	33	20.5	96	68	0
20-12-2020	34	20.8	91	48	0
21-12-2020	33	19	96	76	0
22-12-2020	32	20	91	76	0
23-12-2020	32	20	91	54	0
24-12-2020	34	23.5	91	61	0
25-12-2020	34	23.5	92	61	0
26-12-2020	34	22.5	96	61	0
27-12-2020	32	20	83	83	0
28-12-2020	32	19	92	64	0
29-12-2020	32	21.5	91	56	0
30-12-2020	32	21.5	100	73	0
31-12-2020	33	22.5	91	67	0
01-01-2021	32	23	91	61	0
02-01-2021	34	19.5	91	50	0
03-01-2021	33	24	91	57	0
04-01-2021	33	19	83	61	0
05-01-2021	33	23	96	67	22
06-01-2021	32	23	93	61	0
07-01-2021	32	22	96	79	20
08-01-2021	32	23	96	72	36.6
09-01-2021	32	23	91	70	1.4
10-01-2021	32	23	96	67	0
11-01-2021	32	23	91	66	0
12-01-2021	32	22.5	96	60	0
13-01-2021	33	22	91	59	0
14-01-2021	32	22	96	66	0
15-01-2021	31	24	88	57	0
16-01-2021	32	23	96	65	0
17-01-2021	32	23	96	67	0

18-01-2021	32	23	91	67	0
19-01-2021	31	22.5	96	61	0
20-01-2021	31	21	93	61	0
21-01-2021	34	21	96	61	0
22-01-2021	34	22	96	55	0
23-01-2021	34	24	88	61	0
24-01-2021	34	24	96	64	0
25-01-2021	34	22	96	67	0
26-01-2021	33	20.5	95	56	0
27-01-2021	33	20	91	66	0
28-01-2021	33	20	91	50	0
29-01-2021	32	20.5	91	50	0
30-01-2021	32	20.8	91	50	0
31-01-2021	32	23	92	57	0
01-02-2021	35	20	91	51	0
02-02-2021	33	20	91	59	0
03-02-2021	33	20	94	50	0
04-02-2021	33	22	91	58	0
05-02-2021	32	22.6	95	56	0
06-02-2021	33	21.3	94	64	0
07-02-2021	33	20.9	94	46	0
08-02-2021	33	18	95	32	0
09-02-2021	33	18.2	90	37	0
10-02-2021	33	18.4	91	58	0
11-02-2021	33	19.5	91	61	0
12-02-2021	33	21	93	61	0
13-02-2021	33	22	91	58	0
14-02-2021	33	22	91	64	0
15-02-2021	33	22.3	96	58	0
16-02-2021	33	22	96	58	0
17-02-2021	32	21.5	96	51	0
18-02-2021	33	21	91	53	0
19-02-2021	33	22.5	87	56	0
20-02-2021	33	21	64	61	0
21-02-2021	33	21	91	58	5.8
22-02-2021	33	21.5	87	61	6
23-02-2021	33	21	91	59	0
24-02-2021	34	23	91	56	0
25-02-2021	33	19	92	58	0
26-02-2021	33	22.5	92	64	0
27-02-2021	33	25	92	67	0
28-02-2021	32	25	92	67	0
01-03-2021	33	25	92	64	0
02-03-2021	33	23.5	97	59	0
03-03-2021	34	23	91	67	0
04-03-2021	34	22	92	68	0

05-03-2021	34	21	91	61	0
06-03-2021	37	22.5	87	64	0
07-03-2021	36	23	84	62	0
08-03-2021	35	24.3	93	62	0
09-03-2021	35	24	92	55	0
10-03-2021	34	24.5	88	59	0
11-03-2021	34	24	91	56	0
12-03-2021	34	23.5	89	61	0
13-03-2021	34	24	92	67	0
14-03-2021	34.5	27	85	57	0
15-03-2021	34	24.5	92	59	0
16-03-2021	34	24.5	88	62	0
17-03-2021	34	24	88	62	0
18-03-2021	34	24.5	88	62	0
19-03-2021	34.5	25	88	77	0
20-03-2021	34	25	88	67	0
21-03-2021	34.2	22.8	89	59	1.8
22-03-2021	34	22.8	90	57	0
23-03-2021	34	24.7	88	61	0
24-03-2021	34.5	25.5	85	62	0
25-03-2021	34.7	25.5	88	71	0
26-03-2021	33.8	25.5	90	71	0
27-03-2021	34.6	25.5	88	68	0
28-03-2021	34.5	26	81	68	0
29-03-2021	34.5	26	85	65	0
30-03-2021	34.5	25	51	65	0
31-03-2021	34.5	25	88	62	0
01-04-2021	34	27	88	62	0
02-04-2021	35	24	92	62	0
03-04-2021	33.5	24	92	68	0
04-04-2021	34	24	84	57	0
05-04-2021	35	24	92	73	0
06-04-2021	34	23.5	81	66	0
07-04-2021	34.5	22	75	79	0
08-04-2021	34	28	85	79	0
09-04-2021	34	25	88	63	0
10-04-2021	35	25	88	63	0
11-04-2021	35	28	78	57	0
12-04-2021	35	25	84	57	0
13-04-2021	34.5	24	96	60	0