# Effect of Elevated $CO_2$ and Insecticide Chloropyriphos on the Nitrification Potential of Rhizospheric Soil of Wheat

THESIS

*Submitted to the*

**Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya,**
**Gwalior (M.P.)**

**In partial fulfillment of the requirements for the degree of**

**MASTER OF SCIENCE**

***In***

**AGRICULTURE**

**(ENVIRONMENTAL SCIENCES)**

*By*

**Bhoopendra Singh**

**Department of Environmental Sciences**
**Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya**
**College of Agriculture, Gwalior (M.P)**

**2019**

# CERTIFICATE - I

This is to certify that the thesis entitled **“Effect of elevated $CO_2$ and insecticide chloropyriphos on the nitrification potential of rhizospheric soil of wheat”** submitted in partial fulfillment of the requirement for the degree of **Master of Science in Agriculture (Environmental sciences)** of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of bonafide research work carried out by **Mr. Bhoopendra Singh ID No 923/2009** under my guidance and supervision. The subject of the thesis has been approved by the students Advisory Committee and the Director of instruction.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc) or has been published / published part has been fully acknowledgement. All the assistance and help received during the course of the investigation has been acknowledged by the scholar.

**Dr. Amita Sharma**
(Major advisor and chairman)

**Dr. K. Bharati**
(Co Advisor, IISS, Bhopal)

## THESIS APPROVED BY THE STUDENTS ADVISORY COMMITTEE

Chairman : (Dr. Amita Sharma) …………………………………..

Co Advisor : (Dr. Kollah Bharati) ………………………………….

Member : (Dr. V. B. Singh) ………………………………….

Member : (Dr. Shashi Yadav ) …………………………………..

# CERTIFICATE – II

This is to certify that the thesis entitled **“Effect of elevated $CO_2$ and insecticide chloropyriphos on the nitrification potential of rhizospheric soil of wheat”** submitted by **Mr. Bhoopendra Singh ID No. 923/2009** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfillment of the requirement of **Master Of Science In Agriculture in the Department of Environmental Sciences, College of Agriculture, Gwalior** has been after evaluation approved by the external examiners and by the students committees after an oral examination on the same.

Place : Gwalior

Date : ……………

| | |
|---|---|
| **Dr. Amita Sharma** | **Dr. K. Bharati** |
| (Major advisor and chairman) | (Co Advisor, IISS, Bhopal) |

**MEMBER OF THE STUDENT’S ADVISORY COMMITTEE**

Chairman : (Dr, Amita Sharma) ……………………………………………

Co Advisor : (Dr. Kollah Bharati) ……………………………………………

Member : (Dr. V. B. Singh ) ……………………………………………

Member : (Dr. Shashi Yadav) …………………………………………….

Head of the Department/ Section : ……………………………………………

Dean : ……………………………………………

Director of Instructions : ……………………………………………

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद
Agrisearch with a human touch

ICAR - Indian Institute of Soil Science
Indian Council of Agricultural Research
www.iiss.nic.in

# Certificate

It is certified that the thesis entitled "Effect of elevated $CO_2$ and insecticide chloropyriphos on the nitrification potential of rhizospheric soil of wheat" which is being submitted by Mr. Bhoopendra Singh in partial fulfillment of the requirement for the award of the Degree of Master of Science in Agriculture (Environmental sciences) of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, MP is a record of candidate's own work carried out by him under my supervision and guidance from July 17-June 2018. The matter embodied in this report has not been submitted for award for any other degree.

**Dr. K. Bharati**
(Principal Scientist)
ICAR Indian Institute of Soil Science
Bhopal

Dr K Bharati, Principal scientist, ICAR- Indian Institute of Soil Science, Nabibagh, Berasia Road, Bhopal, 462038, Email –bharatik1@gmail.com, Phone – 0755-2730970 ext 318

# ACKNOWLEDGEMENT

*I humbly bow my head before the Lord for blessing me with the power to complete this endeavour successfully. I feel highly privileged to the Chairman of my Advisory Committee* ***Dr.Amita Sharma****, Department of Environmental sciences, College of Agriculture, Gwalior for his precious guidance, keen interest and encouragement during the course of my research investigation.*

*I am indebted to co-advisor of my advisory committee****Dr. Kollah Bharati****,* ***Senior Scientist,*** *Indian Institute of Soil Science, Bhopal, M.P. for her inspiring guidance, support, constructive criticisms and constant encouragement. Without her help, it would not have been possible to complete this work.*

*I have great pleasure in expressing my sincere thanks to* ***Dr. S.R. Mohanty****,* ***Senior Scientist****, Indian Institute of Soil Science, Bhopal, M.P. without whose benevolent guidance and constant motivation, it would not have been possible to complete this project. His very affectionate behaviour always filled me with vitality and enthusiasm.*

*I am highly grateful to* ***Dr. S.K. Trivedi****,* ***H.O.D****,Department of Environmental Science, RVSKVV, College of Agriculture, Gwalior for his valuable guidance and co-operation.*

*I have great pleasure in expressing my sincere thanks to* ***Dr. Ashok K. Patra****,* ***Director of Indian Institute of Soil Science*** *for giving me this opportunity and allowing me to work under Dr. Kollah Bharati in Institute.*

*I am deeply obliged to all the members of my advisory committee namely,* ***Dr. V. B. Singh****,* ***HOD and Professor****, Department of Agricultural Statistics for their valuable guidance and timely help during the course of this investigation****.***

*I am highly grateful to Senior Research Scholar at I.I.S.S.* ***Dr. Usha Ahirwar*** *and* ***Dr. Garima Dubey*** *for their kind support in my research. I am indebted to them for their special interest and valuable suggestions regarding the present work. It is them, who encouraged me by always being behind me since this work's inception. I will always remember them for their encouragement and invaluable guidance.*

*I am extremely thankful to* ***Dr. Anuradha goel, Asstt. Prof.*** *and* ***Dr. Kiran divediAsstt. Prof.****, Department of Envronmental Science, RVSKVV, College of Agriculture ,Gwalior for providing help, guidance and valuable suggestions from time to time, during the course of investigation.*

*I would like to thank to****Manoj Parihar (scientist)*** *for supporting my endeavours and for encouraging me to do better.*

*Thanks are due to* ***Bhujbal Ahirwar****, for his help in lab which made my work easier for providing all the facilities throughout the study. He is always ready to help with a smile.*

*It gives me immense pleasure to recall the emotional support and strength given by my friends****Mr. Rajbeer TomarMr. Abhishek Yadav, Dr. Vikas Mandloi, Dr. Roopsingh Dangi, Mr. Mahesh Nagar, Mr. Raghvendra Chturvedi, Dr. Dharmendra Tomar, Mr.Rahul Tomar, Mr.Pravendra Kushwah and Mr. Dharmendra Meena*** *who also shared my happiness and grievances with equal interest and importance.*

*I must mention that without the unbounding affection, encouragement and blessing of my Family is namely, my father****Shri Gourishankar Singh Tomar,*** *my mother* ***Smt. Devki Devi Tomar,*** *my brother* ***Mr. Munendra Singh Tomar*** *and my sister- in- law* ***Smt. Priyanka Tomar*** *whose constant inspirations devotion, blessing brought me to success.*

*Many a good soul have had helped me knowingly and unknowingly in this work. For all those individuals I convey my grateful thanks.*

**Place:** Gwalior

***Date:*** ***(BHOOPENDRA SINGH)***

# List of Contents

| **S. No.** | **Contents** | **Page** |
|---|---|---|
| 1 | Introduction | 1-2 |
| 2 | Review of literature | 3-12 |
| 3 | Materials and methods | 13-17 |
| 4 | Results | 18-26 |
| 5 | Discussion, Conclusive Remarks & Summary | 27-32 |
| 6 | References | 33-40 |
|  | VITA | 41 |

# List of Tables and Figures

| Content | S. No | Title | Page |
|---|---|---|---|
| **Table** | 1 | Apparent nitrification rate, abundance of 16S rRNA gene copies of eubacteria, amoA gene copies of nitrifying bacteria. | 21 |
| | 2 | Wheat growth parameters under the influence of $CO_2$, biochar and chloropyriphos. | 23 |
| | 3 | Analysis of variance of the factors and variables to determine interaction effect of $CO_2$, biochar and chloropyriphos on apparent nitrification rate, wheat shoot length, root length, population of eubacteria (16S rRNA gene copies), and ammonia oxidizing bacteria (AOB) in soil | 25 |
| **Figure** | 1 | Influence of $CO_2$, biochar (BC) and chloropyriphos (Chl) on nitrification | 19 |
| | 2 | Linear regression models ($\alpha$ 0.05) of nitrification and different parameters of the study. The parameters were fresh weight of shoot, fresh weight of root, abundance of 16S rRNA gene of eubacteria, and abundance of amoA genes of ammonia oxidizing bacteria | 26 |

# LIST OF SYMBOLS AND ABBREVIATIONS

| **Symbol** | **Legend** |
|---|---|
| AOB | Ammonia Oxidizing Bacteria |
| AOA | Ammonia Oxidizing Archaea |
| AMO/amoA | Ammonia monooxygenase subunit A |
| A | Alfa |
| $NH_4^+$ | Ammonium |
| & | And |
| *et al.* | And others |
| Etc | and the rest |
| B | Beta |
| Cm | Centimeter |
| $^oC$ | Degree Celsius |
| DNA | Deoxyribo Nucleic Acid |
| DAS | Days after sowing |
| D.F. | Degree of Freedom |
| $\Delta$ | Delta |
| $N_2$ | Dinitrogen |
| EC | Electrical Conductivity |
| Fig. | Figure |
| $Fe^{3+}$ | Ferric ion |
| $Fe^{2+}$ | Ferrous ion |
| G | Gram |
| Γ | Gamma |
| H | Hours |
| HSD | Honestly Significant Difference |
| Kg $ha^{-1}$ | Kilogram per hectare |
| L | Litre |
| Mean Sq | Mean sum of square |
| $Mn^{2+}$ | Manganese ion |
| Mg | Milligram |
| M | Molar |
| mM | Millimolar |

| | |
|---|---|
| μM | Micromolar |
| $\mu g\ g^{-1}$ | Microgram per gram |
| N | Nitrogen |
| NO | Nitric oxide |
| $N_2O$ | Nitrous oxide |
| $NO_3$ | Nitrate |
| pH | Power of hydrogen |
| PNR | Potential Nitrification Rate |
| % | Percent |
| ± | Plus or Minus |
| 16S rRNA | 16S ribosomal Ribo Nucleic Acid |
| Ks | Rate Constant |
| Rpm | rounds per minute |
| R.V.S.K.V.V. | Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya |
| Sum Sq | Sum of Square |
| $SO_4^{2-}$ | Sulfate ion |
| $S^{2-}$ | Sulfide ion |

# CHAPTER - I

# INTRODUCTION

There are three major greenhouse gases (GHG) in the atmosphere viz; $CO_2$, $CH_4$ and $N_2O$. Among these three greenhouse gasses $CO_2$ is increasing rapidly. The atmospheric $CO_2$ concentration is increasing at 3% per year and it is predicted that the concentration of $CO_2$ will reach 550–700 ppm by 2050 and 650–1200 ppm by 2100 (Higgins *et al.,* 2015). The rise in atmospheric GHGs will increase the mean global temperature up to 2.5 °C by 2050 and of up to 6.4 °C by the end of this century (IPCC, 2007). Inter Governmental Panel on Climate Change (IPCC) has reported that there will be 10-40% loss in crop production in India along with other countries in South Asia by the year 2080-2100. India could lose 4-5 million tons of wheat production with every 1ºC temperature rise. Simulation models predict that with rising atmospheric GHG there will be adverse impact on the crop production across the world.

Elevated $CO_2$ and temperature are also likely to increase the diversity of pests due to high moisture in the atmosphere. Therefore, climate change will attract intensive use of pesticide in agriculture (Yan *et al.,* 2017). One of the most commonly used insecticide is chloropyriphos (0,0-diethyl-3,5,6-trichloro-2-pyridylphosphorothioate) (Gomez, 2009). Chloropyriphos is broad-spectrum in nature and works effectively against many insects including mosquitoes (larvae and adults), flies, and ecto-parasite of cattle and sheep (Kumar[1] and Kumar, 2007; Liu *et al.,* 2005). Chloropyriphos negatively impact many soil biological properties. The persistence of chloropyriphos in agricultural soil increases with elevated $CO_2$ and temperature (Ahirwar *et al.* 2018). Use of chloropyriphos in future climate may affect soil biological function severely due to longer persistence. Therefore, it is important to assess the impact of climate change and chloropyriphos on soil biological attributes that are important for agricultural point of view.

Biochar is recommended to improve carbon sequestration and mitigate GHG emission. It can enhance plant growth by improving nutrient use efficiency (Kollah *et al.,* 2015). Biochar is prepared by pyrolysis of organic feedstock or biomass (Lehmann, 2007). It has very high cataion exchange capacity (CEC) enabling it to absorb $NH_4$-N and other plant nutrients (Subedi *et al.,* 2013). Biochar adsorbs insecticides and reduce toxicity to the plants (Diez *et al.,* 2013). Biochar stimulates insecticide biodegradation by stimulating heterotrophic microbial groups (Atkinson *et al.,* 2010).

As discussed above climate change will intensify the use of pesticides and biochar in agriculture. Therefore, there is need of urgent attention to study the interactive effect of climate factors, chloropyriphos and biochar on soil biological functions. Nitrification is one of the important soil biological functions in the prospect of plant nutrition, climate change and global nitrogen balance. Nitrification is a two-step process performed by two distinct microbial groups (Alfreider *et al.,* 2017). The first microbial group oxidize $NH_4$ to $NO_2$, and the second oxidize $NO_2$ to $NO_3$ (Weber *et al.,* 2015). Wheat is taken as a model plant for this study as it is cultivated widely in the temperate and tropical countries. Moreover this crop requires intensive input of N fertilizer and insecticide. Experiment was conducted to evaluate the influence of elevated $CO_2$, chloropyriphos and biochar on nitrification and soil microorganisms in the rhizosphere of wheat grown in the vertisols.

## Objectives of the proposed work

1. Evaluation of nitrification potential of rhizospheric soil of wheat under the influence of elevated $CO_2$ and chloropyriphos
2. Effect of elevated $CO_2$ and chloropyriphos on soil microbial abundance in relation to nitrification

# CHAPTER - II

# REVIEW OF LITERATURE

In this chapter, an attempt has been made to bring out review for the topic of interest **"Effect of elevated $CO_2$ and insecticide chloropyriphos on the nitrification potential of rhizospheric soil of wheat"**. A brief resume of the work done in the past by various researchers is cited under appropriate heads.

Climate change seems to be the most threatening environmental problem of the 21st century. The Intergovernmental Panel on Climate Change (IPCC, 2007) in its fourth assessment report in 1997 indicated that global average surface temperature will increase by 1.1–6.0 ºC in this century. During the last 12 years, the average rate of increase in $CO_2$ level is 1.9 μL $L^{-1}$ $years^{-1}$ (Krull *et al.,* 2005) and the IPCC projection for atmospheric $CO_2$ concentration by 2050 is in the range of 463–623 ppm (McCarthy, 2001). The consequence of this rise in $CO_2$, is a rise in earth's average temperature from 2.0°C to 4.5°C (IPCC, 2007).

The change in climatic conditions are favorable for the proliferation of insect pests due to high temperature and humidity. Increase in pest population will bring greater use of chemical pesticides. Chloropyriphos is one of the most prominently used Organophosphorus pesticide in the world. The intrinsic toxic properties of this pesticide allows to control agricultural insect and pests. The annual average global use of chloropyriphos between 2002 and 2006 was 25 million kg active ingredient, of which 98.5% was used for agricultural purposes. Estimated annual U.S. domestic use of chloropyriphos for all purposes was between 13 and 19 million of active ingredient pounds, with 8 and 10 million pounds used for agricultural purposes (Epstein and Zhang, 2014). Chloropyriphos is used broadly in fruits, vegetables, tobacco, cotton, and corns (United States Environmental Protection Agency) (US) and Water, 2002).Chloropyriphos is registered for application on 13 crops by CIBRC (Centeral Insecticide Board & Registration Committee).

## 2.1 Microbial degradation

Various techniques are available that can be used for the detoxification of chloropyriphos pesticides, however, microbial mediated biodegradation which is considered to be one of the most viable options for the removal of organophosphate pesticides from the environment. Recently, the use of genetically modified microorganisms has increased the chances for in-situ bioremediation of contaminated sites. This literature stabilized a link that the biodegradation process can be enhanced by maintaining an effective chloropyriphos-degrading microbial community in the contaminated site and optimizing environmental conditions (Chishti *et al.,* 2013). The bacterial communities have the genes required for degrading pollutants more efficiently in various environmental conditions over a fairly extensive range of pH, temperature, $CO_2$ and various levels of pesticide concentrations. Initially *Flavobacterium* sp. and the *Arthrobacter* sp. were used in chloropyriphos degradation. Both bacterial isolates have the potential to mineralize chloropyriphos in mineral salt medium (Mallick *et al.,* 1999). Subsequently, various other groups are isolated from agriculture soil. The *Enterobacter strain B-14* are one of them that degrade chloropyriphos more efficiently as compared to other bacterial species (Singh *et al.,* 2004). A strain of *Escherichia coli* was developed by cloning of *mpd* gene from chloropyriphos degrading bacterial strains that enhanced the degradation capacity of microbes (Dhanya, 2014), for use in bioremediation of chloropyriphos contaminated soils. *Bacillus sp w*ere reported to show maximum chloropyriphos degradation by metabolizing its first hydrolysis metabolite 3,5,6-trichloro-2-pyridinol (TCP) up to 90% (300 mg $L^{-1}$) within 8 days of incubation (Anwar *et al.,* 2009). A *Bacillus licheniformis* strain degraded chloropyriphos approximately99% (100 mg $kg^{-1}$) after 14 days (Zhu *et al.,* 2010). In the liquid medium *Bacillus sp* showed higher degradation potential. *Bacillus cereus* isolated from a Chinese soil degraded chloropyriphos up to 100 mg $L^{-1}$, (Liu *et al.,* 2012). *Bacillus firmus* isolated from coral surface of the sea, utilized chloropyriphos up to 25 mg $L^{-1}$(Sabdono, 2013). El-Helow *et al.,* 2013 reported another *Bacillus subtilis Y242* strain isolated from agriculture waste water which showed higher degradation potential in liquid medium with 150 $mgL^{-1}$chloropyriphos degradation (95.12%), recorded within 48 h.

*Pseudomonas sp* possess a series of catabolic enzymes and pathways. These bacteria degrade chlorpyrifo into TCP and DETP and finally to non-toxic metabolites as $CO_2$, $H_2O$, and $NH_3$.*Pseudomonas putida* MAS-1 degrade chloropyriphos up to 90% in 24 h (Gilani *et al.,* 2016). In the rhizosphere *Pseudomonas nitroreducens* PS-2 and *Pseudomonas aeruginosa degrade chloropyriphos*up to 50 mg/l, in 28 days (Fulekar and Geetha, 2008; Korade and Fulekar, 2009). Other *Pseudomonas sp* such as *Pseudomonas putida (NII 1117),Pseudomonas stutzeri (NII 1119), Pseudomonas aeruginosa (NII 1120)* degrade up to 500 mg $L^{-1}$ of chloropyriphos and produced metabolites chlopyrifosoxon and Diethylphosphorothioate (C. Sasikala *et al.,* 2012). As a form of consortium *Pseudomonas fluorescence*, *Brucella melitensis*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella* species, *Serratia marcescens* and *Pseudomonas aeroginosa*, showed 75–87% degradation of chloropyriphos(Lakshmi *et al.,* 2008). Some *Pseudomonas strains,* isolated from contaminated soil of Egypt like *Pseudomonas stuzeri, Pseudomonaspseudoalcaligenes, Pseudomonas maltophila* and *Pseudomonas vesicularis,* had the chlorphyrifos degrading ability up to 100-300 $mgL^{-1}$. Among these strains *Pseudomonas stuzeri* was the most potent degrader (Awad *et al.,* 2011). In a study four bacterial consortium were used *including Pseudomonas putida* (NII 1117), *Klebsiella sp*., (NII 1118), *Pseudomonas stutzeri* (NII 1119), *Pseudomonas aeruginosa* (NII 1120) to degrade chloropyriphos up to 500 $mgL^{-1}$ and produce metabolites chlopyrifos-oxon and Diethylphosphorothioate (C. Sasikala *et al.,* 2012). *Pseudomonas putida* was able to show 76% degradation of chloropyriphos(Vijayalakshmi and Usha, 2012).*Pseudomonas* has the potential to degrade chloropyriphos from 25 to 200 mg chloropyriphos/L, and that of TCP from 25 to 100 mg/L. In addition, the kinetic constants (Ks and Vmax) were also calculated. The Ks (half saturation constant) was found to be in the range of 97–142.3 mg/L and Vmax (maximum rate of CP degradation) ranged from 7.4 to 12.1 mg/L/d (Maya *et al.,* 2011).

Other studies also supported the chloropyriphos degradation efficiency of *Pseudomonas fluorescens*, *Bacillus subtilis, Klebsiella sp* and *Klebsiella sp.* (KaviKarunya, 2012). A bacterial consortium obtained by selective enrichment from highly contaminated soils in Moravia (Medellin, Colombia). The consortium was able to degrade 150 mg $L^{-1}$ of chloropyriphos in 120 h. The consortium was identified as

*Acinetobacter* sp, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Stenotrophomonas* sp, *Flavobacterium* sp, *Bacillus* sp, *Proteus vulgaris*, *Pseudomonas* sp, *Acinetobacter* sp, *Klebsiella* sp and *Proteus* sp (Pino and Peñuela, 2011).

A bacterial strain *Stenotrophomonas maltophilia* MHF ENV20 isolated from rhizosperic soil showed the higher degradation potential of chloropyriphos(Dubey and Fulekar, 2012). *Paracoccus sp* TRP isolated from activated sludge sample, degrades 100% of chloropyriphos in 4 days while *Serratia sp* is able to degrade the same in 18 h (Xu *et al.,* 2007, 2008). *Stenotrophomonas sp. YC-1* and *Sphingomonas sp* Dsp-2 isolated from a waste water effluent of a pesticide manufacturing unit are equally efficient in 100% degradation of chloropyriphos within 24 h (Li *et al.,* 2007; Yang et al., 2006). A very efficient *Ralstonia sp* T6 isolated from rhizospheric soil metabolized 100 mg/L TCP in 12 h and 700 mg/L in 80h (Zhu *et al.*, 2010). As a novel strain *Sphingobacterium sp* was able to grow in minimal salt medium (MSM) supplemented with 300 mg $L^{-1}$ of chloropyriphos as sole carbon source(Abraham and Silambarasan, 2013).

*Cupriavidus* strain degrades chloropyriphos and its intermediate metabolite (TCP) and uses these compounds as a sole carbon source. The mpd gene, encoding the enzyme responsible for chloropyriphos hydrolysis to TCP, was cloned and expressed in *Escherichia coli* BL21. *Cupriavidus sp* DT-1 inoculated to the chloropyriphos site which degraded chloropyriphos and TCP of 100% and 94.3%, respectively as compared in un-inoculated soil (Cao et al., 2012; Lu *et al.*, 2013). A chloropyriphos and TCP degrading bacterial strain, *Mesorhizobium* sp. HN3, was isolated and characterized. *Mesorhizobium* sp. HN3 degraded chloropyriphos efficiently up to 400 mg/$L^{-1}$. Subsequently *Mesorhizobium* sp. HN3 converted chloropyriphos into diethylthiophosphate and TCP which was further converted in 3,5,6-trichloro-2-methoxypyridine (Jabeen *et al.*, 2015).

Abraham and Silambarasan, 2016 isolated a bacterial strain *Ochrobactrum* sp. JAS2. The JAS2 strain had the capacity to degrade chloropyriphos upto 300 mg $l^{-1}$ within 12 h of incubation in the liquid medium and it produced the TCP metabolite. However, after 72 h of incubation TCP was also completely degraded by the JAS2 strain. The bacterial consortium consisted of *Staphylococcus warneri* CPI 2*, Pseudomonas putida* CPI 9 and *Stenotrophomonas maltophilia* CPI 15 degraded chloropyriphos more efficiently by the use of a Box–Behnken design it was noticed that this strain was capable of degrading 90% of chloropyriphos (125 ppm) in 8 days of incubation (John *et al*., 2016). A lactobacillus strain isolated from rice straw, showed high degradation in rice straw inoculated with *Lactobacillus casei* WYS3. These strain was shown to bind chloropyriphos up to 33.3-42% and detoxified chloropyriphos via P-O-C cleavage (Wang *et al.*, 2016).

Some biosurfactants are produced by microbes which increase biodegradation rate of chloropyriphos by improving bioavailability of the xenobiotic compound. This causes complete detoxifications of pesticide and minimizes the accumulation of its metabolities. Chloropyriphos degradation was checked in batch as well as continuous bioreactors. It was found that *Pseudomonas* (Iso 1) sp degrade chloropyriphos very efficiently up to 500 mg $L^{-1}$ in bioreactors(Yadav *et al.,* 2014). A bacterial strain of *Acinetobacter calcoaceticus isolated from rhizosphere* of chives contaminated with chloropyriphos. It was found that the strain is capable of utilizing chloropyriphos as the sole source at initial concentration of 100 mg $L^{-1}$ (60.0%). It also showed high degradation capability in sterilized soil, and the degradation reached up to 60.2% after 18 days. A recent study of Singh et al., 2016 used a crude rhamnolipid biosurfactant, which increased the degradation of chloropyriphos with the combination of mix bacterial population. Five potential bacteria belonging to genus *Pseudomonas*, *Klebsiella*, *Stenotrophomonas*, *Ochrobactrum* and *Bacillus* and their mixed culture efficiently degrade chloropyriphos and its intermediate metabolites, TCP and DETP in aqueous medium. These studies showed that there is wide diversity of bacterial species in soil capable of degrading chloropyriphos at varying efficiencies using an array of metabolic pathways.

Fungi has been used as an important biotic component for xenobiotics degradation. In a study the mineralization of chloropyriphos was estimated by introducing *Phanerochaete chrysosporium. This fungal strain degraded chloropyriphos up to*27.5% in 18 d (Bumpus *et al.*, 1993). The fungal strains of *Aspergillus niger* and *Trichoderma viride* were used for the degradation of chloropyriphos. The fungal strain *T. viride* degraded chloropyriphos up to 95.7% as compared to *A. niger* 72.3% in 14d (Mukherjee and Gopal, 1996).

Biodegradation and biotransformation of chloropyriphos is an important phenomenon which is performed by actinomycetes. These groups belong to gram-positive bacteria. Actinomycetes have some stress protein, which help in the survival in extreme condition (Praseetha *et al.*, 2012). Many actinomycetes can degrade different pollutants, including several pesticides. A review of Schrijver and Mot, 1999 showed that the genera *Arthrobacter, Clavibacter, Nocardia, Rhodococcus, Nocardioides,* and *Streptomyces* behave as pesticide degrading Actinomycetes. The metabolic pathway for pesticide degradation by actinomycetes has not been studied extensively; however, it is known that these microorganisms can produce extracellular enzymes that degrade a wide range of complex organic compounds. The presence of monooxygenases and dioxygenases in actinomycetes make this group stronger biodegrade (Larkin *et al.*, 2005). Most recent studies have isolated and characterized different actinomycetes capable of degrading pesticides and many strains of this group are proposed to be used for soil decontamination.

## 2.2 Factors affecting chloropyriphos degradation

Chloropyriphos degradation mainly depends on initial concentration at wide range of temperatures (30–40°C) and pH (6.0–8.0). A laboratory experiment was conducted to study the degradation of 10 and 50 mg $kg^{-1}$ of Chloropyriphos in soil. Degradation rate is indirectly proportional to concentration of Chloropyriphos. Concentration has the greatest effect on the degradation of Chloropyriphos to TCP. The highest concentration of Chloropyriphos applied (1,000 ppm) has the highest amount of Chloropyriphos remaining and the lowest relative percentage of TCP formed. Chloropyriphos degradation was found to be stable in the laboratory condition for a period of one year (Cink and Coats, 1993; Singh *et al.*, 2005; Vischetti *et al.,* 2008). It has been observed that the degradation efficiency of bacteria at higher initial concentration decreases with initial concentration up to 15 to 400 mg/L, respectively.

pH is an important phenomenon which affects the pesticides adsorption rate by increasing or decreasing hydrogen ion concentration. It influences the sorptive behavior of pesticide molecule on clay and organic surfaces. Rate of degradation of Chloropyriphos under different pH has been measured in many studies. According to Singh *et al*., 2003, the kinetics of degradation in five United Kingdom soils varying in pH from 4.7 to 8.4 suggested that dissipation of Chloropyriphos was mediated by the co-metabolic activities of the soil microorganisms. Soils with a pH more than 6.7 are able to maintain this degrading ability even after 90 days after inoculation. Complete degradation of Chloropyriphos was observed at pH 8.0 by *Alcaligenes faecalis* strain (Yang *et al*., 2005).

The effect of temperature on pesticide degradation mainly depends up on the molecular structure of the pesticide. Generally temperature affects adsorption of pesticide, by altering the solubility and hydrolysis of pesticides in soil (Chowdhury *et al.*, 2008). Generally the adsorption process are exothermic and desorption processes are endothermic. It is expected that adsorption will reduce with increase in temperature with a corresponding increase in pesticide solubility (Gebremariam *et al*., 2012). Microbial activity is largely affected by increase in temperature and some ecological groups tend to dominate within certain temperature ranges. The optimal growth and activity of microorganisms in soils occur at a temperature between 25–35 $^0$C(Niti *et al.*, 2013) and the optimal pesticide degradation temperature range is 25–40 $^0$C.

The $CO_2$ concentration is increasing day by day and may be doubled by the 2025. Several theoretical models have predicted that the mean global air temperature will increase 3 to 4 °C with a doubling of the $CO_2$concentration (IPCC, 2007). The rise in $CO_2$ concentration will lead to the rise of atmospheric temperature that may cause various natural disasters. Effect of elevated $CO_2$ has been studied on nitrogenase activity of an alluvial and a laterite rice soil of tropical origin. Soil samples were incubated at ambient (370 µmolmol$^{-1}$) and elevated (600 µmolmol$^{-1}$) $CO_2$concentration at 25ºC, 35ºC, and 45ºC under non-flooded and flooded conditions for 60 days. Elevated $CO_2$ showed significant increase in nitrogenase activities and readily mineralizable C in both alluvial and laterite soils (Das *et al.*, 2011).

Soil moisture generally affect mineralization process of pesticides. The highest percent of mineralization occurred in soil maintained near field capacity (0.30 bar) while the lowest percentage occurred in soil maintained under the driest condition (3.0 bar) (Cink and Coats, 1993). Singh *et al*., 2006 studied the affect of different moisture regime on chloropyriphos degradation. The moisture regimes were adjusted to 20%, 30%, 40%, 50% or 60%. Higher degradation rate of chloropyriphos was observed with influence of moisture. The degradation data at a single soil moisture content (at 60% MWH) was studied by Baskaran *et al.*, 1999 and rapid degradation rate of chloropyriphos was noticed. Effect of pre-incubation and water-holding capacity (WHC) on the biodegradation of chloropyriphos in biomix–andisol, peat and straw has been studied (1:1:2). Data concluded that the biomix, pre-incubated for 15 days with 60% WHC, degraded chloropyriphos efficiently, but was quite slow with 80% WHC (water holding capacity). Higher moisture levels in the soil increases the solubilization of pesticides but is likely to reduce the availability of oxygen.

## 2.3 Biochar in agriculture

Biochar is a type of heterogeneous material produced through pyrolysis, i.e. a thermal process carried out at temperatures ranging from 200 °C to 900 °C and under limited range of oxygen availability, of a wide range of crop residues (Yuan *et al.*, 2011), wood (Spokas and Reicosky, 2009), municipal waste (Mitchell *et al.*, 2013), sewage sludge (Méndez *et al*., 2012), manure (Uzoma *et al.*, 2011), and sometimes it also produced from animal bones (Vassilev *et al.*, 2013). Biochar is basically distinguished from charcoal by its final end use: agriculture and environmental management in the first case, fuel and energy in the latter (Lehmann *et al.*, 2009).

The use of biochar could contribute to the increase of pesticides sorption on soil, decreasing its mobility and reducing the contamination risks of surface and ground waters. The knowledge of biochar properties basically depends upon the feedstock and pyrolysis conditions of biochar.

The effect of biochar on pesticide desorption have been reported by various studies. The first study was done by Yu *et al.*, 2006 in which he described the role of biochar on diuran pesticide desorption in sandy loam soil which was amended by different rates of biochar produced at 450 and 850 ºC. The results showed that sorption irreversibility was greater in the soil amended with the highest rate of BC (850 ºC), which was attributed to the higher micro-porosity of this amendment as compared to BC (450 ºC).

## 2.4 Chloropyriphos and soil enzymatical activities

Soil enzymatical activities are affected in a complex manner due to change in the climatic conditions, due to short-term or long-term weather conditions, and these changes are quite difficult to predict. For example, increase and decrease in global temperature, moisture regimes and elevated $CO_2$ lead to change in the rate of enzymatically-catalysed reactions that affect the soil enzymatical activities (Baldrian *et al.*, 2013). The biological status of the enzymes in the soil are negatively affected with chloropyriphos pesticides. The effect of pesticides on activity of enzymes in soil are not clear due to their multidimensional behavior and its interaction with soil flora. Chloropyriphos which was applied at field rate (0.5 mg $kg^{-1}$ soil) did not affect the enzymatical activities like fluorescein diacetate hydrolysing activity (FDHA), and when the chloropyriphos was applied at higher dosage (50 mg $kg^{-1}$ soil) it significantly decreased the FDHA activity (Dutta *et al*., 2010). Dehydrogenase activities also observed more sensitive till second week at medium dose and till fourth week at higher dose, compared to untreated control. In comparison to control soil, the dehydrogenase activity decreased by 16% in presence of 10 ppm chloropyriphos pesticide, after 14 days treatment. The maximum inhibition (62%) of dehydrogenase activity was noticed in presence of 50 ppm chloropyriphos initially after first day of treatment (Kumar, 2011). Chloropyriphos also reduced the activity of alkaline phosphatase and urease enzyme activity at field application (Jastrzębska, 2011). The addition of biochar in pesticide treated soil improved the soil properties. In addition, it also minimized the negative impact of pesticides on soil properties and on certain microorganisms groups as reported in the Microbial Assay for Risk Assessment (MARA) (Oleszczuk *et al.,*2014; Sun *et al.*, 2014). The response of different enzymes with elevated $CO_2$ (700 ppm) were measured and it was observed that soil enzymatic activities specifically, dehydrogenase, fluorescien diacetate hydrolase, urease, acid phosphatase, and alkaline phosphatase responded positively to elevated $CO_2$ concentrations. However, it showed negative impact on persistence activity of pesticide (Adak *et al.*, 2016). A recent study by Sanchez-Hernandez *et al.,* 2017 described short-term responses of selected soil enzyme activities to chloropyriphos. The study showed two doses (4.8 and 24 kg a.i. $ha^{-1}$) and were evaluated and was concluded that both doses inhibited the activity of enzymes like carboxylesterase (62–78% of controls), acid phosphatase (56–60%)

and β-glucosidase (43–58%) dehydrogenase (47%) and catalase (38%) as compared to control soil. However, only carboxylesterase activity showed a dose-dependent response with the chloropyriphos application rate.

# CHAPTER - III

# MATERIALS AND METHODS

## 3.1 Soil sampling site

For the experiments to be carried out the soil samples were collected from the fields of the Indian Institute of Soil Science, Bhopal, Madhya Pradesh, India (23.30 N, 77.40 E, 485 m asl). The field was cultivated with soybean (*Glycine max* L.) and wheat (*Triticum aestivum* L.) during the summer and winter seasons, respectively during the past 13 years. Wheat variety HI 8498 and soybean variety JS 335 were grown at a spacing (cm) of 22.5 x 5 and 45 x 5 with seeding rates of 100 kg and 80 kg ha$^{-1,}$ respectively. Soils were collected from the control plot that received no fertilizer and sampling was done during June 2017 where there was no crop. A composite sample was prepared by mixing 4 samples from each corners and 1 sample from centre of the field. Then the soils were collected from depth profile of 5-15 cm. The study area has a humid subtropical climate, with a hot summer and a humid monsoon season. It experiences southwestern monsoon rains between July and September. Mean annual temperature is about 25 °C. Highest temperature goes up to 45 °C during the mid summer (May-June). During winter (December-January) the average temperature remains about 15 °C, & the average annual rainfall is 1200 mm with humidity of 65%.

## 3.2 Soil physico-chemical properties

After collecting the soil samples, the soil clods were broken and cleaned of small stones and root parts. Soil was then passed through a 2-mm mesh sieve and used within 2 days of collection. The soil is a heavy clayey Vertisol (Typic Haplustert) characterized with 5.7 g organic C, 225 mg available N, 2.6 mg available P, and 230 mg available K. Organic carbon (OC) was determined by wet digestion method (Walkley and Black, 1934). Available N was determined by alkaline $KMnO_4$ method (Subbiah and Asija, 1956). Available phosphorus was extracted by 0.5 N $NaHCO_3$ solution buffer at pH 8.5 (Olsen, 1954). Available potassium was calculated by neutral normal ammonium acetate (Hanway and Heidel, 1952) and K was determined by flame photometer (Lindsay and Norvell, 1978). The electrical conductivity (EC) was 0.43 dS m$^{-1}$ and the pH was 7.5 (1:2.5 of soil and water in w:v)

(Smith and Doran, 1996). The water holding capacity, bulk density and saturated hydraulic conductivity of the soil were 62% (w/w), 1.45 mg $m^{-3}$, and 7.3 x $10^{-6}$ m $s^{-1}$ respectively. The textural composition of soil was: sand 15.2%, silt 30.3%, clay 54.5%. The soil in the had 863.24 µM $NO_3^-$, 0.01 µM $Fe^{2+}$ and 101.02 µM $SO_4^{2-}$.

## 3.3 Biochar preparation

Biochar was prepared by pyrolysis method pigeon pea (*Cajanus cajan*) stalk grown in the experimental farm located at Central Institute of Agricultural Engineering Institute, Bhopal, India. The sun dried stalks of pigeon pea were shredded to 5-7 cm in length. The system used for biochar preparation was an unconfined insulated chamber made of mild steel. The chamber had inner diameter of 360 mm, height of 500 mm and wall thickness of 2 mm. Chamber temperature was maintained externally and the heating rate to attain the pyrolysis temperature (450 ºC) was 6 ºC $min^{-1}$. Charring process was completed in 4 hours (Gangil, 2014). Characterization of biochar was estimated by standard protocols (Nelson and Sommers, 1982). The pH (1:1.25, $H_2O$), electrical conductivity, ash content and bulk density of the BC were 9.57, 1.95 (dS $m^{-1}$), 15.5% and 239 kg $m^{-3}$ respectively. The total C, N, P and K concentrations (%) of BC were 86.4, 0.40, 0.09, and 0.74 respectively. Total Ca, Mg, and Na concentration (mg $kg^{-1}$) was 92.2, 19.5, and 395 respectively. The BC was grounded manually and passed through 2 mm sieve to attain the particle size less than 2.00 mm.

## 3.4 Microcosms set up

A factorial design was used to analyze the impact of factors (elevated $CO_2$, biochar and insecticide) on nitrification. The factors were $CO_2$ (400 ppm, 800 ppm), chloropyriphos (0 ppm, 10 ppm) and biochar (0 %, 1%), Biochar was applied at the rate of 1-2% as soil amendment (Asai *et al*., 2009). Each factorial combination (2 $CO_2$ x 2 biochar x 2 choropyrifos) was replicated 3 times, for a total of 18 experimental units. Soil of 5 kg weight was put into the pots. Soil was mixed with chloropyriphos stock solution before putting in the pots. A 1000 ppm stock solution of chloropyriphos (Sigma Aldrich, USA) was prepared using HPLC grade acetonitrile (Sigma Aldrich, USA). The chloropyriphos stock of 0.05 ml mixed with 10 ml water was added to soil to represent 10 ppm (w/w) chloropyriphos. Similarly, the soil added with 10 ml of water containing 0.05 ml pure acetonitrile served as treatment of 0 ppm chloropyriphos. Biochar was added to pots at the level of 0% or 1% (w/w). The soil was also mixed with aqueous stock of $NH_4SO_4$, KCl, and $Ca(H_2PO_4)_2$ for a final concentration of 50 mg $NH_4$-N, 25mg $K_2O$ –K, and 25 mg $P_2O_5$-P per kg soil. After adding all constituents soil was mixed thoroughly. Distilled water was added to maintain 60% moisture holding capacity. Pots were kept in plant growth chamber (M/s Genesis, Mumbai, India). The plant growth chamber had 2 compartments for setting 2 different $CO_2$ concentrations (400 ppm & 800 ppm). Wheat (*Triticum aestivum* L) was sown in each pot (two seeds per pot). Plant growth chamber was equipped with $CO_2$ sensor, red green blue LED tube lights, infrared heater, air conditioner, and microwave humidifier. The chamber was set for 18 hours illumination per day, temperature 28±2°C and relative humidity 65±2%. The pots were moistened with water (50 ml) at regular intervals of 2 days to maintain the moisture.

## 3.5 Nitrification measurement

Soil samples were collected regularly from pots (3-5 cm below surface) to evaluate nitrification. Concentration of $NO_3^-$ was estimated after extraction with $CaSO_4$ and reaction by phenol disulphonic acid method (Jackson 1958). Potential nitrification rate (PNR) was determined by estimating the $NO_3^{1-}$ concentration during the whole period of plant growth. Soil sampling and $NO_3^{1-}$ estimation was carried out for 21 days as the concentration of $NO_3^{1-}$ did not change. The slope of the regression line from $NO_3^{1-}$ N and incubation time was used to determine the potential nitrification rate (µg $NO_3^{1-}$ produced $g^{-1}$ soil $d^{-1}$) (Schmidt and Belser 1982).

## 3.6 DNA extraction

DNA was extracted from 0.5 g soil samples using the ultraclean DNA extraction kit (MoBio, USA). The DNA concentrations were determined in a biophotometer (Eppendorf, Germany) by measuring absorbance at 260 nm (A260), assuming that 1 A260 unit corresponds to 50 mg of DNA per µl. DNA extraction was further confirmed by electrophoresis on a 1% agarose gel. The extracted DNA was dissolved in 50 µl TE buffer and stored at −20 °C until further analysis.

## 3.7 Quantification of 16S rRNA gene of eubacteria and amonA gene of ammonia oxidizing bacteria

Real time PCR was performed on a Step one plus real time PCR (ABI, USA) to quantify the 16S rRNA and amoA genes. Reaction mixture was prepared with 2 µl of DNA template, 10 µl of 2X SYBR green master mix (Affymetrix, USA), 200 nM of primer (GCC Biotech, N Delhi). Final volume of PCR reaction mixture was 20 µl. Oligonucleotide primers targeting eubacterial 16S rRNA gene and nitrifying bacterial amoA gene were used for amplification. The primers (5'-3') for eubacteria were 1F (CCT ACG GGA GGC AGC AG) and 518R (ATT ACC GCG GCT GCT GG) (Baek *et al.* 2010) and nitrifying bacteria 1F (GGG GTT TCT ACT GGT GGT) and amoA 2R1 (CCC CTC TGG AAA GCC TTC TTC) (Okano *et al*. 2004). Thermal cycling was carried out by an initial denaturizing step at 94 °C for 4 min, 40 cycles of 94 °C for 1 min, annealing temperature for 30 sec, 72 °C for 45 sec; final extension carried out at 72 °C for 5 min. The annealing temperature for 16S rRNA was 52 ºC, and 50 ºC for amoA. Fluorescence was measured during elongation step. Data analysis was carried out with Step one plus software (ABI, USA) following user's manual. The CT (threshold cycle) values (cycle at which the fluorescence of target molecule number exceeded the background fluorescence) were determined. The quality of PCR amplification products were determined by melting curve analysis with temperature increase of 0.3 °C per cycle. Standard for the 16S rRNA and amoA genes was made from series of 10 fold dilutions of purified amplified products and data presented as number of cells g-1 soil.

## 3.8 Plant growth parameters

Wheat plants were harvested after 60 days of sowing. Plants were carefully removed from soil with minimal damage to roots. Soil particles adhering to root were washed out with tap water. Plants were cut into root and shoot parts. Weight of root and shoot was determined.

## 3.9 Statistical analysis

All statistical analyses were carried out using the "agricolae" and "vegan" packages of the statistical software R (2.15.1) (Ihaka and Gentleman, 1996). Results for the experiments were presented as arithmetic means and standard deviation of triplicate observations. Tukeys honestly significant difference (HSD) test was performed to define the significant difference among treatments at α 0.05. Effect of factors ($CO_2$ concentration, biochar and chloropyriphos) on the variables or parameters (nitrification, abundance of 16 S rRNA gene of eubacteria, amoA gene of ammonia oxidizers, root weight and shoot weight) was tested by analysis of variance (ANOVA) at α 0.05. Estimated data were ln transformed for ANOVA. Linear regression models were developed to predict nitrification from different variables.

# CHAPTER - IV

# RESULTS

## 4.1 Gross nitrification

The initial concentration of $NO_3$-N in the native soil was 12.25 mg kg-1. About 50 mg $NH_4$-N [$(NH_4)2SO_4$)] kg-1 soil was added to study nitrification. The nitrification efficiency of the soil remained within 30 to 66% among various treatments. Nitrification was studied in terms of $NO_3$-N produced out of the $NH_4$-N added. The temporal variation of nitrification in the rhizosphere of wheat under the influence of $CO_2$, biochar and chloropyriphos is given in Fig 1. The $NO_3$-N measurement was carried out for 21 days as the concentration of $NO_3$-N stabilized after 16 to 18 days irrespective of treatments. The highest $NO_3$-N was observed during 8-10 days. During this period the concentration of $NO_3$-N varied from 19.09 to 41.5 mg kg-1 soil. Apparent nitrification rate was determined accounting the period of increasing $NO_3$-N. Apparent nitrification rate varied among the treatments (Table 1). Lowest nitrification rate (0.665 mg $NO_3$-N produced g-1 soil d-1) was in the treatment of $CO_2$ 400 PPM biochar 0% chloropyriphos 10 ppm. Highest nitrification rate (4.525 mg $NO_3$ –N produced g-1 soil d-1) was in the treatment of 800 ppm $CO_2$ biochar 1% chloropyriphos 0 ppm. The result indicated that the elevated $CO_2$ and biochar stimulated the nitrification while chloropyriphos inhibited the nitrification. Elevated $CO_2$ stimulated nitrification rate by factors of 1.95 to 2.84 than ambient $CO_2$. Biochar stimulated nitrification by factors of 14% to 38% than no biochar. Chloropyriphos inhibited nitrification by factors of 59% to 67% than no chloropyriphos.

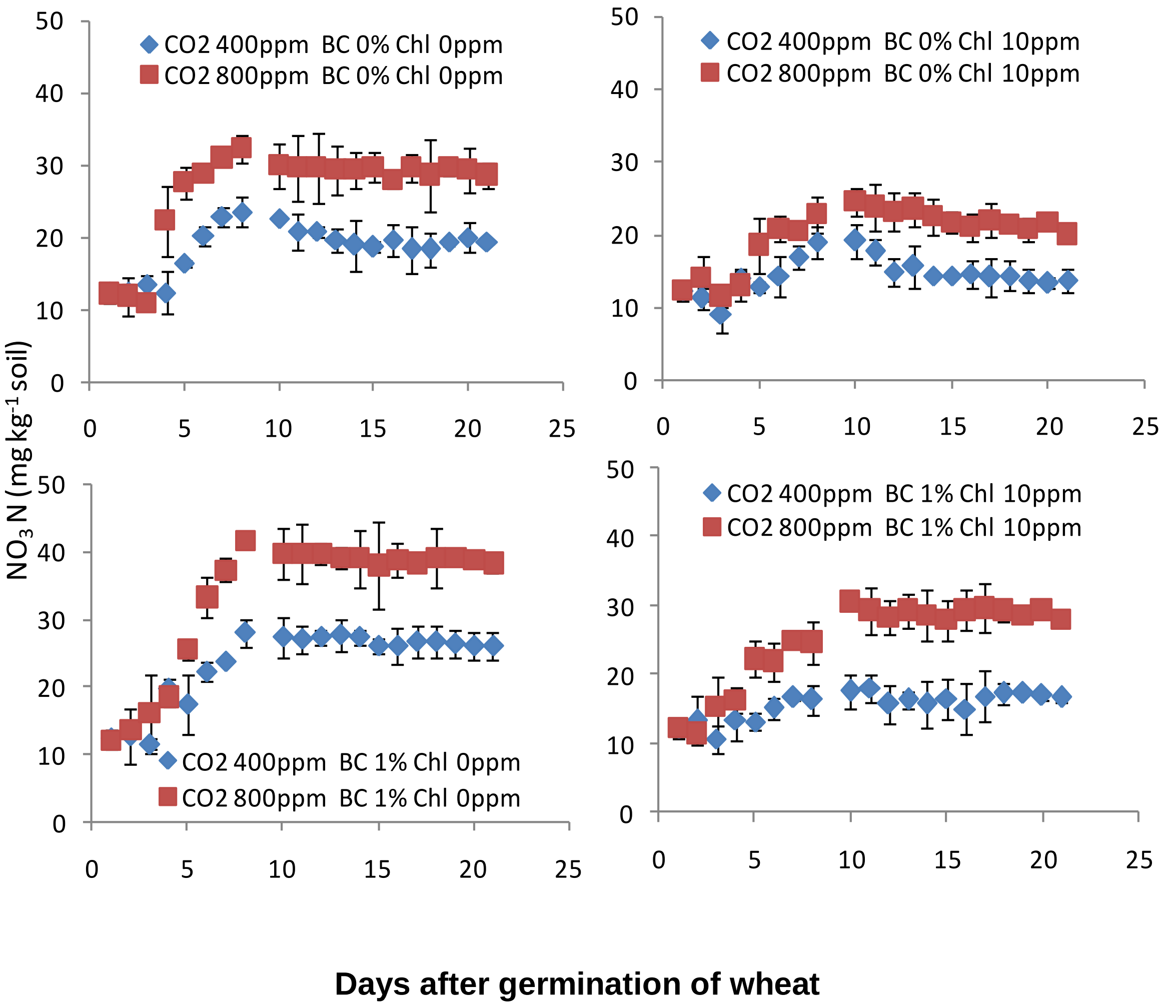

**Fig 1.** Influence of $CO_2$, biochar (BC) and chloropyriphos (Chl) on nitrification. $NO_3$ concentration in the rhizopheric soils of wheat was estimated. Wheat plants were grown under controlled environment with different $CO_2$ concentration (400 ppm , 800 ppm), biochar (0%, 1%), and chloropyriphos (0 ppm, 10 ppm). $NH_4$-N ($NH_4SO_4$) was applied to soil at 100kg N ha$^{-1}$ basis.  Soils were planted with wheat and incubated under in the rhizosphere of wheat grown in soil (vertisol) under the. Each data point represents arithmetic mean and standard deviation as error bar of three replicated observations. X axis represents incubation period and Y axis represents $NO_3$ (mM).

## 4.2 Microbial abundance

Abundance of eubacteria and ammonia oxidizing bacterial population was estimated during the peak nitrification phase (Table 1). The gene copies of 16S rRNA of eubacteria varied from 25±3.00 x $10^6$ $g^{-1}$ soil to 75±4.04 $10^6$ $g^{-1}$ soil. Highest number of eubacteria was in the treatment of 800 ppm $CO_2$ biochar 1% with no chloropyriphos. Lowest number of eubacteria was in the treatment of 400 ppm $CO_2$ without biochar and with chloropyriphos 10 ppm. The abundance nitrifies (amoA gene copies of ammonia oxidizing bacteria) varied in from 8.33±0.577 x $10^5$ cells $g^{-1}$ soil to 1.43±0.404 x $10^5$ cells $g^{-1}$ soil. Highest number of ammonia oxidizer was in the treatment of 800 ppm $CO_2$ biochar 1% and no chloropyriphos. Lowest number of ammonia oxidizer was in the treatment of 400 ppm $CO_2$ 0% biochar and 10 ppm chloropyriphos. Result showed that both elevated $CO_2$ and biochar acted as stimulator to eubacteria and ammonia oxidizers. Contrastingly, chloropyriphos inhibited the proliferation of these microbial groups. Elevated $CO_2$ stimulated the abundance of eubacteria by factors of 1.35 to 1.58. Similarly elevated $CO_2$stimulated the abundance of ammonia oxidizing bacteria by factors of 2 to 2.50 fold. Chloropyriphos inhibited both groups by 17% to 58% than no chloropyriphos.

**Table 1.**Apparent nitrification rate (mM $NO_3$ produced $g^{-1}$ soil $d^{-1}$), abundance of 16S rRNA gene copies of eubacteria, amoA gene copies of nitrifying bacteria. Soils were amended with chloropyriphos (0 ppm, 10 ppm), biochar (0%, 1%), and incubated under $CO_2$ (400 ppm, 800 ppm). Soils were planted with wheat. Each data represents arithmetic mean ±standard deviation of three replicated observations

| **$CO_2$ (ppm)** | **Biochar (%)** | **Chloropyriphos (%)** | **Nitrification rate (mg $NO_3$-N produced $g^{-1}$ soil $d^{-1}$)** | **16S rRNA gene (x $10^6$ cells $g^{-1}$ soil)** | **amoA gene of nitrifying bacteria (x $10^5$ cells $g^{-1}$ soil)** |
|---|---|---|---|---|---|
| 400 | 0 | 0 | 1.808±0.276$^{e}$ | 34±4.04$^{e}$ | 3.33±0.577$^{ef}$ |
| | | 10 | 0.665±0.152$^{h}$ | 25±3.00$^{g}$ | 1.43±0.404$^{g}$ |
| | 1 | 0 | 2.368±0.187$^{c}$ | 39±3.78$^{d}$ | 3.66±0.577$^{e}$ |
| | | 10 | 0.760±0.069$^{g}$ | 30±1.73$^{f}$ | 3.03±0.058$^{f}$ |
| 800 | 0 | 0 | 3.815±0.407$^{b}$ | 74±2.51$^{a}$ | 7.83±0.764$^{b}$ |
| | | 10 | 1.562±0.293f | 44±5.29$^{c}$ | 4.33±0.577$^{d}$ |
| | 1 | 0 | 4.525±0.585$^{a}$ | 75±4.04$^{a}$ | 8.33±0.577$^{a}$ |
| | | 10 | 2.157±0.100$^{d}$ | 54±4.04$^{b}$ | 6.06±0.902$^{c}$ |
| **Tukeys HSD (p 0.05, df error 23)** | | | **0.061** | **1.88** | **0.076** |

## 4.3 Plant growth attributes

Growth of wheat was evaluated as fresh weight of shoot and root after 60 days of germination (Table 2). Shoot weight varied from 43.0±3.7 g to 23.0±2g while the root weight varied from 25.4±2.33g to 9.6±0.577g. Plant growth (both shoot weight and root weight) was highest in the treatment of 800 ppm $CO_2$ biochar 1% and chloropyriphos 0%. Lowest growth was observed in the treatment of 400 ppm $CO_2$ biochar 0% and chloropyriphos 10 ppm. Elevated $CO_2$ and biochar stimulated the growth of wheat while chloropyriphos inhibited the growth of wheat. Elevated $CO_2$ stimulated root growth more than shoot growth. Elevated $CO_2$ stimulated shoot weight by a factor of 1.23 than ambient $CO_2$. Similarly, elevated $CO_2$ enhanced the root weight by a factor of 2.52 than ambient $CO_2$. Effect of biochar on plant growth parameters varied from 1 % to 38% than no biochar. Contrastingly chloropyriphos inhibited plant growth parameters (both root weight and shoot weight) by 17% to 42%.

**Table 2.** Wheat growth under the influence of $CO_2$, biochar and chloropyriphos. Soils were amended with chloropyriphos (0 ppm, 10 ppm), biochar (0%, 1%), and incubated under $CO_2$ (400 ppm, 800 ppm). Soils were planted with wheat and the growth of plants was measured as shoot weight (g) and root weight (g) after 45 days of sowing. Each data represents arithmetic mean ±standard deviation of three replicated observations. Mean values followed by same letters are not significantly different at $p < 0.05$.

| **$CO_2$ (ppm)** | **Biochar (%)** | **Chloropyriphos (%)** | **Shoot weight (g)** | **Root weight (g)** |
|---|---|---|---|---|
| 400 | 0 | 0 | $27.6 \pm 1.637^{e}$ | $12.6 \pm 0.346^{de}$ |
| | | 10 | $23.0 \pm 2.000^{f}$ | $9.6 \pm 0.577^{f}$ |
| | 1 | 0 | $30.7 \pm 0.755^{d}$ | $17.3 \pm 1.429^{c}$ |
| | | 10 | $23.3 \pm 1.155^{f}$ | $10.0 \pm 0.001^{e}$ |
| 800 | 0 | 0 | $41.6 \pm 2.887^{b}$ | $21.6 \pm 0.577^{b}$ |
| | | 10 | $29.3 \pm 5.038^{d}$ | $14.6 \pm 3.055^{d}$ |
| | 1 | 0 | $43.0 \pm 3.700^{a}$ | $25.4 \pm 2.335^{a}$ |
| | | 10 | $34.3 \pm 0.577^{c}$ | $16.3 \pm 2.309^{c}$ |
| **Tukeys HSD (p 0.05, df error 23)** | | | **1.12** | **1.58** |

## 4.4 Statistical analysis

One way ANOVA revealed that the factors ($CO_2$, biochar, and chloropyriphos) significantly ($p<0.01$) influenced the parameters (nitrification rate, shoot weight, root weight, abundance of eubacteria and abundance of ammonia oxidizers) (Table 3). The interaction of $CO_2$ and chloropyriphos ($CO_2$ x chloropyriphos) significantly influenced the nitrification rate ($p < 0.001$), abundance of eubacteria ($p < 0$) and ammonia oxidizers ($p< 0.001$). The interaction of biochar and chloropyriphos (biochar x chloropyriphos) significantly influenced root weight ($p<0.01$) and abundance of ammonia oxidizers ($p<0.001$). The interaction among $CO_2$, biochar and chloropyriphos ($CO_2$ x biochar x chloropyriphos) had no significant effect on any of the parameters. Linear regression models indicated that nitrification rate was linearly fitted with the shoot weight, root weight, abundance of eubacteria and ammonia oxidizers (Fig 2). Shoot weight fitted linearly as 5.428 x nitrification rate +19.64. Root weight fitted linearly as nitrification rate x 3.914 +7.332. Abundance of eubacteria linearly fitted as nitrification rate x 13.10 +18.23. Similarly, the abundance of ammonia oxidizers fitted as nitrification x 1.608 +1.207.

**Table 3**. Analysis of variance (ANOVA) of the factors and variables to determine interaction effect of $CO_2$, biochar and chloropyriphos on apparent nitrification rate, wheat shoot length, root length, population of eubacteria (16S rRNA gene copies), and ammonia oxidizing bacteria (AOB) in soil

| **Factors** | **Nitrification** | **Shoot** | **Root** | **Eubacteria** | **AOB** |
|---|---|---|---|---|---|
| $CO_2$ (C) | 6.57e-10*** | 2.37e-08*** | 1.82e-08*** | 1.08e-12*** | 5.31e-11*** |
| Biochar (BC) | 0.00117** | 0.0374* | 0.00149** | 0.00279** | 0.00532** |
| Chloropyriphos (Chl) | 9.17e-11*** | 1.02e-06*** | 5.09e-08*** | 3.01e-09*** | 2.72e-07*** |
| C x BC | 0.20990 | 0.5032 | 0.90087 | 0.82825 | 0.48649 |
| C x Chl | 0.00172** | 0.0518. | 0.05163. | 4.72e-05*** | 0.00461** |
| BC x Chl | 0.26191 | 0.8498 | 0.03007* | 0.14221 | 0.00260** |
| C x BC x Chl | 0.49171 | 0.1610 | 0.41457 | 0.14221 | 0.33988 |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

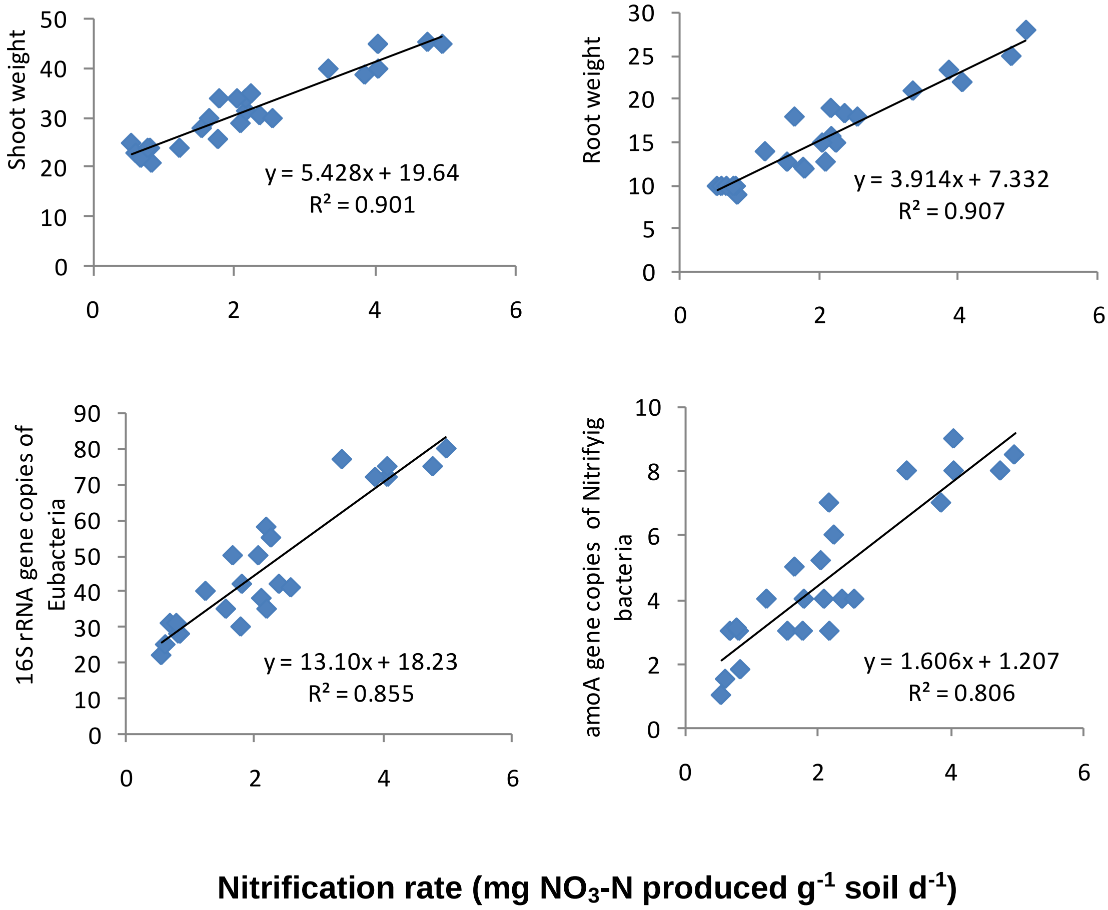

**Fig 2.** Linear regression models (α 0.05) of nitrification and different parameters of the study. The parameters were fresh weight of shoot, fresh weight of root, abundance of 16S rRNA gene of eubacteria, and abundance of amoA genes of ammonia oxidizing bacteria. Y axis represents different parameters while the X axis represent nitrification rate.

# CHAPTER - V

# DISCUSSION

The experiment was framed to generate useful information on the impact of elevated $CO_2$, biochar and chloropyriphos on nitrification potential under controlled laboratory environment to avoid variations which often originate from the environmental artefacts under field situations. The elevated $CO_2$ concentration was kept at 800 ppm as it is predicted that the concentration of atmospheric $CO_2$ will increase from present 410 ppm to 1000 ppm by the end of this century (O'Neill et al. 2017). Biochar is recommended for field application to improve soil function and to mitigate greenhouse gas emissions (Kollah *et al*. 2015). The insecticide chloropyriphos is recommended to apply to crops at 1-5 ppm level. However, the frequency and dose of its application is predicted to increase due to change in climate (Ahirwar *et al.* 2018). Therefore, in this study the chloropyriphos was evaluated at 10 ppm level. Wheat is grown all over the world with application of high dose of N to enhance crop yield. Nitrogen is basically applied in the form of $NH_4$-N. In soil the $NH_4$-N undergoes nitrification for plant uptake. Therefore, nitrification is an important process in agricultural soil ecosystem. To evaluate nitrification, $NO_3$ was estimated from soil samples at regular intervals because the end product of nitrification is $NO_3$.

The concentration of $NO_3$-N increased steadily soon after the addition of $NH_4$-N. The concentration of $NO_3$-N was highest when crop was 10-12 days old. This indicated that the soils were active and had high abundance of microbial groups which help in nitrification. Subsequently the concentration of $NO_3$-N remained at a steady state. Probably the soils attained the maximum nitrification potential and resulted plateau level of $NO_3$-N. The wheat N uptake is about 20-50 kg $ha^{-1}$ at maximum tillering, stem elongation and flowering stages (McGuire *et al*. 1998). In addition to the plant's $NO_3$-N uptake, soil microorganisms also assimilate considerable amount of $NO_3$-N. In soil ecosystem microbes assimilate both $NH_4$-N and $NO_3$-N. Heterotrophic microbes assimilate less $NH_4^+$ than $NO_3^-$ because of competition by nitrifiers (Burger and Jackson 2003). Therefore, the uptake of $NO_3$-N both by plant and microbes caused a saturation curve in the nitrification. It was also observed that out of 50 mg $NH_4$-N $kg^{-1}$ only 30-66 % was nitrified. This could be due to the effect of different factors on the nitrifiers and variation in N uptake by plant and microorganisms.

The factors (elevated $CO_2$, chloropyriphos and biochar) influenced the nitrification, plant growth and microbial abundance. In general the elevated $CO_2$ exerted highest impact followed by the impacts of chloropyriphos and biochar. Elevated $CO_2$ and biochar exhibited positive impact on the parameters while the chloropyriphos exterted negative impact. For example, the positive impact of elevated $CO_2$ on nitrification was twice than that of the ambient $CO_2$. Elevated $CO_2$ stimulated root weight and shoot weight. High root biomass improves root exudation leading to high microbial abundance than that of low root biomass. Under such scenario, the high microbial abundance probably stimulated the chloropyriphos degradation effecting nitrification. Similar result has been observed in a tropical rice soil. In the rice field experiment biodegradation of chloropyriphos under the influence of elevated $CO_2$ was explored. About 88.4% of initially applied chloropyriphos was degraded from the rice soil maintained under elevated $CO_2$ (700 ppm) by 5 day, whereas the 80.7% of the chloropyriphos was degraded under ambient $CO_2$(Adak et al. 2016). Thus the elevated $CO_2$ reduced the negative impact of chloropyriphos under elevated $CO_2$ and stimulated nitrification. Secondly, elevated $CO_2$ increased microbial abundance including nitrifiers which in turn stimulated nitrification. Positive effect of elevated $CO_2$ on nitrification can be explained by two means based on the microbial abundance and plant growth. Nitrifiers are autotrophs and obligatory aerobic in nature. Nitrifiers use $CO_2$ as substrate for their growth and metabolism. Therefore, elevated $CO_2$ stimulated nitrifying microbial population. Elevated $CO_2$ increased the plant biomass as well as root biomass. This effect was due to the $CO_2$-C fertilization. High growth of plant biomass also causes high root exudation as discussed before. In a free air carbon dioxide ($CO_2$) enrichment (FACE) field experiment Spring wheat (*Triticum aestivum* L. cv. TRISO) was grown for three consecutive seasons in order to examine the effects on crop yield and grain quality. Elevated $CO_2$ promoted the aboveground biomass (+11.8%) and grain yield (+10.4%) (Högy et al. 2009). In another experiment the effect of elevated $CO_2$ on the root structure and function and agronomic properties of two wheat (*Triticum aestivum*L.) cultivars was studied. Elevated $CO_2$ improved the N uptake and enhanced the allocation of N to grains (Tausz *et al.* 2017). Elevated $CO_2$ influences microbial structure and function through increased root exudation in plants (Wang et al. 2017). High soil organic carbon due to increased root exudation stimulated microbial activity (nitrification) and aided in plant's N uptake.

Biochar stimulated the nitrification potential of soil irrespective of other factors. In a study it was reported that biochar promoted soil ammonia-oxidizer populations (bacterial and archaeal nitrifiers) and accelerated gross nitrification rates more than two-folds (Prommer *et al*. 2014). The positive effect of biochar is linked to its properties. Biochar is a porous material and has high surface area (Atkinson *et al.*, 2010). Both the physical properties are favourable for the growth of aerobic microorganisms. Ammonia oxidizing bacteria are obligate aerobes and were thus stimulated in the soil amended with biochar. Biochar provides shelter to microorganisms too within its pores. Therefore, the abundance of microorganisms (both heterotrophic bacteria and ammonia oxidizers) was significantly ($p<0.05$) higher in the biochar amended soils. Chemically biochar is rich in minerals (Zhao *et al*. 2013). Ammonia oxidizers are chemolithoautotrophs and possibly the inorganic minerals favoured nitrification. Biochar has a higher cation exchange capacity (CEC) (Steinbeiss *et al*. 2009). High CEC allows adsorption of positively charged molecules like $NH_4$+. Higher availability of $NH_4$ favoured the ammonia oxidizers residing in the pores of biochar. Biochar stimulates the decomposition of chloropyriphos and it positively stimulates biodegradation of several xenobiotics. In this study biochar stimulated the population of heterotrophic bacteria. Heterotrophic bacteria like *Acenetobacter*, *Enterobacter* biodegrade the organic pollutants including chloropyriphos (Singh *et al*. 2004). Biochar basically stimulate CH4 oxidation by enhancing the abundance of aerobic methanotrophs, heterotrophs and actinomycetes (Ahirwar *et al.* 2018). Methanotrophs possibly enhanced the nirification (Kollah *et al.* 2015).

The concentration of chloropyriphos was within the range of recommended field dose (Posey *et al.*, 2006). Abundance of eubacteria and ammonia oxidizers was estimated at the peak nitrification period. Chloropyriphos inhibited the abundance of eubacteria and nitrifiers. In an earlier study it was observed that chloropyriphos inhibited methanotrophs (Ahirwar *et al.* 2018). Chloropyriphos inhibits bacterial enzymes including hydrolases (carboxylesterase, acid phosphatase, β-glucosidase, urease and protease) and oxidoreductases (dehydrogenase and catalase)(Sanchez-

Hernandez *etal.*, 2017). Heterotrophic bacterial population represent the overall microbial activity of a soil and many heterotrophs degrade chloropyriphos (Singh *et al*. 2004). Change in the abundance of both eubacteria and nitrifiers varied according to nitrification potential. Probably, the favourable factors (biochar and elevated $CO_2$) and stress factors (chloropyriphos) influenced bacterial abundance parallel to nitrification.

One way ANOVA revealed that the factors ($CO_2$, biochar and chloropyriphos) as an individual & interactively influenced ($p<0.01$) the parameters like nitrification, microbial abundance and plant growth. This was due to the significant effect of the factors on parameters. However, the interaction of factors did not exhibit significant influence on the parameters. Wheat shoot and root weight exhibited significant ($p<0.01$) positive relation with the nitrification rate. The negative effect of chloropyriphos on wheat growth could be due to the inhibition of soil bacteria (both heterotrophic eubacteria and nitrifiers). The negative effect of chloropyriphos was negated by biochar. Biochar stimulates degradation of many pesticides due to its high CEC, porosity and carbonaceous nature (Khorram *et al.* 2016). Biochar also adsorbs pesticides and reduces bioavailability for plant uptake and enhances the growth of the plant (Yu *et al.* 2009). Biochar also absorbs pesticides and channelize to the inhabiting microbes for degradation (Jing *et al.* 2018). Considering these facts it is assumed that biochar can be used to manage chloropyriphos contamination and improve crop growth under climate changing conditions.

# Conclusion

Experiment after analysis depicted the impact of chloropyriphos, biochar and climate factors on the nitrification activity in the rhizosphere of wheat. Soil was treated with chloropyriphos at 10 ppm, biochar was amended at 1% w/w. The climatic factor considered was elevated $CO_2$ (800 ppm). Soil was added with $NH_4$-N at 50 mg $kg^{-1}$ soil. Wheat seeds were sown in pots and plants were grown in a plant growth chamber under controlled conditions (temperature and humidity). Nitrification was measured by estimating the $NO_3$-N concentration periodically. Abundance of 16S rRNA gene of eubacteria and amoA gene of ammonia oxidizing bacteria were estimated. Plant growth attributes comprising shoot weight and root weight were measured. Results highlighted that elevated $CO_2$ and biochar stimulated nitrification while, chloropyriphos inhibited nitrification. Microbial abundance varied in parallel with the nitrification. Among the different factors undertaken the elevated $CO_2$ exerted maximum effect followed by chloropyriphos and biochar. Nitrification was positively related with all the parameters. Based on this experiment it can be concluded that the negative effect of chloropyriphos on nitrification can be subsidized by elevated $CO_2$ and biochar addition. The elevated $CO_2$ acts as C fertilizer and increased root and shoot biomass. Probably, the high root exudates due to increased plant root biomass stimulated the eubacteria and ammonia oxidizing bacteria and nitification. Biochar was effective in regulating the negative impact of chlorpyifos due to its physico chemical nature. As predicted by IPCC the increasing atmospheric $CO_2$ is likely to have positive effect on nitrification in the rhizosphere of wheat. However, there is need of experiments considering the effect of rising temperature for a holistic understanding on the interaction of climate, biochar and chloropyriphos on nitrification.

# SUMMARY

There is a prediction that as the climate will change, there will be increase in pesticide application in agriculture. Now a days biochar is recommended for soil application to improve carbon sequestration, climate mitigation, and crop production. Nitrification plays key role in plant's nitrogen uptake. However, it is not clearly understood how the climate factors, pesticide and biochar will influence nitrification. Experiment was carried out to study nitrification in the rhizosphere of wheat under the influence of elevated $CO_2$, chloropyriphos and biochar. The experimental factors were $CO_2$ (400 ppm, 800 ppm), biochar (0%, 1%), and chloropyriphos (0 ppm, 10 ppm). The parameters considered were nitrification rate, abundance of 16S rRNA gene of eubacteria, abundance of amoA gene, plant shoot and root biomass. It was estimated by measuring soil $NO_3$-N in the early vegetative growth of wheat. Nitrification rate was lowest (0.665±0.152 mg $NO_3$-N produced $g^{-1}$ soil $d^{-1}$) in soil under $CO_2$ 400 ppm biochar 0% chloropyriphos 10 ppm. Highest nitrification rate (4.525±0.585 mg $NO_3$-N produced $g^{-1}$ soil $d^{-1}$) was in $CO_2$ 800ppm biochar 1% chloropyriphos 0 ppm. Abundance of 16S rRNA gene copies (x $10^6$ cells $g^{-1}$ soil) of eubacteria varied from 25±3.00to 75±4.04. Abundance of ammonia oxidizing bacteria (x $10^5$ cells $g^{-1}$ soil) varied from 1.43±0.404 to 8.33±0.577. Shoot weight of wheat ranged from 23.0±2.00 g to 43.0±3.700 g. Similarly root weight of wheat varied from 9.6±0.577 g to 25.4±2.335 g. Results exhibited that elevated $CO_2$ and biochar stimulated the nitrification process, microbial abundance and plant growth. On the other hand, chloropyriphos inhibited these parameters. The impact of elevated $CO_2$ was highest in chloropyriphos followed biochar. Study concluded that elevation in atmospheric $CO_2$ may increase the negative impact of chloropyriphos on nitrification and soil microbial abundance. However, the use of biochar may alleviate these negative impacts and aid in retaining soil function.

# REFERENCES

Abraham J, Silambarasan S (2013) Biodegradation of chloropyriphos and its hydrolyzing metabolite 3, 5, 6-trichloro-2-pyridinol by Sphingobacterium sp. JAS3. *Process Biochem* 48:1559–1564

Abraham J & Silambarasan S. (2016) Biodegradation of chloropyriphos and its hydrolysis product 3, 5, 6-trichloro-2-pyridinol using a novel bacterium Ochrobactrum sp. JAS2: A proposal of its metabolic pathway. *Pestic Biochem Physiol* 126:13–21

Adak T, Munda S & Kumar U. (2016) Effect of elevated $CO_2$ on chloropyriphos degradation and soil microbial activities in tropical rice soil. *Environ Monit Assess* 188:1–12

Ahirwar U, Dubey G & Singh N. (2018) Interactive effect of climate factors, biochar and insecticide chloropyriphos on methane consumption and microbial abundance in a tropical Vertisol. *Ecotoxicol Environ Saf* 157:409–416

Alfreider A, Baumer A & Bogensperger T. (2017) CO2 assimilation strategies in stratified lakes: Diversity and distribution patterns of chemolithoautotrophs. *Environ Microbiol*

Anwar S, Liaquat F & Khan QM. (2009) Biodegradation of chloropyriphos and its hydrolysis product 3, 5, 6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. *J Hazard Mater* 168:400–405

Asai H, Samson B K & Stephan HM. (2009) Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Res* 111:81–84

Atkinson CJ, Fitzgerald JD & Hipps NA. (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *PlantSoil* 337:1–18

Awad NS, Sabit HH, Abo-Aba SE & Bayoumi RA. (2011) Isolation, characterization and fingerprinting of some chloropyriphos-degrading bacterial strains isolated from Egyptian pesticides-polluted soils. *Afr J Microbiol Res* 5:2855–2862

Baek KH, Park C & Oh H-Ml. (2010) Diversity and abundance of ammonia-oxidizing bacteria in activated sludge treating different types of wastewater. *J MicrobiolBiotechnol* 20:1128–1133

Baldrian P, Šnajdr J & Merhautová V. (2013) Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. *Soil Biol Biochem* 56:60–68

Baskaran S, Kookana RS& Naidu R. (1999) Degradation of bifenthrin, chloropyriphos and imidacloprid in soil and bedding materials at termiticidal application rates. *Pestic Sci* 55:1222–1228

Bumpus J A, Kakar S N & Coleman RD. (1993) Fungal degradation of organophosphorous insecticides. *Appl Biochem Biotechnol* 39:715–726

Burger M & Jackson LE. (2003) Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. *Soil Biol Biochem* 35:29–36

Cao L, Liu H& Zhang H. (2012) Characterization of a newly isolated highly effective 3, 5, 6-trichloro-2-pyridinol degrading strain Cupriavidus pauculus P2. *CurrMicrobiol* 65:231–236

Chishti Z, Hussain S & Arshad KR. (2013) Microbial degradation of chloropyriphos in liquid media and soil. *J Environ Manage* 114:372–380

Chowdhury A, Pradhan S, Saha M & Sanyal N. (2008) Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. *Indian J Microbiol* 48:114–127

Cink JH & Coats JR. (1993) Effect of concentration, temperature, and soil moisture on the degradation of chloropyriphos in an urban Iowa soil. *Pestic Urban Environ* 522:62–69

Das S, Bhattacharyya P & Adhya TK. (2011) Impact of elevated CO2, flooding, and temperature interaction on heterotrophic nitrogen fixation in tropical rice soils. *Biol Fertil Soils* 47:25–30

Dhanya MS (2014) Advances in microbial biodegradation of chloropyriphos. *J Environ Res Dev* 9:232

Diez M C, Levio M & Briceño G. (2013) Biochar as a partial replacement of peat in pesticide-degrading biomixtures formulated with different soil types. J Biobased Mater Bioenergy 7:741–747

Dubey K K & Fulekar MH. (2012) Chloropyriphos bioremediation in Pennisetum rhizosphere by a novel potential degrader Stenotrophomonas maltophilia MHF ENV20. *World J Microbiol Biotechn*ol 28:1715–1725

Dutta M, Sardar D, Pal R & Kole RK. (2010) Effect of chloropyriphos on microbial biomass and activities in tropical clay loam soil. *Environ Monit Assess* 160:385–391

El-Helow ER, Badawy ME & Mabrouk ME. (2013) Biodegradation of chloropyriphos by a newly isolated *Bacillus subtilis* strain, Y242. *Bioremediation J* 17:113–123

Epstein L & Zhang M. (2014) The impact of integrated pest management programs on pesticide use in California, USA. In: Integrated Pest Management. *Springer*, pp 173–200

Fulekar MH & Geetha M. (2008) Bioremediation of Chloropyriphos by Pseudomonas aeruginosa using scale up technique. *J Appl Biosci* 12:657–660

Gangil S (2014) Thermogravimetric Evidence for Better Thermal Stability in Char Produced Under Unconfined Conditions. *Environ Eng Sci* 31:183–192

Gebremariam SY, Beutel MW & Yonge DR. (2012) Adsorption and desorption of chloropyriphos to soils and sediments. In: Reviews of environmental contamination and toxicology. *Springer,* pp 123–175

Gilani RA, Rafique M & Rehman A. (2016) Biodegradation of chloropyriphos by bacterial genus Pseudomonas. *J Basic Microbiol* 56:105–119

Gomez LE (2009) Use and benefits of chloropyriphos in US agriculture. *Dow AgroSciences* LLC

Hanway JJ & Heidel H. (1952) Soil analysis methods as used in Iowa state college soil testing laboratory. *Iowa Agric* 57:

Higgins JA, Kurbatov AV &Spaulding NE. (2015) Atmospheric composition 1 million years ago from blue ice in the Allan Hills, Antarctica. *Proc Natl Acad Sci* 112:6887–6891

Högy P, Wieser H & Köhler P. (2009) Effects of elevated $CO_2$ on grain yield and quality of wheat: results from a 3year freeair $CO_2$ enrichment experiment. *Plant Biol* 11:60–69

Ihaka R & Gentleman R. (1996) R: a language for data analysis and graphics. *J ComputGraph Sta*t 5:299–314

IPCC (2007) Climate Change: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Jabeen H, Iqbal S, Anwar S. (2015) Biodegradation of chloropyriphos and 3, 5, 6trichloro2pyridinol by a novel rhizobial strain Mesorhizobium sp. HN3. *Water Environ J* 29:151–160

Jackson ML (1958) Soil Chemical Analysis. Prentice- Hall, Inc, Englewood, Cliffs, New Jersey

Jastrzębska E (2011) The effect of chloropyriphos and teflubenzuron on the enzymatic activity of soil. *Pol J Envron Stud* 20:903

Jing X, Wang T & Yang J. (2018) Effects of biochar on the fate and toxicity of herbicide fenoxaprop-ethyl in soil. *R Soc Open Sci* 5:171875

John EM, Sreekumar J, Jisha MS. (2016) Optimization Of Chloropyriphos Degradation By Assembled Bacterial Consortium Using Response Surface Methodology. *Soil Sediment Contam In*t J 00–00

KaviKarunya S (2012) Biological degradation of chloropyriphos and monocrotophos by bacterial isolates*. Int J Pharm Biol Arch 3:*

Khorram MS, Zhang Q & Lin D. (2016) Biochar: a review of its impact on pesticide behavior in soil environments and its potential applications. *J Environ Sci* 44:269–279

Kollah B, Dubey G & Parasai P. (2015) Interactive effect of biochar size and organic amendments on methane consumption in a tropical vertisol*. Soil Use Manag* 31:52–59

Korade DL & Fulekar MH. (2009) Rhizosphere remediation of chloropyriphos in mycorrhizospheric soil using ryegrass. *J Hazard Mater* 172:1344–1350

Krull ES, Skjemstad JO & Burrows WH. (2005) Recent vegetation changes in central Queensland, Australia: Evidence from δ 13 C and 14 C analyses of soil organic matter. Geoderma 126:241–259

Kumar S (2011) Fluctuation of soil bacterial dehydrogenase activity in response to the application of endosulfan and chloropyriphos*. J Cell Tissue Res* 11:2847

Kumar[1] M & Kumar A. (2007) Application and Health Effects of Pesticides Commonly Used in India

Lakshmi CV, Kumar M & Khanna S. (2008) Biotransformation of chloropyriphos and bioremediation of contaminated soil. *Int Biodeterior Biodegrad* 62:204–209

Larkin MJ, Kulakov LA & Allen CC. (2005) Biodegradation and Rhodococcus–masters of catabolic versatility. *Curr Opin Biotechnol* 16:282–290

Lehmann J (2007) Bio-energy in the black*. Front Ecol Environ* 5:381–387

Lehmann J, Czimczik C, Laird D & Sohi S. (2009) Stability of biochar in soil. *Biochar Environ Manag Sci Technol* 183r206

Li X, He J & Li S. (2007) Isolation of a chloropyriphos-degrading bacterium, Sphingomonas sp. strain Dsp-2, and cloning of the mpd gene. *Res Microbiol* 158:143–149

Lindsay WL & Norvell WA. (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper1. *Soil Sci Soc Am J* 42:421–428

Liu H, Xu Q, Zhang L & Liu N. (2005) Chloropyriphos resistance in mosquito Culex quinquefasciatus. *J Med Entomol* 42:815–820

Liu ZY, Chen X, Shi Y & Su ZC. (2012) Bacterial degradation of chloropyriphos by Bacillus cereus. Adv Mater Res 356:676–680

Lu P, Li Q & Liu H. (2013) Biodegradation of chloropyriphos and 3, 5, 6-trichloro-2-pyridinol by Cupriavidus sp. DT-1. *Bioresour Technol* 127:337–342

Mallick K, Bharati K & Banerji A. (1999) Bacterial degradation of chloropyriphos in pure cultures and in soil. *Bull Environ Contam Toxicol* 62:48–54

Maya K, Singh RS, Upadhyay SN & Dubey SK. (2011) Kinetic analysis reveals bacterial efficacy for biodegradation of chloropyriphos and its hydrolyzing metabolite TCP. *Process Biochem* 46:2130–2136

McCarthy JJ (2001) Climate change 2001: impacts, adaptation, and vulnerability: contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press

McGuire AM, Bryant DC & Denison RF. (1998) Wheat yields, nitrogen uptake, and soil moisture following winter legume cover crop vs. fallow. *Agron J* 90:404–410

Méndez A, Gómez A, Paz-Ferreiro J & Gascó G. (2012) Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. *Chemosphere* 89:1354–1359

Mitchell PJ, Dalley TS, Helleur RJ. (2013) Preliminary laboratory production and characterization of biochars from lignocellulosic municipal waste. *J Anal ApplPyrolysis* 99:71–78

Mukherjee I & Gopal M. (1996) Degradation of chloropyriphos by two soil fungi Aspergillus niger and Trichoderma viride. *Toxicol Environ Chem* 57:145–151

Nelson DW & Sommers LE. (1982) Total carbon, organic carbon and organic matter. Page Eds Methods Soil Anal Part 2 Chem Microbiol Prop Agron Monogr Am Soc Agron Soil.*Sci Soc Am MadisonWI USA* 9:539–576

Niti C, Sunita S, Kamlesh K & Rakesh K. (2013) Bioremediation: An emerging technology for remediation of pesticides. *Res J Chem Env* 17:88–105

Okano Y, Hristova KR & Leutenegger CM. (2004) Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Appl Environ Microbiol* 70:1008–1016

Oleszczuk P, Jośko I & Futa B. (2014) Effect of pesticides on microorganisms, enzymatic activity and plant in biochar-amended soil. *Geoderma* 214:10–18

Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *United States Department Of Agriculture; Washington*

O'Neill BC, Oppenheimer M & Warren R. (2017) IPCC reasons for concern regarding climate change risks. *Nat Clim Change* 7:28–37

Pino N & Peñuela G. (2011) Simultaneous degradation of the pesticides methyl parathion and chloropyriphos by an isolated bacterial consortium from a contaminated site. *Int Biodeterior Biodegrad* 65:827–831

Posey FR, White WH & Reay-Jones FPF. (2006) Sugarcane borer (Lepidoptera: Crambidae) management threshold assessment on four sugarcane cultivars. *J Econ Entomol* 99:966–971

Praseetha PK, Sugunan VS, Shabi R & Jegadambikadevi S. (2012) Stress response of Actinomycetes to toxins in their bioremediation processes*. J Microbiol Biotechnol Res* 2:

Prommer J, Wanek W & Hofhansl F. (2014) Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. *PloS One* 9:e86388

Sabdono A (2013) Biodegradation of chloropyriphos by a marine bacterium Bacillus firmus strain by6 associated with branching coral Acropora sp. *J Coast Dev* 10:115–123

Sanchez-Hernandez JC, Sandoval M & Pierart A. (2017) Short-term response of soil enzyme activities in a chloropyriphos-treated mesocosm: Use of enzyme-based indexes. *Ecol Indic* 73:525–535

Sasikala C, Jiwal S, Rout P & Ramya M. (2012) Biodegradation of chloropyriphos by bacterial consortium isolated from agriculture soil. *World J Microbiol Biotechnol* 1–8

Schmidt EL & Belser LW. (1982) Nitrifying bacteria. *Methods Soil Anal Part* 2:1027–1042

Schrijver AD & Mot RD. (1999) Degradation of pesticides by actinomycetes. *Crit Rev Microbiol* 25:85–119

Singh BK, Walker A, Morgan JAW & Wright DJ. (2004a) Biodegradation of chloropyriphos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. *Appl Environ Microbiol* 70:4855–4863

Singh BK, Walker A, Morgan JAW & Wright DJ. (2003) Effects of soil pH on the biodegradation of chloropyriphos and isolation of a chloropyriphos-degrading bacterium. *Appl Environ Microbiol* 69:5198–5206

Singh BK, Walker A, Morgan JAW, Wright DJ (2004b) Biodegradation of chloropyriphos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol 70:4855–4863

Singh BK, Walker A & Wright DJ. (2006) Bioremedial potential of fenamiphos and chloropyriphos degrading isolates: influence of different environmental conditions. *Soil Biol Biochem* 38:2682–2693

Singh BK, Walker A & Wright DJ. (2005) Cross-enhancement of accelerated biodegradation of organophosphorus compounds in soils: Dependence on structural similarity of compounds. *Soil Biol Biochem* 37:1675–1682

Singh P, Saini HS & Raj M .(2016) Rhamnolipid mediated enhanced degradation of chloropyriphos by bacterial consortium in soil-water system. *Ecotoxicol Environ Saf* 134:156–162

Smith JL & Doran JW. (1996) Measurement and use of pH and electrical conductivity for soil quality analysis. In: Methods for assessing soil quality. *Soil Science Society of America Madison, WI*

Spokas KA & Reicosky DC. (2009) Impacts of sixteen different biochars on soil greenhouse gas production. *Ann Environ Sci* 3:4

Steinbeiss S, Gleixner G & Antonietti M. (2009) Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol Biochem* 41:1301–1310

Subbiah B & Asija GL. (1956) Alkaline permanganate method of available nitrogen determination. *Curr Sci* 25:259

Subedi R, Kammann C & Pelissetti S. (2013) Use of biochar and hydrochar to reduce ammonia emissions from soils fertilized with pig slurry.

Sun Z, Bruun EW & Arthur E. (2014) Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils. *Biol Fertil Soils* 50:1087–1097

Tausz M, Bilela S & Bahrami H.  (2017) Nitrogen nutrition and aspects of root growth and function of two wheat cultivars under elevated ($CO_2$). *Environ Exp Bot* 140:1–7

(US) NC. Water USEPAO of (2002) Leasing and restoration of submerged lands: Strategies for Community-based, watershed-scale conservation. *Nature Conservancy*

Uzoma KC, Inoue M & Andry H. (2011) Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use Manag* 27:205–212

Vassilev N, Martos E & Mendes G. (2013) Biochar of animal origin: a sustainable solution to the global problem of high-grade rock phosphate scarcity. *J Sci Food Agric* 93:1799–1804

Vijayalakshmi P & Usha MS. (2012) Optimization of chloropyriphos degradation by Pseudomonas putida. *J Chem Pharm Res* 4:2532–2539

Vischetti C, Monaci E & Cardinali A. (2008) The effect of initial concentration, co-application and repeated applications on pesticide degradation in a biobed mixture. *Chemosphere* 72:1739–1743

Walkley A & Black IA. (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid extraction method. *Soil Sci* 37:29–38

Wang P, Marsh EL & Ainsworth EA. (2017) Shifts in microbial communities in soil, rhizosphere and roots of two major crop systems under elevated $CO_2$ and $O_3$. *Sci Rep* 7:15019

Wang Y-S, Wu T-H & Yang Y. (2016) Binding and detoxification of chloropyriphos by lactic acid bacteria on rice straw silage fermentation. *J Environ Sci Health Part B* 51:316–325

Weber EB, Lehtovirta-Morley LE, Prosser JI & Gubry-Rangin C. (2015) Ammonia oxidation is not required for growth of Group 1.1 c soil Thaumarchaeota. *FEMS Microbiol Ecol* 91:fiv001

Xu G, Li Y & Zheng W. (2007) Mineralization of chloropyriphos by co-culture of Serratia and Trichosporon spp. *Biotechnol Lett* 29:1469–1473

Xu G, Zheng W & Li Y. (2008) Biodegradation of chloropyriphos and 3, 5, 6-trichloro-2-pyridinol by a newly isolated Paracoccus sp. strain TRP. *Int Biodeterior Biodegrad* 62:51–56

Yadav M, Srivastva N & Singh RS. (2014) Biodegradation of chloropyriphos by Pseudomonas sp. in a continuous packed bed bioreactor. *Bioresour Technol* 165:265–269

Yan Y, Wang Y-C & Feng C-C. (2017) Potential distributional changes of invasive crop pest species associated with global climate change. *Appl Geogr* 82:83–92

Yang C, Liu N, Guo X & Qiao C. (2006) Cloning of mpd gene from a chloropyriphos-degrading bacterium and use of this strain in bioremediation of contaminated soil. *FEMS Microbiol Lett* 265:118–125

Yang L, Zhao Y & Zhang B. (2005) Isolation and characterization of a chloropyriphos and 3, 5, 6-trichloro-2-pyridinol degrading bacterium. *FEMS Microbiol Lett* 251:67–73

Yu X-Y, Ying G-G & Kookana RS. (2006) Sorption and desorption behaviors of diuron in soils amended with charcoal. *J Agric Food Chem* 54:8545–8550

Yu X-Y, Ying G-G & Kookana RS. (2009) Reduced plant uptake of pesticides with biochar additions to soil. *Chemosphere* 76:665–671

Yuan J-H, Xu R-K & Zhang H. (2011) The forms of alkalis in the biochar produced from crop residues at different temperatures. *Bioresour Technol* 102:3488–3497

Zhao L, Cao X & Wang Q. (2013) Mineral constituents profile of biochar derived from diversified waste biomasses: implications for agricultural applications. *J Environ* Qual 42:545–552

Zhu J, Zhao Y & Qiu J. (2010) Isolation and application of a chloropyriphos-degrading Bacillus licheniformis ZHU-1*. Afr J Microbiol Res* 4:2410–2413

# *VITA*

*The author of this thesis Mr.Bhoopendra singh s/o Shri Gouri Shankar was born on 20th of janvary, 1990 at District – Bhopal (M.P). He passed his high school (10th) in the year 2005 from City montesury H.S.S., Morena (M.P.), with 68.8% marks and higher secondary (12th) in the year 2007 from A.K. public school with 71.33% marks.*

*He successfully completed B.Sc. (Agriculture) in the year 2014 from College of Agriculture, Gwalior, Rajmata Vijayaraje Sindia Krishi Viswa Vidyalaya, Gwalior (M.P.) with 66.67% marks.*

*The author thereafter, carried his post graduation studies from the Department of Environmental Science, College of Agriculture Gwalior, Rajmata Vijayaraje Sindia Krishi Viswa Vidyalaya, Gwalior (M.P.). This thesis is a bonafide research being submitted by him as a partial fulfillment for the award of M.Sc. (Ag.) Degree.*

*During all the period of his education, from schooling to post graduation he was very sincere and honest towards his studies and works.*

***