Interactive Ability of Carbamates With Essential Metals of Soil

A

Thesis

Submitted to

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DOCTOR OF PHILOSOPHY (Ph.D)

in

(CHEMISTRY)

By

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DECLARATION

I hereby declare that the thesis entitled, **"Interactive Ability of Carbamates With Essential Metals of Soil"** submitted for Ph.D.Chemistry Degree to Department of Chemistry, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for the award of any other degree.

Sukhmanpreet Kaur
Reg. No. 11014217

CERTIFICATE

This is to certify that **Ms. Sukhmanpreet kaur** has completed the Ph.D. Chemistry titled **"Interactive Ability of Carbamates With Essential Metals of Soil"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma.

The thesis is fit for the submission for the partial fulfilment of the condition for the award of degree of Ph.D. in Chemistry.

Signature of Supervisor

(Dr.Niraj Upadhyay)
Assistant. Professor
Department of Chemistry
Dr Harisingh Gour Central University

ABSTRACT

As tittle suggest, the thesis addresses interactive ability of carbamate (specifically carbofuran, thiophanate methyl, thoidicarb, carbendazim and methomyl) with essential trace metal ions of soil. The work is consequential to unwind the impact of such interaction to the real world,as, metal ions are important component of the soil fertility and their interaction with pesticides (like carbamates) directly influence soil, which is fundamental for our survival. Salient features of the work included:1) Investigation of the factors resulting into the interactions of five carbamatepesticides with essential trace metal ions (Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)).2) Mode of bonding of carbamate with metal ion and stability (thermal) determination of the formed product after the interactions of carbamates (pesticide of investigation) with trace essential metal ions (Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)). 3) Effect of five carbamates pesticides on the siderophore production ability of plant growth promoting rhizobacterial strains. 4) Effect of five carbamates pesticides on maize growth and metal ions content of seeds of the *Zea mays* plant.

The designed experiments lead to the astonishing disclosure, which are explained as follows:

It was observed that in the presence of soil components, the rate of interaction of carbamate pesticide is rapid with metal ions. Investigation in liquid medium, showed increase rate of metal- pesticide interaction with the metal ions at high temperature and high pH (basic medium), suggesting rate of metal- carbamate complex formation would be higher in hot countries and in basic soil.UV-vis spectrophotometric technique helped in investigation of rate of interaction on the basis of the amount of the ligand consumed in the reaction. It was found that most of the pesticide interacts with the metal ions soon after coming in contact and almost all reaction in liquid medium completes within the 5-6 hours of the reaction. Minimum time was consumed by the thiophanate methyl with Cu(II) ion, which get completed within 1 hour. The maximum time (8h) for the interaction with the each metal ions was used by the thoidicarb pesticide. Carbamate doesn’t interact uniformly with the entire metal ions and interaction of carbamate with metal ion was found dependent on hard soft acid base interaction.

IR and NMR spectrophotometer, helped in finding site of interaction of carbamate with metal ion, by which site of interaction was observed through the donor sites like (O or N atom) with metal ions (Mn(II),Fe(II),Co(II),Ni(II),Cu(II) and Zn(II)). It was also affirmed by the mass spectrometer.TGA analysis suggested that, most of the formed complexes between the

carbamates pesticides and the metal ions are highly stable and doesn't easily decompose. The most stable product of each of the carbamate doesn't completely decompose below 850°C.SEM analysis revealed that formed metal complexes are nanosized and may exist in the soil for long time in the form of nano- particle.

In the rhizosphere, the metal ion uptake is regulated by siderophores produced by soil microorganisms, particularly by plant growth promoting rhizobacteria. In the current study, the selection of plant growth promoting rhizobacteria was done on the basis of their ability to produce siderophores. The selected bacteria and the siderophores produced by them areas follows:*Rhizobium leguminosarum* (Trihydroxamate); *Pseudomonas fluorescens* (Hydroxamate); *Bacillus brevis* (Bacillibactin); *Azotobacter vinelandii* (Azotobactin); *Salmonella typhimurium* (Enterobactin).In the siderophore production assay, with the applications of pesticides, minimum adverse effect (15-78%) has analyzed on the siderophore production ability of *Rhizobium leguminosarum*, and highest adverse effect of (26-86%) has been observed on *Bacillus brevis*. Among the remaining strains, the inhibition of siderophore production is as: 19-83% for *Azotobacter vinelandii*, 24-81% for *Pseudomonas fluorescens*, and 21-81% for *Salmonella typhimurium*. The decreasing order of adverse effect of the pesticides on siderophore production ability was found as, methomyl> carbendazim > thoidicarb >thiophanate methyl>carbofuran. This order is linear with toxicity of pesticides. Hence, we can say that pesticides inhibit the siderophore production of soil microorganisms by two ways: 1) through the direct effects where the toxic chemicals kill these microorganisms before the siderophore production and 2) through the indirect effects, where the chelating carbamate inhibit the siderophore production or other plant growth promoting activities by competing with siderophore produced by these microorganisms. Hence, pesticides can inhibit the metal ions uptake through both ways.

Among the five pesticides, at different dose levels, the order of adverse effect on the *Zea mays* plant growth and total metal ions content in the seeds in decreasing order was found as:methomyl> carbendazim > thoidicarb >carbofuran>thiophanate methyl. This order is linear with the chelating ability of pesticides under study. With the applications of additional amount of metal ions (Mn(II), Fe(II), Cu(II) and Zn(II)), 5-24% increase in plant growth and metal ions content has achieved with respect to carbamate effected plant. Thus, foreground the adverse effects of pesticides usage and its ability to restrict the metal ions transportation for the healthy development of plants.

Entire study help to conclude that carbamate trap the essential trace metal ions and restrict them for plant growth. If we continue, application of carbamates on soil, long term soil infertility may cause and therefore this the alarming time to switch towards a new generation pesticide.

Acknowledgement

"Relying on your mercy, Dear **Waheguru**, I have been able to compile the work", I bow my deepsense of gratitude for same.

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and my husband **S.Jashandeep Singh**, without your constant motivation and sacrifices it would merely remain as dream for me to complete my work. I am also thankful to my stress relievers, my pet dogs **Sikander** and **Scobby** for their support.

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(Sukhmanpreet Kaur)

Table of Contents

LIST OF TABLES

LIST OF FIGURES

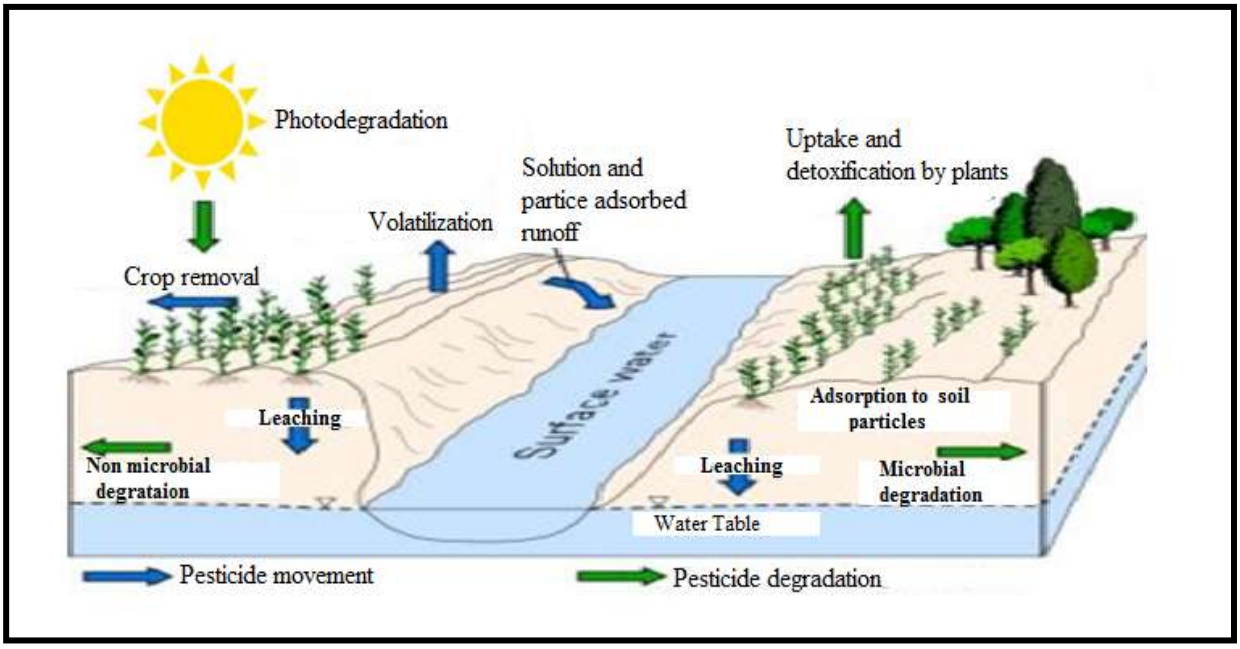
ABBREVIATIONS AND SYMBOLS

CZ	Carbendazim
CF	Carbofuron
M	Methomyl
TC	Thoidicarb
TM	Thiophanate methyl
M(II)	Divalent Metal ions (Mn, Fe, Co, Ni, Cu, Zn etc.)
HSAB	Hard Soft Acid Base
AChE	Acetylcholinesterase
MSDS	Material Safety Data Sheet
WHO	World Health Organisation
LD	Lethal Dose
Z	Atomic Number
PGPR	Plant Growth Promoting Rhizobacteria
Da	Dalton
US$	United State Dollar
°C	Degree Centigrade
H	Hour(s)
mL	Millilitre
mM	Millimolar
mg	Milligram
rpm	Rotation Per Minute
UV	Ultraviolet
FTIR	Fourier Transformation Infrared
NMR	Nuclear Magnetic Resonance
TGA	Thermal Gravimetric Analysis
KBr	Potassium bromide
Cm	Centimetre
NCL	National Chemical Laboratory
NCIM	National Collection of Industrial Microorganisms
Nm	Nanometre
€	Molar Absorption Constant
DMSO	Dimethyl Sulfoxide
TFA	Triflouroacetic Acid
MHz	Mega Hertz
ppm	Parts Per Million
m/z	Mass to Charge Ratio
TG	Thermal Gravimetric
DTG	Differential Thermal Gravimetric
Min	Minute(s)

g/L	Gram/ Litre
CFU	Colony Formation Unit
ICP-AES	Inductive Coupled Plasma Atomic Emission Spectroscopy
ANOVA	Analysis of Variance
v	Stretching Frequency
δ	Bending Frequency
j	Coupling Constant
s	Singlet
d	Doublet
t	Triplet
q	Quartet
ʎmax	Absorption Maxima
ES-MS	Electron Spray MassSpectrum
FESEM	Field Emission Scaning Microscopy

Chapter-1

A Brief Review on Carbamates- Physical and Chemical properties with their impact on Environment



1.1. DESCRIPTION OF TOPIC

It is the precept that, "healthy soils" produce the nutritious food and sequentiallyensures healthy human beings and animals.[1] Soil possesses not only a nucleus position for existence of living being but also safeguards their future existence.[2] In addition, soil plays the vital functions to sustain plant productivity and maintain environmental quality.[3]

Prior to industrial revolution, agricultural practices were ecofriendly, crop yields in agricultural system depended on internal resources, recycling of organic matter, rainfall patterns and used crop rotation to maintain the soil nutrients. However, the increase in population leads to the scarcity of the food and desperate attempts were made to modernize the traditional agriculture.[4] For that purpose, technologies and strategies were borrowed from the west and it foster the impressive results by increasing the crop production and economic margin.[5] The key factor involved in the agricultural transformations was the application of the chemical pest control. Furthermore, its unrestrained usage progressed under the adage "if little is good, a lot more will be better" has lead to the deterioration of the soil health.[6]

As the attention was not devoted to the fact that, whenever a part of the system is manipulated ripple effects are often felt beyond the point of interruption. The exacerbate consequences were achieved, which reported that the soil in almost every country become zinc deficient. Besides, the deficiencies of copper, molybdenum and manganese were found between the range of 10-15% and Fe deficiencies were observed 3% in developing countries. Thus, together adversely affected in total of the 40% of all the soil.[7,8] The reflection of the micronutrients deficiency in the soil is also observed in our food. The scientist examined the changes in the nutrient content on the 27 varieties of the vegetables and found the 76% loss of the copper and 59% loss of the Zinc in the present vegetables.[9] Another study suggested that, decrease in the concentrations of the Zn, Fe, Cu and Mg in the wheat grains were observed only after the mid 1960s.[10] The low nutritional quality of the food caused severe Fe deficiency in the 3.7 billion people.[11] In addition 35% of all the children suffer from the Zn deficiency and 19% of all the death before the age of the 5 years is attributed to both Fe and Zn deficiencies.[12,13]

While scrutinizing the root cause of the situation, some of the shocking revelation suggested that usage of the pesticides has adverse effect on the metal ions.The best documented examples are the facts, which reported that the application of Glyphosate pesticide (one of the organophosphates pesticide) has caused substantial decrease in concentration of Fe and Mn in leaves of the Sunflower (*Helianthus annuus* L) Plants.[14] In a

similar manner, reported study on the seeds of the soybean plant depicted the decrease in the concentration of the Ca, Mg, Fe and Mn after the application of glyphosate.[15] The study concluded that glyphosate pesticide has bind with metal ion which leads to their immobilization. The supporting evidence for the same have been found in another report which explained that glyphosate ability to form complex with the Mn and Zn has affected the plant uptake for the trace element.[16] Taking into the consideration, irreversible cholinesterase activity of organophospahtes which lead to severe toxic effects on the human being they were replaced by the carbamates.[17] The Carbamates are representative of one of the main category of synthetic organic pesticides. Ideally, they are designed due to its very low bioaccumulation potential and short term toxicity as well as for a versatile class of compounds.[18] As, explained through below:

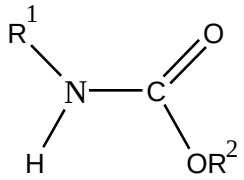


Figure 1.1: General structure of carbamate

where, R^2 is an aromatic or aliphatic moiety. The pesticide behavior of carbamate varies with the nature of the substituent group:

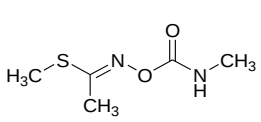
(a) Carbamate insecticides; if R^1 is a methyl group;

(b) Carbamate herbicides; if R^1 is an aromatic moiety; and

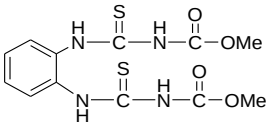
(c) Carbamate fungicides; if R^1 is a benzimidazole moiety.[19, 20, 21]

On the other hand their possibility to interact with the metal ions was totally ignored. As, the study that was conducted on [oxamyl 1,1 {methyl-2-(dimethylamine)-N-[(methylamino) carbonyl))oxy-]-2- oxoethanimidothioate (I); and [{N- Phenyl (ethylcarbamoyl) propyl carbamate (III)] carbamates found that application of doses of the pesticides decreased the yield of edible part of the tomato plants.[22]Moreover, two of the carbamates (thiophanate methyl and methomyl) were reported for their interaction with Co(II) and Cu(II) for thiophanate methyl and Fe(III) for methomyl and their interaction has been used in the field of sensing.The interaction reported in data mainly occurs due to electron donating ability of the sulphur in thiocarbonyl group (in case of thiophanate methyl), oxygen of the carbonyl (in case of methomyl) and electron accepting ability of Co(II),Cu(II) and Fe(III) metal ion.[23,24] Such facts have evoked the curiosity for monitoring the behavior of the carbamates pesticides in presence of the essential metal ions (Mn, Fe, Ni, Co, Cu and Zn). Simentanously, there is

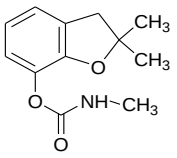
possibility that the interactive ability of the pesticides could adversely affect the soil microflora and the plant crops.For that purpose, their impact on the plant (Zea Mays) as well as plant growth promoting bacteria (*Rhizobium leguminosarum* (Trihydroxamate); *Pseudomonas fluorescens* (Hydroxamate); *Bacillus brevis* (Bacillibactin); *Azotobacter vinelandii* (Azotobactin); *Salmonella typhimurium* (Enterobactin) were checked. The study was conducted on the five carbamates (as shown in figure 1.2) which were selected on the basis of their wide application in the agriculture field and their susceptibility for showing metal interaction.



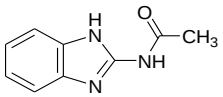
Methomyl (M)



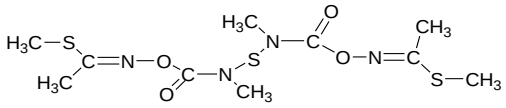
Thiophanate methyl (TM)



Carbofuran (CF)



Carbendazim(CZ)



Thoidicarb (TC)

Figure 1.2: Showing structures of Methomyl (M), Thiophanate methyl (TM), Carbofuran (CF), Carbendazim (CZ) and Thiodicarb (TC) carbamates.

1.2 LITERATURE SURVEY

1.2.1 Origin of Carbamates

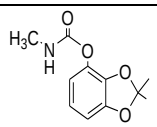
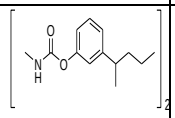
Carbamates pesticides are derived from carbamic acid and have been used for the plant protection services since 1950 and onwards. The first carbamate compound known as physostigmine (eserine alkaloid), was extracted from the Calabar beans (ordeal poison) of a perennial plant found in West Africa.[25] The seeds of physostigmine are the only known naturally occurring carbamate esters. Above that, the synthesis of carbamates as pesticides was initiated by the researches of Hans Gysin in Switzerland and Robert Metcalf and the co-workers in the Unites States. Carbaryl was the first carbamate synthesized in 1956 to be used as insecticides.[26] The urgency to develop the new range of the pesticides occurred, when the greatest strengths of these early chemicals (D.D.T and Organophosphates) environment

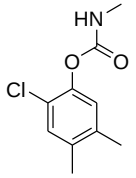
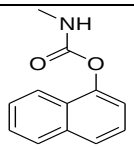
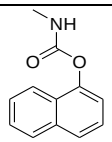
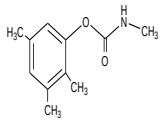
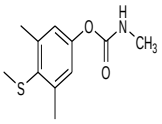
stability and broad spectrum activity become their greatest weaknesses. As, their non specificity killed the beneficial organisms and pesticides residues were found to accumulate in body fat of non-target organisms resulted in the disastrous impact on the food chain and ecological balance. To minimize the adverse effect, pesticides were replaced with the less persistence and less toxic products which are famously known as Carbamates.[27,28]

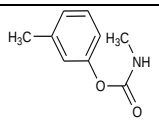
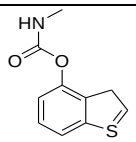
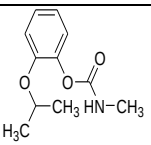
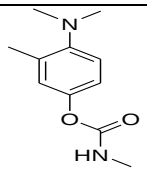
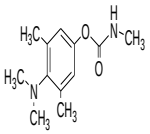
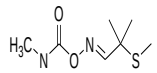
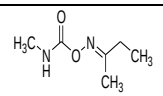
1.2.2 Physical and Chemical Properties of Carbamates.

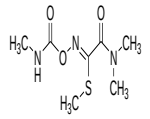
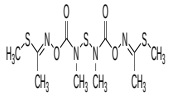
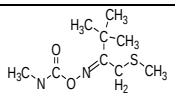
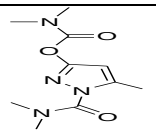
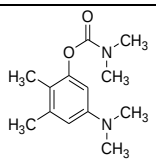
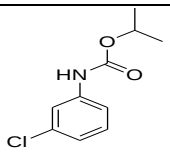
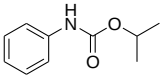
Carbamates owned broad spectrum usage from their different structural derivatives. Therefore, it is prerequisite to comprehend their knowledge to gain insight on them. They are divided into the nine principle groups, namely as: N-methylcarbamates, amino phenyl N-methyl carbamates, oxime N-methylcarbamates, N,N dimethyl carbamates, N-phenyl carbamates, benziimidiazole carbamates, thiocarbamates, dithiocarbamates and ethylene bisdithiocarbamates. Each of the carbamates physical and chemical properties (including the physical form, structure, melting point, vapor pressure, and solubility in different solvents) are incorporated under their classification in tabular form below as Table 1.[29]

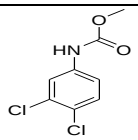
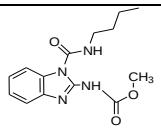
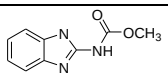
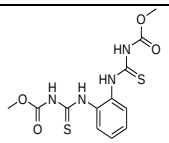
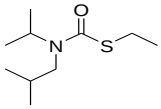
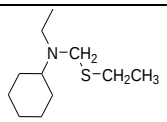
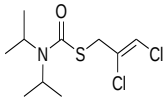
Table 1- Physical and chemical properties of carbamate.

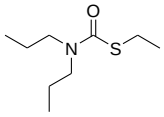
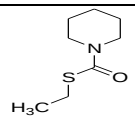
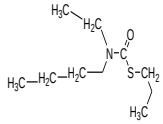
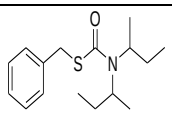
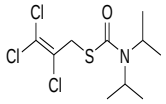
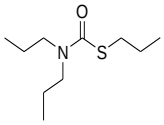
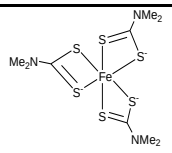
Common Name Other Name Trade Name	**Chemical Structure, Formulae and Activity**	**Physical Form Melting Point (^{0}C) Vapor Pressure(25^{0}C)**	**Solubility at 25^{0}C Water Acetone Benzene Ethanol/Methanol n-Hexane**	**Toxicity to Mammals LD_{50}Acute (mg/Kg)** **Oral Dermal**
N-methylcarbamates				
Bendiocarb Bendiocarbe Ficam	 $C_{11}H_{13}NO_4$ Insecticide	Colorless crystals 124.6–128.7 4.6 mPa	0.28 g/L (20°C) 150–200 g/L 40 g/L 40/75–100 g/L 0.225 g/L	40–156 566–600
Bufencarb Bux Metalkamate	 $C_{26}H_{38}N_2O_4$ Insecticide	Yellow amber solid 26–39 4.0 mPa (at 30°C)	<0.005% – – –/Very high -	87 680

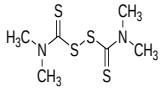
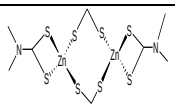
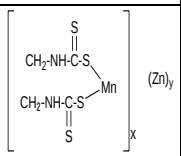
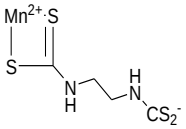
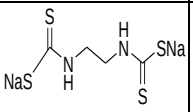
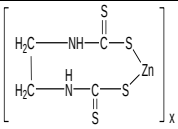
Carbanolate Banol Chlorxylam	 $C_{10}H_{12}ClNO_2$ Insecticide	White crystals 130–133 –	– – – –/– –	30–55 -
Carbaryl SevinR, Dicarbam Carbicide	 $C_{12}H_{11}NO_2$ Insecticide	Colorless crystals 142 41 µPa (23.5°C)	120 mg/L (20°C) 200–300 g/kg – – Readily soluble	500-850 >4000 >2000 (rabbit)
Carbofuran FuradanR NIA-10242	 $C_{12}H_{15}NO_3$	Colorless crystals 153–154 0.031 mPa	320 mg/L (20°C) 15% 4% 4%/–	8–14 >3000 (rabbit)
Landrin	 $C_{11}H_{15}NO_2$ Insect icide	Buff crystals 105–114 5 × 10−5 mmHg (23°C)	60 mg/L (23°C) – – – –/–	208 2500 (rabbit)
Methiocarb MesurolR Metmercapturo n, Mercaptodimeth ur	 $C_{11}H_{15}NO_2S$ Insecticide Acaricide Bird repellent	Colorless crystals 119 0.015 mPa	27 mg/L (20°C) – – – –/–	100 350–400

Metolcarb	 $C_9H_{11}NO_2$ Insecticide	Colorless solid 76–77 145 mPa (20°C)	12.6 g/L (30°C) – – –/ 880g/kg–2 g/L (20°C)	498–580 –
Moban MCA-600	 $C_{1O}H_9NO_2S$ Insecticide	White crystals 128 1 × 10−8 mmHg (25°C)	<0.1% – – – –/–	20–125 –
Propoxur BaygonR Blattanex, Unden, Sendran	 $C_{11}H_{15}NO_3$ Insecticide	Colorless crystals 90 2.8 mPa	1.9 g/L (20°C) Soluble – Soluble 1–2 g/L (20°C)	128 >5000
Aminocarb Matacil	 $C_{11}H_{16}N_2O_2$	Tan crystals 93–94 Nonvolatile	Slight Moderate – Soluble –	
Mexacarbate ZectranR	 $C_{12}H_{18}NO_2$ Insecticide	White crystals 85 <0.1 mm Hg (139°C)	– – – –/– –	24 >500
Aldicarb TemikR UC-21149	 $C_7H_{14}N_2O_2S$ Insecticide Acaricide Nematicide	Colorless crystals 98–100 13 mPa (20°C)	6 g/L 350 g/kg 150 g/kg –/– –	0.9 20 (rabbit)
Methomyl Lannate Methavin	 $C_5H_{10}N_2O_2S$	Colorless crystals 78–79 6.65 mPa	57.9 g/L 730 g/kg – 420/1000 g/kg	17–24 >5000 (rabbit)

	Insecticide Acaricide		Sparingly	
Oxamyl Vydate DPX-1410	 $C_7H_{13}N_3O_3S$ Insecticide	Colorless crystals 100–102 31 mPa	280 g/L 670 g/kg – 330/1440 g/kg –	5.4 >2000 (rabbit)
Thiodicarb **Larvin**	 $C_{10}H_{18}N_4O_4S_3$ Insecticide	Colorless crystals 173–174 5.7 mPa (20°C)	35 mg/L 8 g/kg – 5 g/kg	66 >2000 (rabbit)
Thiofanox Dacamox Thiofanocarb	 $C_9H_{18}N_2O_2S$ **Insecticide**	Colorless crystals 56.5–57.5 22.6 mPa	5.2 g/L (22°C) Soluble Soluble –/–	8.5 39 (rabbit)
Dimetilan Snip	 $C_{10}H_{16}N_4O_3$ Insecticide	Colorless crystals 68–71 1 × 10−4 mmHg (20°C)	24% Readily soluble Readily/readily –	<50 >2000
Pirimicarb Pirimor Aphox, Fernos	 $C_{11}H_{18}N_4O_2$ Insecticide	Colorless crystals 90.5 0.97 mPa	3.0 g/L (20°C) 4.0 g/kg Readily 2.5 g/kg/readily	147 >500
N-phenylcarbamates				
Chlorprophan CIPC Chloro-IPC	 Herbicide	Colorless solid 38.5–40 10−5 mm Hg (25°C)	89 mg/L Soluble Soluble Readily soluble	5000 2000 (dog)
Propham IPC Banhoe, Tuberit	 $C_{10}H_{13}NO_2$ Herbicide	Colorless crystals 87–87.6 –	250 mg/L (20°C) Soluble Soluble Soluble –	5000 6800 (rabbit)

Swep	 $C_8H_7ClNO_2$ Herbicide	White solid 112–114 –	– Soluble – –/– –	552 -
Benomyl Arylate, Benlate, and Tersan	 $C_{14}H_{18}N_4O_3$ Fungicide	Colorless crystals 140 <4.9 μPa	4 mg/kg (pH 3–10) 18 g/kg – 4 g/kg/– –	>10000 10000 (rabbit)
Carbendazim Carbendazime, Derosal, Carbendazol, Bavistin	 $C_9H_9N_3O_2$ Fungicide	Crystalline powder 302–307 0.09 mPa (20°C)	29 mg/L (pH 4) 0.3 g/L 0.036 g/L 0.3 g/L/– 0.0005 g/L	>15000 >2000
Thiophanate Methyl. Methylthiofanate Mildothane	 $C_{12}H_{14}N_4O_4S_2$	White powder 172 0.095 mPa	26.6mg/l 58.1mg/l - 29.2 mg/l 43 mg/l	>5000 >2000
Butylate Sutan	 $C_{11}H_{23}NOS$ Herbicide	Colorless liquid – 1.73 Pa	36 mg/L (20°C) Miscible Miscible Miscible -	5366 >5000 (rabbit)
Cycloate Ro-Neet Hexylthiocarbam	 $C_{11}H_{21}NOS$ Herbicide	Colorless liquid 11.5 2.13 mPa	75 mg/L (20°C) Miscible Miscible Miscible –	3160 >5000 (rabbit)
Diallate Avadex	 $C_{10}H_{17}Cl_2NOS$ Herbicide	Yellowish oily liquid – 1.5 × 10−4 mmHg	14 mg/L Soluble Soluble Soluble –	395 >2000 (rabbit)

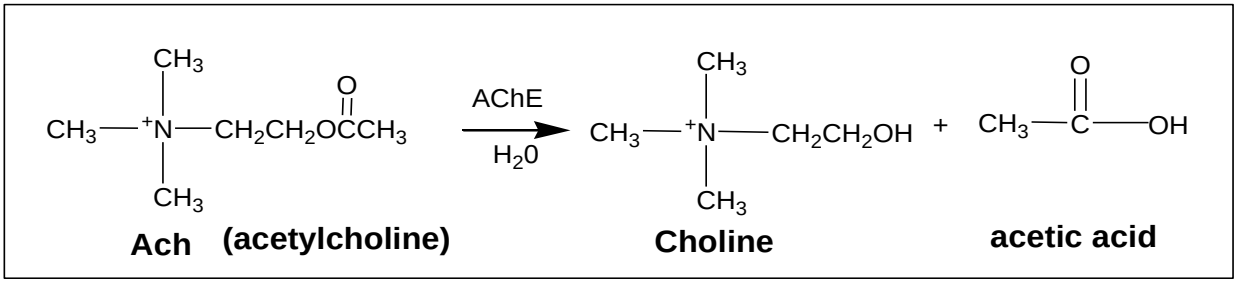
EPTC Eptam	 $C_9H_{19}NOS$ Herbicide	Colorless liquid −30 0.01 mPa	375 mg/L Miscible Miscible Miscible –	1367 >2000
Molinate Ordram	 $C_8H_{15}NOS$ Herbicide	Clear liquid – 746 mPa	88 mg/L (20°C) Miscible Miscible Miscible –	369–45 >4640 (rabbit)
Pebulate Tillan	 $C_{10}H_{21}NOS$ Herbicide	Colorless or yellow liquid – 9 Pa (30°C)	60 mg/L (20°C) Miscible Miscible Miscible –	1120 4640 (rabbit)
Tiocarbazil	 $C_{16}H_{25}NOS$ Herbicide	Colorless liquid – 93 mPa (50°C)	2.5 mg/L (30°C) Miscible Miscible Miscible Miscible	>10,000 >1200
Triallate Triallate Avadex BW	 $C_{10}H_{16}Cl_3NOS$ Herbicide	Oily, amber liquid 29–30 16 mPa	4 mg/L Readily soluble Readily soluble Readily soluble Soluble	1100 8200 (rabbit)
Vernolate Vernam	 $C_{10}H_{21}NOS$ Herbicide	Clear liquid – 1.39 Pa	90 mg/L (20°C) Miscible – Miscible –	1500 >5000 (rabbit)
Ferbam Fermate	 $C_9H_{18}FeN_3S_6$ Fungicide	Black powder decomposes >180°C Negligible (20°C)	130 mg/L Soluble – – –	>4000 –

Thiram Thirame Thiuram, TMTD	 $C_6H_{12}N_2S_4$ Fungicide	Colorless crystals 155–156 2.3 mPa	18 mg/L 80 g/L (20°C) – <10 g/L/– 0.04 g/L	780 >1000
Ziram Milbam Zerlate	 $C_6H_{12}N_2S_4Zn$ Fungicide	White powder 246 <1 μPa	0.03 mg/L (20°C) Moderately soluble – Insoluble –	320 >6000
Mancozeb Dithane M-45 Manzeb	 $C_8H_{12}MnN_4S_8Zn$ Fungicide	Grayish- yellow powder decomposes without melting Negligible	6–20 mg/L – – – –	>5000 >10,000 (rabbit)
Maneb Dithane M-22 Manzate	 $C_4H_6MnN_2S_4$ Fungicide	Yellow crystalline solid Decomposes without melting Negligible	Slightly soluble Insoluble Insoluble Insoluble Insoluble	6750 >5000
Nabam Dithane D-14 Parzate, nabame	 $C_4H_6N_2Na_2S_4$ Fungicide Algicide	Colorless crystals Decompose without melting Negligible	200 g/L Insoluble Insoluble Insoluble Insoluble	395 –
Zineb Dithane Z-78 Parzate	 $C_4H_6N_2S_4Zn$ Fungicide	Light-colored powder Decomposes without melting <0.01 mPa (20°C)	10 mg/L Insoluble Insoluble Insoluble Insoluble	>5200 >6000

1.2.3 MODE OF ACTION AND ACUTE TOXICITY

1.2.3.1 Mechanism of inhibition

Carbamates elicit acute intoxication by virtue of their ability to inactivate the enzyme acetyl cholinesterase. Chiefly, acetyl cholinesterase acts as regulating agent by catalyzing the hydrolysis of acetylcholine into choline and acetic acid (as shown in eq1).[30] When acetyl cholinesterase is inactivated in case of carbamates by carbamylation, the enzyme is no longer able to hydrolyze the acetyl choline (mechanism shown in Figure 1.3).[31] Thus, causing the accumulation of acetylcholine at the nerve endings of all cholinergic nerves which could ultimately end in death due to respiratory failure.



Eq.1: Conversion of acetylcholine into acetic acid and choline in presence of acetylcholinesterase.

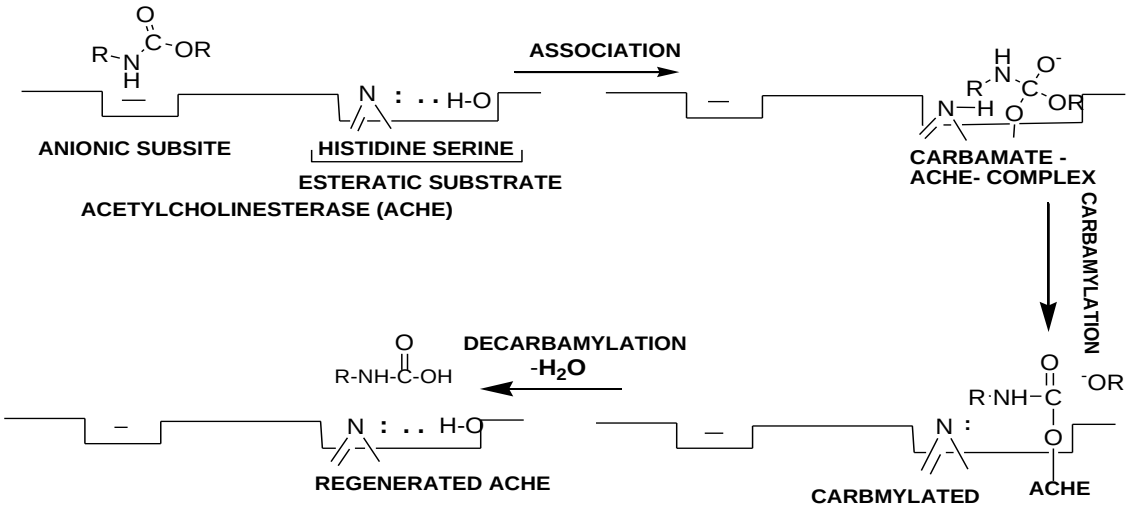
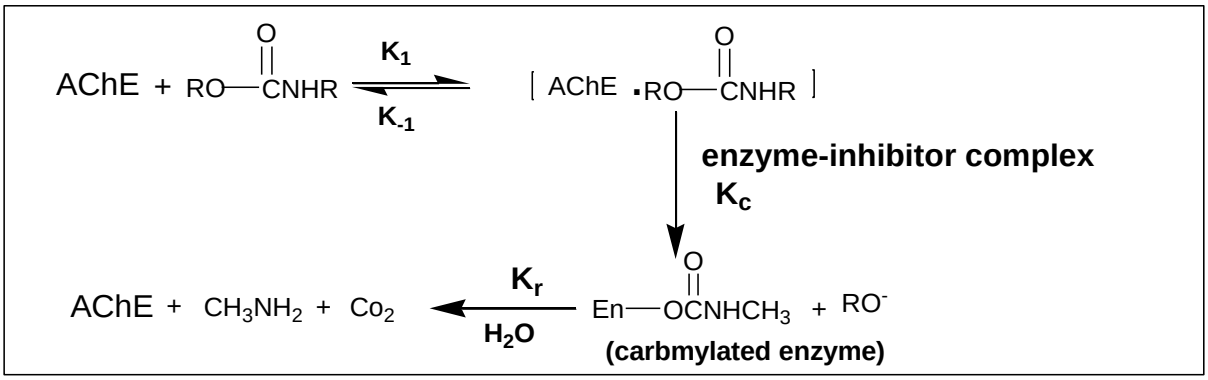


Figure 1.3: Steps involved in the reversible inhibition of Ache (carbmylation).

The inhibition of AChE by carbamates takes place via chemical reaction in which carbamylation of the serine hydroxyl leads to the development of the enzyme inhibitor complex forming carbmylated enzyme.Besides, occurs the regeneration step (Kr) in which the carbmylated enzyme spontaneously regenerates to active enzyme, methyl amine and carbon dioxide (as depicted in Eq.2).[32]



Eq.2: Steps involved in reaction mechanism of carbamates depicting the inhibition of acetylcholinesterase enzyme.

Where: k_1 = Second order rate constant for formation of complex;

k_{-1}= First order rate constant for breakdown of complex starting materials;

k_c= First order rate constant for carbamylation of the enzyme;

k_r = First order rate constant for hydrolyses of the carbamylated enzyme.

1.2.3.2 Factors influencing mechanism of inhibition.

Inactivation of the acetyl cholinesterase by carbamates depends upon the formation of the enzyme-inhibitor complex prior to carbmylation. Certain factors played the crucial role for the formation of the complex such as:

(a) Electron donating and withdrawing group attached to carbamates.

(b) Size of the substituted group.

(c) Attachment of the substituent group.

(d) Equilibrium constant.[30,31,32]

Primarily, electron donating and withdrawing groups attached with carbamates played the major role. As, introduction of nitro substituent (electron withdrawing group) into the phenyl ring of the phenyl methyl carbamates results in a compound of such high reactivity that it hydrolytically degraded, before it has an opportunity to inhibit the enzyme. Whereas, electron donating substituents enhance the complex formation required for inhibition by increasing the electron density in the neighborhood of the oxygen atom at the complex forming site. Moreover, size of acetylcholine exhibit spatial similarities with strong anticholinesterase carbamates. As,10 fold increase in anticholinesterase activity is found by increasing size from hydrogen, methyl, ethyl to isopropyl .Attachment of the substituent group also affecting inhibition, which is best advocated by taking example of carbofuran and propoxur. Both methyl carbamates are closely related through structure .But, carbofuran is more potent in

inhibiting the acetyl cholinesterase due to rigidly fixed gem-dimethyl group at the optimum level from the carbamate moiety .On the other hand, in case of propoxur, isopropoxy moiety is bridged to ring by a methylene group. Another important factor is the value of the equilibrium constant for dissociation of enzyme inhibitor complex. From the reported studies, it was observed that carbamates with larger value of equilibrium constant exhibit the lesser ability of inhibiting acetyl cholinesterase and smaller value of equilibrium constant exhibit the higher ability of inhibition.

1.2.4 PERSISTENCE AND DEGRADATION OF CARBAMATES PESTICIDES

1.2.4.1 Stability of Carbamates.

Carbamates when applied are likely to enter various compartments of environment through leaching by water, volatilization into atmosphere, or sorption to various surfaces.[33] The fate of carbamates existence in the soil is decided by the principle factors like adsorptive forces (such as chemical bonding, physical binding and hydrogen bonding which helped in attaching the pesticides to the soil) and soil type (which is compiled by taking into consideration soil pH, soil moisture, soil temperature , organic matter and clay colloids in soil).[34,35] It was found in the studies conducted on the carbamates that, they generally persist in neutral , acidic medium and mild alkaline medium.[36] Moreover, being less water soluble they are plausible to be adsorbed on the soil via coordination and protonation which could upheaved the persistence of carbamates in soil.[37] The principle factor attributed to the stability of selected carbamates in envoironment are showed in tabular form as Table 2. [38,39,40]

Table 1.2: Carbamates and their stability in the envoironment.

Name of Carbamates Pesticides	Stabilty of Carbamates in Envoironment
Aldicarb	Generally found stable in neutral, acidic, and weakly alkaline media. Also, found stable at higher temperature upto 100^0C. Aldicarb has been shown to decompose more slowly in soils than in plants, and to have a $t^{1/2}$ of 7 to 12 days which varies according to soil type.
Aminocarb	Through reported studies it was found that in environmental conditions like (stream water and pond water) $t^{1/2}$ found was 8.7 days (pH 7.1) and 4.4 days

	(pH 5.5).
Bendiocarb	Primarily found stable to light and heat. Moreover, hydrolyzed more slowly in neutral and acid media. Reported $t^{1/2}$ in(water at 25^0C) is 4 days (pH 7). $t^{1/2}$ for (soil) ranges from 0.5 to 10 days depending upon soil type, moisture and temperature.
Benfuracarb	It is found stable in neutral and weakly alkaline media, but unstable in acid and strongly alkaline media. Decomposes only at higher temperature of 225^0C. $t^{1/2}$ in(water) is 3h;and (soil) 4 to 28 h.
Benomyl	In the neutral pH it is found to be highly stable and decomposed only by strong acids and strong alkali. One of the salient feature of benomyl is rapidly converted into carbendazim in the environment, with a $t^{1/2}$ of 2 and 19 h in water and in soil, respectively.
Bufencarb	Mostly found stable in neutral and acidic medium and decompose only in highly alkaline medium.
Butylate	It is found stable in neutral medium. Also, found thermally stable up to 200^0C. $t^{1/2}$(soil) 1.5 to 10 weeks.
Carbanolate	Neutral and acidic medium help in persisting the stability whereas highly alkaline media leads to unstability.
Carbaryl	Neutral and weakly acid conditions leads to the stability of it as well as found stable to light and heat. $t^{1/2}$ in (sea water: 20^0C) is 4 days at (pH 8.0),(river water) 4.6 days (pH 7.5). Under aerobic conditions, carbaryl (1 mg/l) degrades with $t^{1/2}$ 7 to 14 days in sandy loam and 14 to 28 days in clay loam.
Carbendazim	Chiefly found stable in acids and more importantly found. stable for at least 2 years below 50^0C. $t^{1/2}$ in (water) 350 days (pH 5 and pH 7), 124 days (pH 9).

	$t^{1/2}$ (soil) 8 to 32 days under outdoor conditions.
Carbofuran	Acid and neutral media contribute towards the stability of it. Thermally, they are found stable upto 150^0C. $t^{1/2}$ in (river water: environmental conditions) 13.5 days (pH 7.5), and (pond water: 26 to 30^0C) 2.3 days (pH 7.8 to 8.5), and (deionized water: 27 ± 2^0C) 36 days (pH 7), and (deionized water: 27 ± 2^0C) 1.2 h (pH 10). $t^{1/2}$(soil) 30 to 60 days.
Chlorpropham	It is found stable in acidic and alkaline media and in UV light. Decomposes above 150^0C. $t^{1/2}$in (distilled water) 4 weeks. $t^{1/2}$in(soil) 65 days (15^0C), 30 days (29^0C).
Cycloate	Strong acids and alkali medium may lead to instability of it. However, normal and mild alkali medium contributed towards stability of it. Thermally, found stable upto (120^0C). $t^{1/2}$(soil) 4 to 8 weeks.
Diallate	Its $t^{1/2}$ reported in (soil: heavy clay) 5 to 6 weeks, (soil: loam) 4 weeks. Generally, found stable in neutral and acidic medium.
Dimetilan	It persisted in neutral medium and only hydrolyzed by boiling with strong acids and alkalis leads to decomposition.
EPTC	It is found stable in neutral and mild alkali medium upto 200^0C. $t^{1/2}$(soil: heavy clay) 4 to 5 weeks, (soil: loam) 4 weeks.
Ferbam	Stable to storage in closed containers. It tends to decompose on exposure to moisture and heat, and on prolonged storage.
Landrin	$t^{1/2}$ (water: 38^0C) 42 h (pH 8).
	It is found stable under normal, dry storage

Mancozeb	conditions. From the reported studies $t^{1/2}$ in (water: 25^0C) 20 days (pH 5) and 34 h (pH 9).$t^{1/2}$ in(soil) 6 to 15 days.
Maneb	It is especially found stable to light. $t^{1/2}$ found in (soil) is 25 days (loamy sand in dark, aerobic conditions).
Methiocarb	Normal, acidic and mild alkaline media attributes towards the stability. But found, unstable in highly alkaline media. $t^{1/2}$ in (water: 22^0C) . 1 year (pH 4), 35 days (pH 7), 6 h (pH 9). Degradation in soil is rapid.
Methomyl	At room temperature, aqueous solutions undergo slow decomposition. Highly unstable in alkaline medium. $t^{1/2}$ (ground water) < 5 h.
Mexacarbate	$t^{1/2}$ (non sterile river water: 20^0C) 9.1 days (pH 8.2), (sterile river water: 20^0C) 6.2 days (pH 8.2 to 8.4), (buffered water: 12 to 138C) 2 weeks (pH 7.4),(buffered water: 20^0C) 25.7 days (pH 7.0), (buffered water: 12 to 13^0C) 2 days (pH 9.5).
Mobam	Neutral and acidic media leads to the stability of it .While, found unstable in alkaline media. It is also found stable to heat up to 100^0C.
Molinate	It is found stable for at least 2 years at room temperature and at least 2 months at 120^0C. Relatively stable to acidic and alkali medium (pH 5 to 9) at 40^0C. $t^{1/2}$(aerobic soil: pH 5 to 6) 8 to 25 days, (flooded soil) 40 to 160 days.
Nabam	Chiefly found stable as an aqueous solution. It is found to form a continuous film on plant surfaces, which is said to become insoluble in water. On aeration, aqueous solutions deposit yellow mixtures of which, the main fungicidal components are sulfur and

	etem.
Oxamyl	Primarily, stable in acidic medium than that of neutral and alkaline medium. $t^{1/2}$ in soil is 4 to 20 days which varies according to the soil. $t^{1/2}$ in (water) is 31 days (pH 5), 8 days (pH 7), 3h (pH 9).
Pebulate	It is found to resisit heat up to 200^0C., $t^{1/2}$ in(water: 40^0C) 11 days (pH 4 and pH 10), 12 days (pH 7). $t^{1/2}$ in(soil: heavy clay and loamy) 2 to 3 weeks.
Pirimicarb	It is found stable in neutral medium and can be kept for more than 2 years under normal storage conditions.$t^{1/2}$ in (soil) 7 to 234 days, depending on soil type.
Propham	Thermally, found stable up to 100^0C and also stable to neutral medium. Lower temperature helped in the persistence of propham. Moreover, not sensitive to light. $t^{1/2}$ found in (soil) 15 days (16^0C), 5 days (29^0C) and $t^{1/2}$ (distilled water) 8.5 weeks.
Propoxur	It is highly water soluble and has a high potential for groundwater penetration. $t^{1/2}$in soil 28 days. The effect of pH is found prominent on the $t^{1/2}$valueasin (river water: environmental conditions) 16.1 days (pH 7.5), (buffered water: 20^0C) 16 days (pH 8.0), (buffered water: 20^0C) 1.6 days(pH 9.0), (buffered water: 20^0C) 4.2 h (pH 10.0).
Swep	It is found stable in neutral medium while, hydrolyzed slowly in acid and alkaline media.
Thiobencarb	Primarily found stable at pH 5 to 9 for 30 days at 21^0C. $t^{1/2}$ in (soil) was reported 2 to 3 weeks in (aerobic conditions) or 6 to 8 months (anaerobic conditions).
	It is found to be stable at pH 6 however, rapidly

Thiodicarb	hydrolyzed at pH 9 and slowly at pH 3. Thermally, found stable up to 60^0C. $t^{1/2}$ found in (soil) varies from 3 to 18 days according to the type of the soil.
Thiofanox	Stable under normal storage conditions. Relatively stable to hydrolysis at pH 5 to 9 (under 30^0C). Decomposed by strong acids and alkalis.
Thiophanate Methyl	Generally stable under acidic condition as $t^{1/2}$ at 25°C is 867 days (pH 5), 36 days (pH 7), 0.7 days (pH 9) and stable for 14 days at 54°C; for 3 years at room temperature.
Thiram	It is found to get easily decomposed in acid media. Some deterioration on prolonged exposure to heat, air or moisture. $t^{1/2}$ in (water: 22^0C) 128 days (pH 4), 18 days (pH 7), 9 h (pH 9) and $t^{1/2}$ (sandy soil: pH 6.7) 12h.
Tiocarbazil	It is found stable at pH 5.6 to 8.4. Slightly decomposed after 30 days at 40^0C in aqueous ethanol at pH 1.5.Strongly adsorbed in soil and $t^{1/2}$ in (soil/water of a rice field) found is 8 to 15 days.
Triallate	Triallate exhibited the ability to adsorb well to loam and clay soils and is not readily dissolved in water (4ug/ml). It is found stable under normal storage conditions and its decomposition temperature is 200^0C. $t^{1/2}$ in (soil: heavy clay and loamy) 10 to 12 weeks and 8 to10 weeks.
Vernolate	Generally found, stable in neutral media, and relatively stable in acid and alkaline media. It could resist the heat up to 200^0C. $t^{1/2}$(water: 40^0C) 13 days (pH 7). $t^{1/2}$(soil) 8 to 16 days (27^0C) and 2 months (48^0C).
Zineb	It is found to be adsorbed strongly in soil particles and usually does not move below the upper layer of soil.

	Its bioactive $t^{1/2}$ in the field is 16 days. When precipitated from a concentrated solution, a polymer is formed which is more persistence and less fungicidal.
Ziram	In the reported studies, $t^{1/2}$ of 30 days has been estimated for ziram in field. If ziram gets to the bottom of bodies of water, it might persist for months.

1.2.4.2 Degradation of Carbamates.

Breakdown of carbamates pesticides into the nontoxic or less harmful compounds in the environment is achieved by three types of degradation, classified as chemical degradation, photo degradation and microbial degradation.[41]

i. Chemical degradation - It is the process in which breakdown of pesticides took place without the participation of living organisms. The mechanism involved in chemical degradation of carbamates varies according to their sub classes. In case of N-Methylcarbamates, successive oxidation was reported in soil which resulted in formation of Co_2 as the final product. While in case of oxime N methyl carbamates, such as (oxamyl and methyoml) rapid degradation is reported by a redox pathway involving Fe(II).[42] Benzimidazole carbamates like (carbendazim and benomyl) was found to be slowly hydrolysed to 2-aminobenzimidazole.[43]

ii. Photo degradation- In this process of degradation, breakdown of pesticides is done by sunlight. Carbamate pesticides undergo aliphatic side-chain oxidation, thioether oxidation, hydrolysis, methylation, N-dealkylation, and rearrangement reactions when exposed to light.[44] In the presence of UV light, carbamate pesticides in water undergo cleavage of the ester bond (or N-O bond) and hydroxylation of the methyl which further undergo photodecomposition to form a number of products. The pesticides like (Carbaryl or propoxur) undergo cleavage of the ester bond, which resulted in the production of the phenol or heterocyclic enol of the carbamate ester.[41]

iii. Microbial degradation – In such kind of degradation, microorganisms acted as protagonist. The pesticide is absorbed into the cell membrane of the microbe and the enzymes present in the microbe breakdown the pesticide into smaller fragments with minerals as the final end-product. It is mainly of two type aerobic and anaerobic , the first layer of soil generally undergo the degradation by aerobic mechanism while below the first layer anaerobic mechanism persisted.[41]

Carbamates pesticides are transformed in general via oxidation, hydrolysis and through conversion of conjugated compounds to hydroxy products into the more water-soluble molecules and degradable products. In specific, oxime N- methyl carbamates (Aldicarb, methomyl, and oxamyl) undergo hydrolytic metabolism to form pesticide oxime as a major product, further degraded to carbon dioxide in soil.[45] While in case of N-phenylcarbamates, such as (propham and chlorpropham) degradation process undergo three successive steps from hydroxylation, oxidation and hydrolysis to convert into the hydroxy acetanilide.[42] Furthermore, degradation of N-methyl carbamates was found to be occurred through hydroxylation.[46] Most important microbes which are used as degraders for carbamates are found within the genuses Arthrobacter, Aspergillus, Alcaligenes, Bacillus, Corynebacterium, Flavobacterium, Fusarium, Nocardia, Penicillium, Pseudomonas, and Trichoderma.[47,48]

1.2.5 TOXICOLOGICAL EFFECTS OF CARBAMATES PESTICIDES

Carbamates are intentionally toxic and any agent designed to kill pests is of potential danger to other non-target organisms, such as humans, avians and wild mammalians. The deleterious impacts of carbamate could be perceived by apprehending, the harmful impact of cholinesterase-inhibiting ability of the carbamates.[49, 50]

1.2.5.1 Threatening Impacts of Cholinesterase-inhibiting ability of Carbamates.

The hazardous impacts of cholinesterase inhibiting chemicals on the non- target species predominately occurred by inhalation, ingestion and dermal exposure of the carbamates. In case of human, dose frequency and length of exposure of carbamates caused the varied effect ranging from acute toxicity, intermediate syndrome and chronic toxicity.[51, 52]

i. Acute Toxicity: The acute poisoning with carbamates pesticides chiefly occurred because of the overstimulation of the both muscarinic and nicotinic Ach receptors by accumulated acetylcholine (Ach) resulted from AchE inactivation.[53] Moreover, the cholinesterase inhibiting abilty of carbamates causing the varied disastrous impact on the central nervous system, immunology and reproductive system. Exposure to doses of the carbamates leads to the origin of injurious impact on the central nervous system which included, tremors, mental disturbances, incoordination, cyanosis and coma.[54]

As, carbamates mode of action is based on the inhibition of serine hydrolase activity which is linked to the proper functioning of several immune functions. Therefore, they were found altering the lymphocytic cholinergic signals via inhibition of acetylcholinesterase.[55] The

outcome of the impact could be the overstimulation or the downregulation of cholinergic receptors which provoked the risk of inflammation and cancer. In addition, the hyperactivity of the cholinergic receptors induced the oxidative stress which could disturb the various parts of the cellular signaling. Furthermore, carabamtes were reported profoundly altering the hormonal levels by effecting the cortisol secretion from adrenal gland and gonadotropin-releasing hormone GnRH- receptor signaling. Hence, leading to the malicious endocrine disruption.[56]

Alterations that occurred at the hormonal level could extend their adverse effect on the male and female reproductive system. As, menstruation, pregnancy, and lactation in female and sperm production as well as androgen synthesis in male are dependent on the secretion of gonadotropin-releasing hormone (GnRH) . Other harmful effects associated with reproductive organ, is insufficient neurodevelopmental in child and childhood leukemia.[57]

Other side effects are associated with muscarinic and nicotine receptors which included the symptoms like hypersalivation, gastrointestinal cramps, urinary incontinence, miosis and bradycardia (related to muscarinic signs) and tremors, muscle weakness, blurred vision, paralysis and muscle fasciculation (related to nicotine signs).[58,59]

ii. Intermediate Syndrome: The intermediate syndrome (IMS) is a delayed-onset of muscular weakness occurred after the acute cholinesterase inhibitor poisoning. It is named so because it can occur between 24-96 hours (1-4 days) after resolution of the acute cholinergic toxidrome.[60] The IMS is characterized by the weakness in several cranial motor nerves, neck flexors, and extraocular, palatal, nuchal , proximal limb as well as acute paralyses. The root cause of the intermediate syndrome could be the defect at the neuromuscular end plate and post synaptic level involving nAChRs.[53]

iii. Chronic toxicity : Prolonged exposure of the carbamates pesticides are suggested to cause the skin and eye irritation, hemopoietic alterations, degeneration of the liver, kidneys and testes. In addition some carbamates exhibited the histopathologic changes in the nervous system and also produced the tetragenic effects.[51, 53]

In a similar manner, carbamates are believed to adversely affect the muscarinic and nicotinic receptors of the birds as well as wild mammals by causing the accumulation of acetylcholine at the sites of cholinergic receptors. The harmful impact on the muscarinic receptors will directly cause the damaging of the exocrine gland, smooth and voluntary muscles in the lungs, gastrointestinal tract and eye.[61] Moreover in case of nicotine receptor, skeletal muscles and sympathetic ganglia are badly affected. Birds and mammals have A esterase enzyme which is

used to hydrolyze the carbamates, is also inhibited by it. Thus, reducing their ability to metabolize or detoxifying the carbamates.[62] In addition, carbamates could also alter multiple neurotransmitters which are essential in functioning of the body in an integrated manner. As, alteration in central nervous system totally disrupted the endocrine, reproductive, behavioral and immune system. It was also examined, peculiarly in case of birds that singing ability is controlled by cholinergic system and their inhibition by carbamates impact their ability to sing. Therefore, decrease its chances of successfully attracting a mate or establishing a territory.[63]

Toxicity of the carbamates is not only exhibited through its anticholinesterase ability, but their unmanaged used for agricultural purposes is also of the great concern. As, it created the several anomalies in soil health. For that purpose it is essential to unveil this essential aspect.

1.2.6 ECOLOGICAL EFFECTS OF CARBAMATES.

Ever since, the usage of the chemical pesticides increased in the environment the negative emanation also raised up. Exhausted from the years by the practice of chemical pest, soil quality deteriorated and its devastated effects become clearly visible. As, it led the decimation of the beneficial microorganism as well as the morphological parameters and the nutritional value of the food crop. It ultimately affected the whole food chain and negatively influenced the ecosystem. The facts ascertaining above statements for carbamates are highlighted as follows.

1.2.6.1 Adverse effect of the carbamates on the soil microorganism.

A renowned microbiologist, Jacob once remarked," a soil devoid of the microorganism is the dead soil". Primarily, microorganisms acted as a cook and serve the platter of the humus for the plants. Humus consisted of the organic matter containing dead microbes, plant, human and animal waste decomposed by the soil microorganisms.[64] Apart from providing nutrition to the plant, humus helped soil to absorb and retain moisture and promotes the formation of good soil structure. The plant required carbohydrates for the respiration and they used photosynthesis to produce it from the inorganic substances. In general, even the rapid growing plants like corn and sugar cane only fix a maximum of six to seven percent of the sun's energy.[65] Alternatively, there are photosynthetic bacteria like (*Rhodopseudomonas* spp), lactic acid bacteria, (*Lactobacillus* spp) and yeasts (*Saccharomyces* spp) which fulfill their requirement for it.These micro-organisms are special types of bacteria that contain light

absorbing pigments and reaction centers which make them capable of converting light energy into chemical energy.[66]

In a similar manner, biological nitrogen fixation contributes to 60% of the nitrogen fixed on earth, which mainly relied upon the *Cyanobacteria*, *Actinomycetes* and *Rhizobium* species. Above that, the metal ions such as iron, copper, zinc, and manganese, are involved in many crucial processes of the plants are absorbed using the strains like *Bacillius*, *Arthobacter*, *Pseudomonas* and *Klebsiella.*[67] However, the usage of the pesticide caused the negative impact on the growth of the microorganism which can be well received by taking into consideration its effect on Microbial biomass and Micro biochemical reaction.[68]

i. Microbial Biomass: The extensive use of carbamates has the ability to adversely affect the proliferation of beneficial soil microorganisms. The growth of the microorganisms in the soil provides direct assessment of the linkage between its activity and other ecological processes. On investigating, population dynamics of soil microorganisms under the effect of carbamates such as (carbofuran, methyomyl, oxamyl and carbetamide). It was observed that, initially all the pesticides after application decrease the population of fungi and actinomycetes. Whereas in case of bacteria, population at lower dose of application initially increased and thereafter decreased at higher dose of pesticides. While in case of protozoa, population at both the studied concentration for all the studied pesticides decreased with time[69]. Specifically, the retardation in growth of bacteria like *Pseudomonas*, *Staphylococcus*, *Micrococcus* and *Klebsiella* and fungi like *Fusarium*, *Humicola* and *Rhizopus* was observed in presence of the carbofuran pesticides.[70, 71]

In a similar manner study reported, the application of the carbendazim carbamate decreased the diversity of the soil bacterial community from the1.43 in the control to 1.29 in treated soil. Its harmful effect found increased with the repeated applications.[72] Another study also reported, the over usage of the carbendazim has lead to the alteration in bacterial community composition and its negative impact is highly observed in γ-*proteobacterium species*.[73] The effect of the carbamates namely TMTD, sevin and dymid on the *Rhizobium* strain suggested that, pesticides concentration at particular incubation temperature has affected their strains.[74] Also, five carbamate insecticides effect was examined on the three cyanobacteria, *Anabaena* flos-aquae, *Microcystis* flos-aquae, and *Mirocystis* aeruginosa, and five green algae, *Selenastrum* capricornutun, *Scenedesmus* quadricauda, *Scenedesmus* obliquus, *Chlorella* vulgaris, and *Chlorella* pyrenoidosa.The acute toxicity of

the carbamate insecticides to the cyanobacteria and the green algae was in descending order carbaryl>carbofuran, propoxur, metolcarb >carbosulfan.[75]

ii. Microbial Biochemical Reaction: Microorganism's functions as a living catalyst that enables the vast number of the chemical processes to occur in soil. Although, they uses different metabolic strategies but in particular they depend upon the organic matter of the soil to achieve them. Such microorganisms mediated reaction constitutes the most significant aspect of the geochemistry and are also called as biogeochemical cycles.[76] For example, their ability to degrade organic carbon from the biomass, petroleum and xenobitic sources and returning to the atmosphere as the CO_2..

In a similar manner, they help to balance the nitrogen, oxyen , sulphur and phosphorous in the atmosphere. In addition, binding the atmospheric nitrogen and converting ammonia to nitrates for the plant utilization. They also secreted the high affinity chelating compound having the ability to bind with metal ions of the soil and transport it to the rhizosphere of the plant for their proper growth.[77]

The usage of the carbamate pesticides not only retarded the growth of microorganisms but also harmfully impacted the biotransformation associated with them. For instance, carbamates like(1-naphthol, sevin, dimetilan, trematan, NaDDC,dymid ,carbendazim, imazetapir, carbofuran and thiram) were found detrimental for the Rhizobia strains like (R. *leguminosarum*, R. *japonicum*, L. *corniculatus* and R. *meliloti*). As well as, they exhibited ability to reduce the nitrogenase activity in them. Therefore, inhibited their nodulation and nitrogen fixing capacity in plants.[78] Reported study also suggested that presence of the specific group like (ethyl groups than that of methyl group) attached to carbamates found more effective for inhibition.[79] Similar type of the effect was found in case of the *Pisum sativum* and *Vigna sinensis* plant, where nodule development was negatively affected under the influence of the carbamate pesticides.[80,81] Another emphasized point is its adverse effect on the dehydrogenase enzyme. It is consider as the important enzyme for the microbial respiratory processes. When examined under the influence of carbofuran, it was observed that pesticide strongly inhibited the dehydrogenase system in the bacteria like (Bacilius *subtilis* and Bacilius *sphaerics*).[82,83] Another study reported, the application of the carbofuran carbamate reduces the cell growth of the E.*gracillis* algae. It is one of the algae which acquire vitamin B_{12}(cobalamine) through symbiotic relationship with bacteria.[84,85]

1.2.6.2 Adverse effect of carbamates on the food crop.

Nutrients are involved in every step of plant life, their functions ranges from being structural unit to redox-sensitive agents.There are 25 elements which are regarded as essential or beneficial for the survival of plants in which carbon, nitrogen, hydrogen, and oxygen are regarded as four major elements which are taken up through both air and water, while remaining elements accumulate through the plant roots from the soil.The macronutrients include potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulfur (S); these are generally found in plants at concentrations greater than 0.1% of dry tissue weight. Each of them has the significant role to play for the development of the plant like nitrogen (N) play important part in the metabolic process involved in the synthesis and transfer of energy also, nitrogen, phosphorous (P) and magnesium (Mg) are essential component of the chlorophyll that is responsible for photosynthesis. In a similar manner, potassium (K) and sulphur (S) helps in the building of protein and calcium (Ca) is essential part of the cell wall structure.[86]

The micronutrients include iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), chlorine (Cl), molybdenum (Mo) and nickel (Ni); these generally are found at concentrations less than 0.01% of dry tissue weight.Their importance for the plants can be complied as ,iron (Fe) is important for the formation of the chlorophyll, Zinc(Zn) regulates the consumption of sugar, manganese (Mn) is involved with enzyme system used for the breakdown of the carbohydrates, copper (Cu) is used for the reproductive growth, boron (B) essential for the seed and fruit development, cholrine (Cl) aid plat metabolism, molybdenum (Mo) helps in the use of the nitrogen and nickel (Ni) is used as catalyst in enzyme used to help legumes plant for nitrogen fixation. Additional minerals which are found essential for the plants are ,cobalt (Co), sodium (Na), silicon (Si), selenium (Se), iodine (I) and vanadium (V).[87]

To acquire and sense the metal ions from the soil, different acquisition strategies have been adopted by the plants. Primarily, the symbiotic association is build by the plants with fungus which are termed as mycorrhiza, hypha and mycelium.[88] Secondly, chelates are used by the plant which is synthesized inside the plant. Such biosynthetic chelates, are classified as protein chelates (mugenic acid, histidine,nicotianamine, phytochelatins and metallothioneins), and non-protein chelates(organic acids and phytate). Despite of such associations and mechanisms, pesticide usage has declined the plant health. The harmful effects are not only concise to growth reduction, but also led to the metal ion depletion in the plant.[89]

i. Effect on the morphological parameters of the plants: The reported study depicted, that the usage of the carbaryl carbamate on the three aquatic plant species *Ipomoea* aquatica, *Pistia stratiotes* and *Hydrocharis* dubia has decreased its chlorophyll concentration.Moreover, chlorosis and necrosis occurred at the leaf margin of the treated plants and then they extended into the inner portion of the leaf blade. Finally, the leaves decayed and the plants died.[90] Similary, the delay of germination and growth process was observed in the tomato (*Solanum* lycopersicum) plant after the treatment with the primicarb carbamate.[91] The treatment of the thiophanate methyl at different concentration of the(0.25%, 0.50%, 0.75% and 1%) on the seeds of the *Vigna* radiata has led to decrease trends of germination percentage, seedling survival, plants height, number of branches per plant, number of pods per plant with increasing percentage of the pesticides. The consequences were fond same on treating the *Vigna* radiata plant with the carbofuran at varies concentration of the 5, 10, 25 and 50 ppm. While the 100ppm of carbofuran concentration, was found to be fatal for the growth of the plants.[92]

On applying the carbendazim carbamate on the seeds of the black gram (Phaseolus mungo) as per reported study, it was found that increase in concentrations of the fungicide decreased seed germination and growth parameters of black gramand it was more in 24 h treated seeds than that of 6 h treated seeds.[93] Another study related to carbendazim carbamate, also reported that germination percentage of the seed and biomass production of the crop plants (Cicer arietinum and Zea mays) plant has been affected by its usage. Even the report related to methomyl carbamates, affirmed that its usage reduced the photosynthesis and transpiration rate of the lettuce leaves.[94]

ii. Effect on the nutrients of the plant: The wide use of carbamates pesticides in the soil not only adversely affected the plant morphology. Also, equally found involved in hampering the micronutrient essential for growth of food crop. As reported study suggested, that higher doses of the carbofuran pesticides decreased the trace metal ion concentration of (Zn, Cu, Mn, Fe, Cr, Ni and Pb) in tomato, brinjal, corn and carrot plant . Also the high field rate application of the carbofuran, decreased the nutrients likeammonium (NH_4), nitrogen (N), nitrate (NO_3), phosphorous (P) and potassium (K) in the fields of the tomato plants.[95,96] Likewise, higher doses carbamates pesticides ([oxamyl 1,1 {methyl-2-(dimethylamine)-N-[(methylamino) carbonyl))oxy-]-2- oxoethanimidothioate (I); and [{N- Phenyl (ethylcarbamoyl) propyl carbamate (III)] ,carbamates) were found negatively influencing the metal ion concentration of (Zn, Cu, Mn, Fe, Cr, Ni and Pb) in tomato plant.[22]

Similarly, when the carbendazim with a purity of 100% was applied at three different rates of 1.3 mM, 2.6mM and 5.2 mM to the tobacco plant (Nicotiana tabacum). It was found that, increased in concentration has decreased the nutritional value of the plants.[97]Also, in case of the rice crop (Oryza sativa) the usage of the carbendazim carbamate has decreased the reducing sugars and free amino acid content of the plant.[98] When the effect of the aldicarb was observed on the chemical composition of the tobacco plant, it was observed that pesticide has decreased the concentration of its nicotine and crude protein content. Also, the potassium , total ash , water soluble and insoluble ash contents were decreased.[99]

1.3 Conclusive Statement and literature gap

Carbamates are purposefully introduced in the agricultural sector. As their mode of action, is well perceived and it successfully prevails in agriculture sector due to its short term toxic effect (occur due to anticholinesterase activity) than that of organochlorines and organophosphates pesticides. On the flip side, when applied to soil found generally persists in the acidic and mild alkaline medium in between the pH range of 5-7.The particular range of pH is also optimum for the plant growth. Above that, their persistence in soil intervened the health of the food crops. By making the micronutrients unavailable required for the proper plant growth. The kind of the carbamates activity which prevents the micronutrient to reach the plant is still not known. It laid the foundation to investigate the interaction of the pesticides with the essential metal ions. Moreover, carbamates are also known to adversely affect the microorganisms. Their harmful effect is not only limited up to restrict their population but also negatively influence the microbial biochemical reaction (such as nitrogen fixing capacity of Rhizobacteria).As, many biochemical reaction are associated with hmicroorganisms including their ability to uptake the metal ions from soil to make it available to the plant. Therefore, it encouraged to unearth the effect of the carbamates pesticides on the plant growth promoting microorganisms and their metal uptake ability for the plants. The key facts extracted are represented on next page. (Figure 1.4)

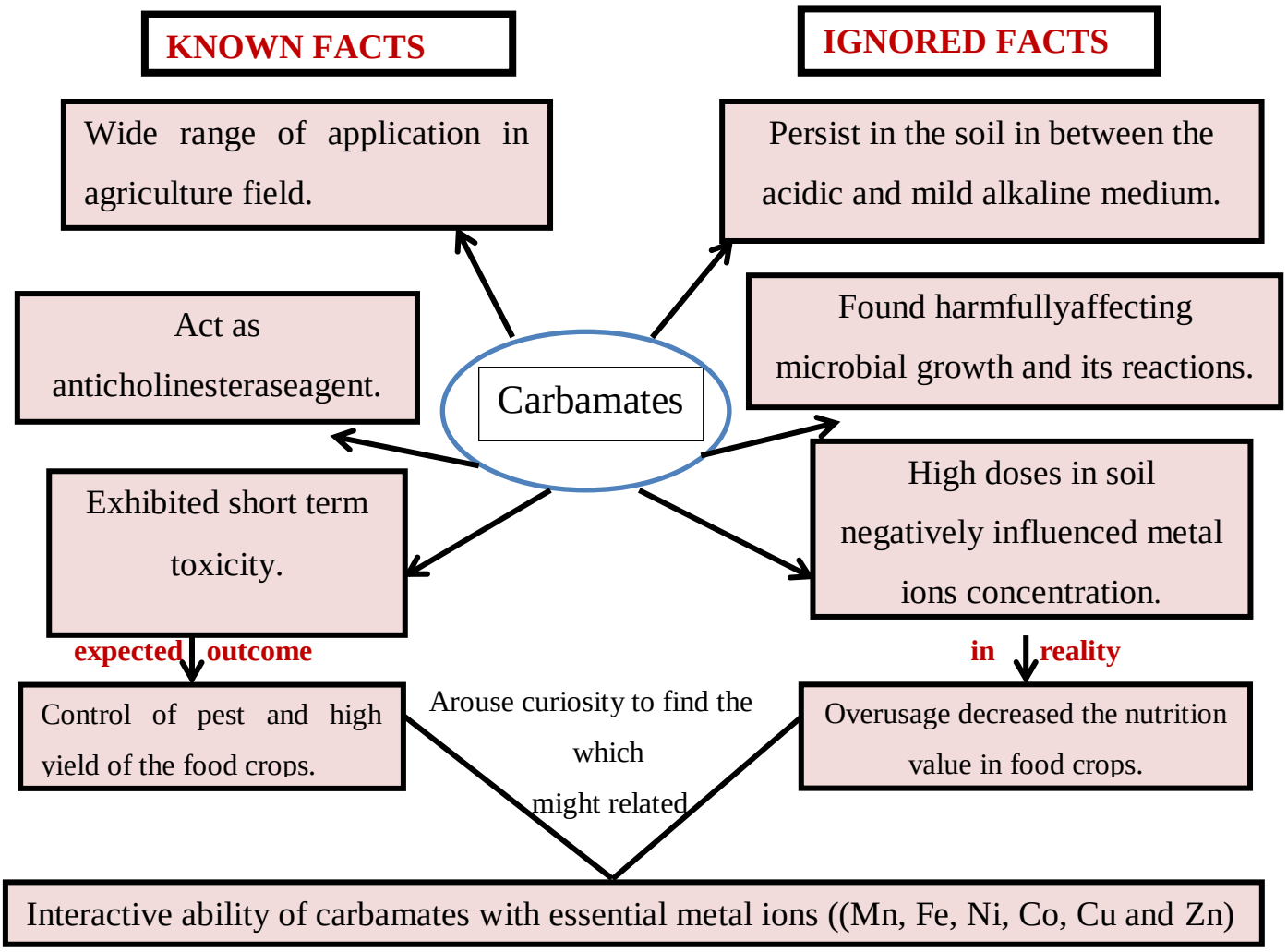
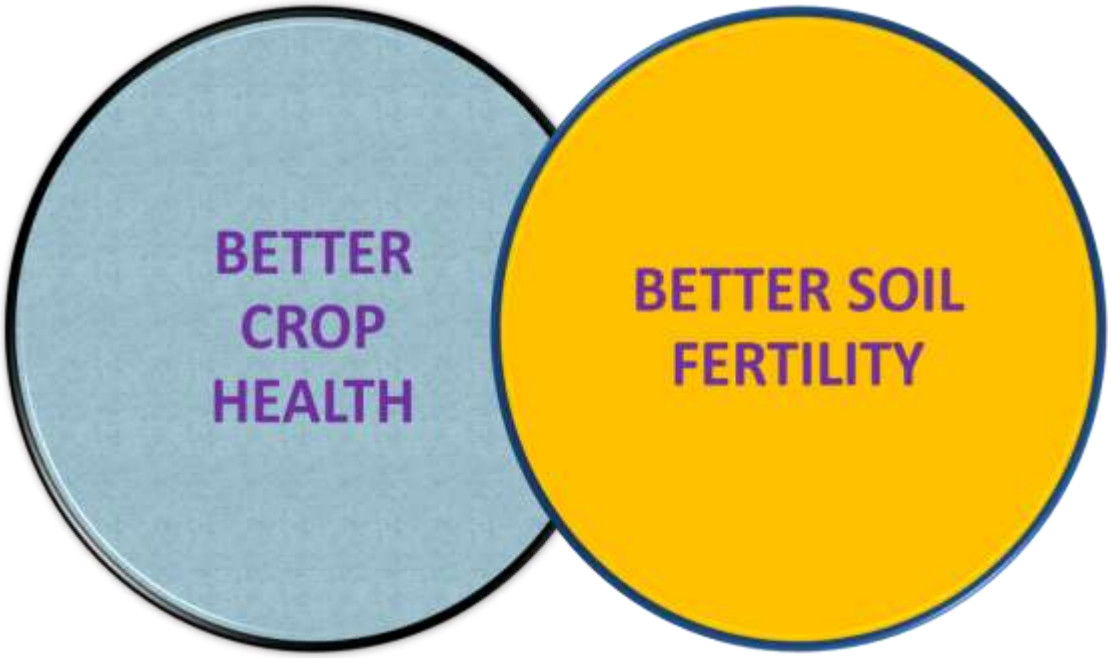


Figure 1.4: Schematic highlights of the key factors and the missing link.

1.4 SCOPE OF THE STUDY

When pesticides are applied on the soil only the certain amount of applied pesticide reach the target molecule while the negative externalities are raised from the remaining amount. These externalities included the adverse effect on the soil physical and chemical properties as well as the biological components (in particular microorganisms). The consequences would lead to the deterioration of the soil health, which ultimately affect the soil fertility. The plants grown under such circumstances would definitely remain deprived from the essential micronutrients. On top of that, might badly affect the health of living organisms consuming them including (animals and human being). Hence, would give rise to the devastated ecological balance. To evade such circumstances, it is requisite to evaluate the pesticides using the new parameter. For that purpose, maiden attempt is made to check the pesticides on the basis of their interaction ability with essential metal ions using carbamates. The new

specification is essential for optimizing the harmful effects of the pesticides. In addition, act as the foundation for the new horizon whose scopes lies in providing,



1.5 AIM & OBJECTIVES

1) To check the complexation ability of carbamates with essential metal ions present in the soil.

2) To determine the rate of formation of complexes.

3) To determine the effect of medium on rate of formation of metal –pesticide complexes.

4) To characterize the metal complexes using the various spectroscopic techniques.

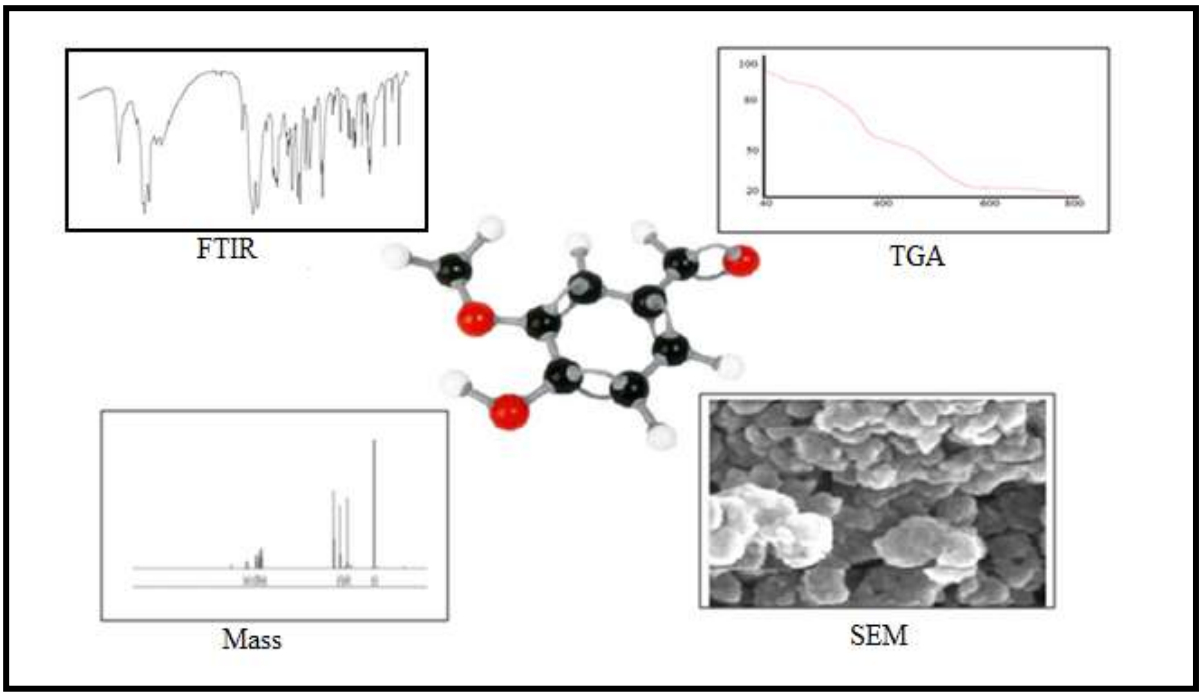
5) To determine the thermal/photochemical stability of these complexes.

6) To determine the effect of pesticides on the plant growth in vitro.

7) To determine the effect of pesticides on quality of farm products.

Chapter-2

Analysis of the interactive behavior of the carbamates with the essential metal ions



2.1. INTRODUCTION

When carbamates are applied over plant, it reaches to soil and get adsorbed over it. Adsorption of the pesticides on the soil particle is one of the chief components which determine their ability to retain and express their mode of action in soil. During this tenure carbamate may interact with soil organic material and from there, it can be transported or can effect transportation of organic/ inorganic material.

Study reported that adsorption occurred either via coordination, protonation, hydrogen bonding, dipole attractions and oxygen of >C=O group of pesticides.[100,101] The adsorption of the pesticides increased with the addition of the organic matters in the soil and tends to decrease the pesticide leaching.[102] Organic matter also displayed the ability to strongly interact with the trace metal ions chemically.[103] In such scenario, it could also be the possibility that the pesticides which exhibited the interaction with organic matters might also coordinate with metal ions. But, very little is known about the pesticides coordinating ability with essential metal ions. Knowledge is still lacking about the impact of such interaction on the environment.

In such scenario, we found it very- very interesting to see the impact of carbamate on essential metal ions via chelation/ complexation. By the study of interaction of carbamate with essential metal ions, we tried to answer the following questions in this chapter: 1. Whether carbmates interact with essential metal ions or not? 2. If yes, in which medium (in liquid medium or over soil surface) 3. How fast the interaction is? 4. In what kind of soil (acidic/ basic/ neutral) and temperature condition interaction will be more? 5. If metal complexes of carbamate are formed, how stable they are? 6. If metal complexes of carbamate forms, in which form it can exist over soil?

To unfold the fact, carbamates (Carbofuran, Carbendazim ,Thiodicarb , Thiophanate methyl and Methomyl) were interacted with metal ions (Mn (II), Fe (II),Ni(II), Co(II), Cu(II) and Zn (II)) in different ratios at different temperature range and pH. At the end, mode of their bonding with the different metal ions were analyzed using different spectroscopic (I.R, U.V and ^{1}H N.M.R) and spectrometry (Mass analyses) technique. Thermal stability of these compounds was analyzed using the thermogravimetry (TGA).

2.2. EXPERIMENTAL

2.2.1. Materials

For experimentation, technical grade (98% pure) Carbofuran, Carbendazim, Thoidicarb, Thiophanate methyl and Methomyl was provided by Gautmi Ltd, Hyderabad (India), which were recrystallized in appropriate solvents (Carbofuran in 1:10 :: isopropanol: water mixture; Carbendazim in DMF:diethyl ether mixture 1:20 (v/v); Thiodicarb in 1:10 acetone : water mixture (v/v), methomyl in 1:20 water-diethyl ether mixture and thiophanate methyl in 1:10 acetone- water mixture). The other laboratory chemicals of AR grade were purchased from Loba Chemie which included NaOH, HCl, DMSO, triethyl amine, acetic acid, and metal salts(zinc(II) acetate, copper(II) acetate, nickel(II) acetate, cobalt(II) acetate, iron(II) chloride, and manganese(II) acetate).

2.2.2. Instrumentation

Shimadzu-1800 UV-vis spectrophotometer in the wavelength range between 200-800nm and cubed length 1.0cm was used for the entire experimental process. Shimadzu-8400 FTIR instrument was used for determining IR spectra of the compounds and comparing the changes occurred in the functional groups of the selected carbamate pesticides after their interaction with the metal ions. For the analyses, 1.5mg of the compound to be characterized is mixed with 200mg of the potassium bromide in the entire experiments. Avance-II (Bruker)FT NMR Spectrophotometer was used for investigating the specific environment of the ^{1}H atom present in the molecule formed after the interaction of the selected carbamates with the trace essential metal ions (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}). For that purpose 5.0mg compounds were prepared in 600 µL d_6-dimethylsulfoxide (d_6-DMSO). All the NMR samples were capped well and put into NMR Instrument automated ballet for analysis. Waters,Q-Tof.Micromass Mass spectrometer instrument was used for analyzing the molecular mass and degradation pattern of the synthesized compound (formed after the interaction of the selected carbamate pesticide with the metal ions of study) by dissolving the appropriate quantity in dimethylsulfoxide. Perkin Elmer STA 6000Thermogravimetry (TGA) instrument was used for the thermal analyses of the compounds. The analysis was carried out over the temperature range from $40^{0}C$ to $850^{0}C$ in a flow of the nitrogen atmosphere with 30mL/min flow rate along with heating rate of $10^{0}C\ min^{-1}$.

2.2.3. Effect of pesticide on metal adsorbed silica.

Solution of 100 mg individual metal ion (of present study) in 40 mL water was thoroughly mixed with 40 g of neutral silica- gel (for column chromatography/ 60-120 mesh size by magnetic stirring for 15 minutes and then the mixture was left for 1 hour and then filtered. The metal impregnated silica were then dried in hot air oven at 60^0C for 24h and then filled in a column (30 cm × 2.5 cm), through which 20 mM pesticide in appropriate solvent (mostly acetone) were passed and changes were monitored. The product that comes out from the column were characterized. Expected changes on the surface of silica were analysed by Field-Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X- ray Spectroscopy (EDX) for each of the products.

2.2.4 Determination of rate of interaction of pesticide with metal ion in specific medium at different temperature and pH.

To determine rate of interaction of pesticide with metal ion in a solvent medium, 1.0 mmol of pesticide was dissolved in 50mL methanol, to which 0.5mmol of metal ion (Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)) were added and stirred on a magnetic stirrer along with heating mantle. The proceeding of reaction were monitored at all possible combinations of three different temperature (15^0C, 30^0C and 45^0C) and three different pH (pH = 5.0, 7.0 or 9.0) by using HCl and triethyl amine specifically for acidic and basic purposes.

2.2.5. Optimization of stoichiometry.

The optimization of the stoichiometric ratios of carbamates pesticides (Carbofuran, Carbendazim , Thoidicarb , Thiophanate methyl and Methomyl)with different metal ions were done by using the Job's method.[104] The Job's method was performed by taking different mole fractions of carbamate pesticide (mole fractions, 0.1 to 0.9) with specific metal ion in 50 mL of methanol (for thiophanate methyl, thoidicarb and methomyl and ethanol for carbofuran and carbendazim) solvent from the stock solution of 0.1 mM (of metal ion and pesticide both) at 25±1°C. The pH of all the experiments was 7.0±0.05, maintained by adding adequate amount of $(C_2H_5)_3N$/ HCl. At constant volume, the changes in the absorbance/ concentration of pesticide (Carbofuran, Carbendazim, Thoidicarb, Thiophanate methyl and Methomyl) was noticed by using UV-visible spectrophotometer. Final molar or stoichiometric ratio was observed by plotting Absorbance (at Y-Axis) vs Mole Fraction (at X-Axis). The lowest absorbance corresponds to mole fraction (X), directly related to the stoichiometric ratio, and it

was calculated as; X = [n/ (1 + n)], here X = mole fraction at lowest absorbance value, and n = stoichiometric ratio.

2.2.6. Synthesis of Carbamates (Carbofuran, Carbendazim ,Thoidicarb , Thiophanate methyl and Methomyl) complexes.

To synthesize the metal - pesticide complexes, 1.0mM of metal ion and known amount of pesticides (obtained by applying Job's method) were dissolved in 25mL of solvent (ethanol for carbofuran and carbendazim, methanol for thiophanate methyl, thoidicarb and methomyl) and continuously stirred in a round bottom flask. The product precipitated after completion of reaction (24h) was separated from reaction mixture using vacuum filtration through G4 crucible followed by thorough washing with five portions of each of 20mL methanol and water. Products were dried in a hot air oven at 60ºC for 12h and then kept in desiccator for 2-3 days before the characterization of the procured samples. Yield of the obtained product was found between 80 -85%. Examination of the dried product was done using I.R, UV-vis, ^{1}HNMR spectra and Mass fragmentation pattern.

2.2.7 Stability determination of the complexes.

For stability study, thermal degradation pattern for each of the product were obtained using TGA pattern.

2.3. RESULT AND DISCUSSION

2.3.1. Interaction of pesticide with essential metal ion adsorbed over silica.

Soil is the medium from which the plant takes up the metal ions. They are sorbed by the plant using different organic/inorganic natural ligand exist in the soil. Simultaneously, it is the same soil surface on which the pesticides are applied. Carbamates has the ability to interact with the soil matter through coordination, protonation, hydrogen bonding, dipole attractions and oxygen of >C=O group of pesticides.[100,101] It aroused the curiosity to check the interactive ability of the pesticide with soil sorbed metal ions. For that purpose, simulative method was used in which metal ions (single metal ion at a time) were adsorbed on silica and packed in column as the stationary phase. Pesticide dissolved in a solvent (acted as a mobile phase), when passed over metal adsorbed silica, showed evident color change. For example, when a saturated solution of thiophanate methyl was passed over Cobalt(II) acetate adsorbed over silica, color quickly changes from light orange to dark brown (Figure 2.1).

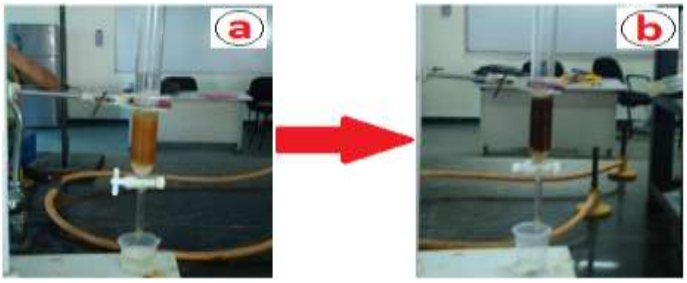


Figure 2.1: Represent the color change form yellow to dark brown after thiophanate methyl passed on Co(II) acetate adsorbed silica.

Expected changes on the surface of silica were analysed by Field-Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X- ray Spectroscopy (EDX) for each of the products.

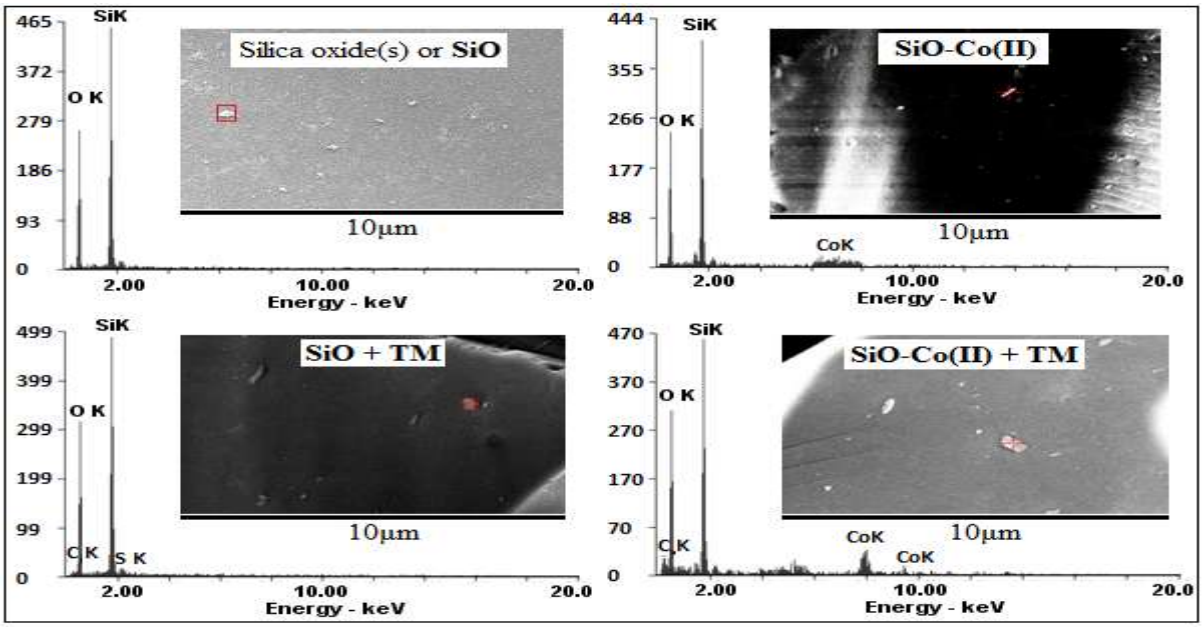


Figure 2.2: Representation of SEM-EDAX analysis of, silica oxide(s) (SiO); Co(II) acetate adsorbed silica (SiO-Co(II); after thiophanate methyl passed on silica oxide(s) (SiO + TM); after thiophanate methyl passed on cobalt(II) acetate adsorbed silica (SiO-Co(II) + TM).

In a similar manner, interaction of soil adsorbed metal ion (Co(II),Zn(II) and Fe(II)) with the pesticides carbofuran, thiodicarb and carbemdazim is shown in Figure 2.3.

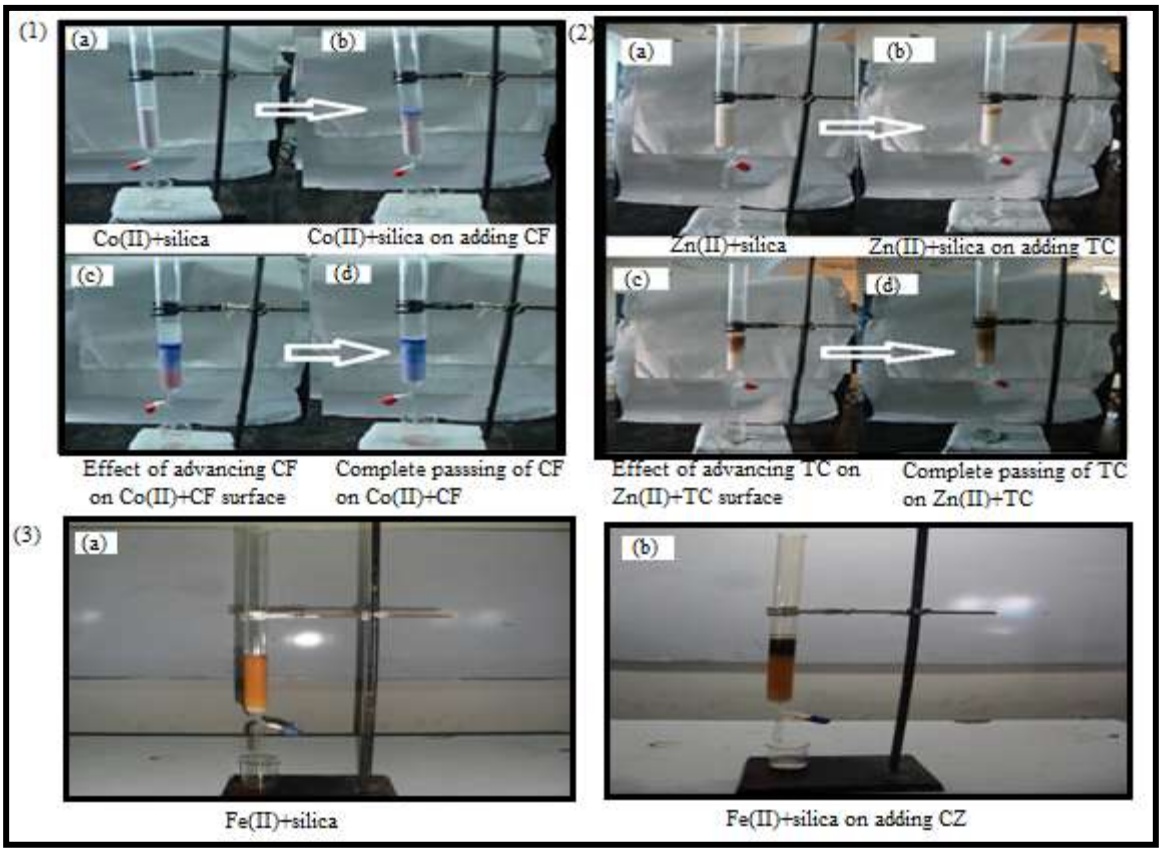


Figure 2.3: showing the changes in (a) Co(II) metal ion adsorbed silica on adding carbofuran (b) Zn(II) metal ions adsorbed silica on adding thoidicarb carbamate (c) Fe(II) metal ion adsorbed silica on adding carbendazim .

2.3.2. Optimization of Stoichiometry.

Metal ions are also present in the liquid medium and could show the possibility to interact with pesticides in that medium too. For that purpose, the interaction of the metal ion with the pesticides in the liquid medium was observed. To evaluate the interaction, primarily the stoichiometry was optimized. The observed results revealed that, at the maximum interaction of the carbamates (carbofuran, thoidicarb, thiophanate methyl, methomyl and carbendazim) pesticides with the metal ions (Mn(II),Fe(III),Co(II),Ni(II),Cu(II) and Zn(II)) absorbance value decreased to minima of the plot (as shown in Figure2.4) . For the different carbamates pesticides, the ratio of interaction was found different. The reported study suggested, that the curve indicated near 0.5 mole fraction symbolized the formation of the 1:1 metal-pesticide complex while at 0.62 and 0.75 indicated the formation of the 1:2 and the 1:3 complexes [105]. In general, it was deduced from the observation that trace essential metal ions mostly interact hwith carbofuran in 1:2 ratio,with carbendazim in 1:2 ratio and with thoidicarb in 1:2 (where 1 for metal and 2 for ligand), while, methomyl and thiophanate methyl should react in 1:1 ratios.

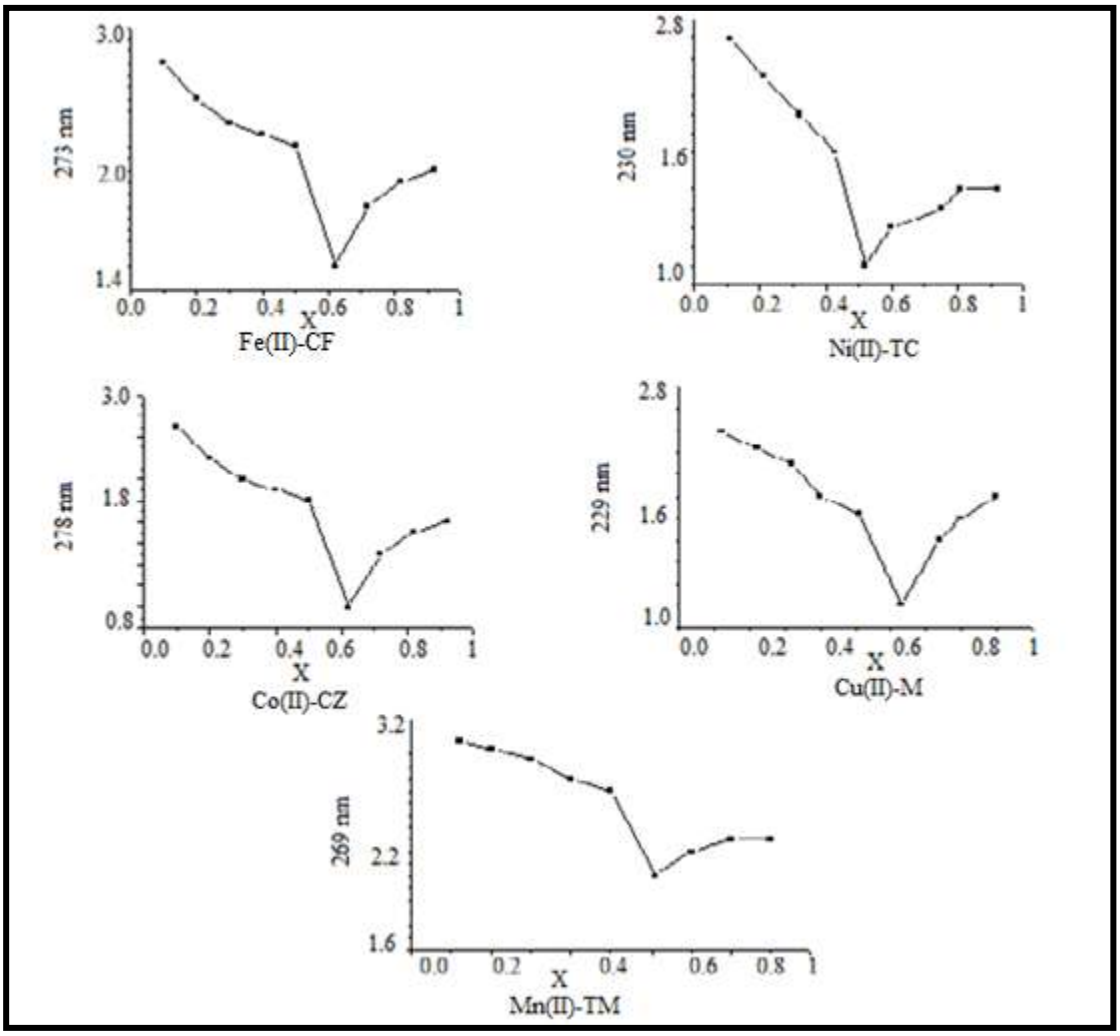


Figure 2.4: Job's plot for (a) carbofuran interaction with Fe(II) metal ion, (b) thoidicarb interaction with Ni(II) metal ion, (c) methomyl interaction with Cu(II) metal ion, (d) thiophanate methyl interaction with Mn(II) metal ion and (d) carbendazim interaction with Co(II) metal ions.

The interaction of the carbamates with the metal ions and the concentration at which interaction takes place is tabulated below:

Table 2.1: Stoichiometric ratio and the concentration of the metal ions as well ligand for the complex formation.

Metal ions	Metal to ligand stoichiometric ratio	Metal to ligand concentration (in mg) in 25 mL solvent
CF Ligand		
Mn(II)	1:3	0.017:0.066
Fe(II)	1:2	0.024:0.066
Co(II)	1:2	0.037:0.066
Ni(II)	1:2	0.026:0.066
Cu(II)	1:2	0.027:0.066
Zn(II)	1:2	0.027:0.066
CZ ligand		
Mn(II)	1:2	0.025:0.057
Fe(II)	1:2	0.024:0.057
Co(II)	1:2	0.037:0.057
Ni(II)	1:2	0.026:0.057
Cu(II)	1:2	0.027:0.057
Zn(II)	1:2	0.027:0.057
TC ligand		
Mn(II)	1:1	0.051:0.10
Fe(II)	1:1	0.048:0.10
Co(II)	1:1	0.074:0.10
Ni(II	1:1	0.053:0.10
Cu(II)	1:1	0.054:0.10
Zn(II)	1:1	0.055:0.10
TM ligand		
Mn(II)	1:1	0.051:0.10
Fe(II)	1:1	0.048:0.10
Co(II)	1:1	0.074:0.10
Ni(II)	1:1	0.053:0.10
Cu(II)	1:1	0.054:0.10
Zn(II)	1:1	0.055:0.10
M ligand		
Mn(II)	1:2	0.025: 0.048
Fe(II)	1:2	0.024: 0.048
Co(II)	1:2	0.037: 0.048
Ni(II)	1:2	0.026: 0.048
Cu(II)	1:2	0.027: 0.048
Zn(II)	1:2	0.027: 0.048

2.3.3. Rate of interaction of pesticide with metal ion at different pH and temperature.

Pesticides constitute an important component in agriculture development; their interaction with the metal ions would prominently affect the agricultural crop. To check the possibility of their interaction, progress of reaction were monitored at different temperature and pH by taking the basis of the agricultural aspects. It is evident from the above stated data, that the carbamates displayed the interactive ability with metal ions. As carbamates are applied to agriculture field, it is essential to check the factors which would affect its interaction ability with metal ions. Prior to the study, it is important to consider that healthy plant could only grow with in the pH range of 4.0 to 9.0 and temperature range of the 15^0C-45^0C.On inspecting the factors, interactive ability of the carbamates with metal ions was observed at pH 4.0,7.0 and 9.0 and the temperature 15^0C,30^0C and 45^0C. To make the solution acidic20µl of (0.3mM) of HCl and basic 20µl of (0.3mM) of triethyl amine was used. Observation revealed pronounced effect of temperature and pH, as rate of complex formation increases with increase of temperature and pH. The observed data is depicted in Figure 2.5.

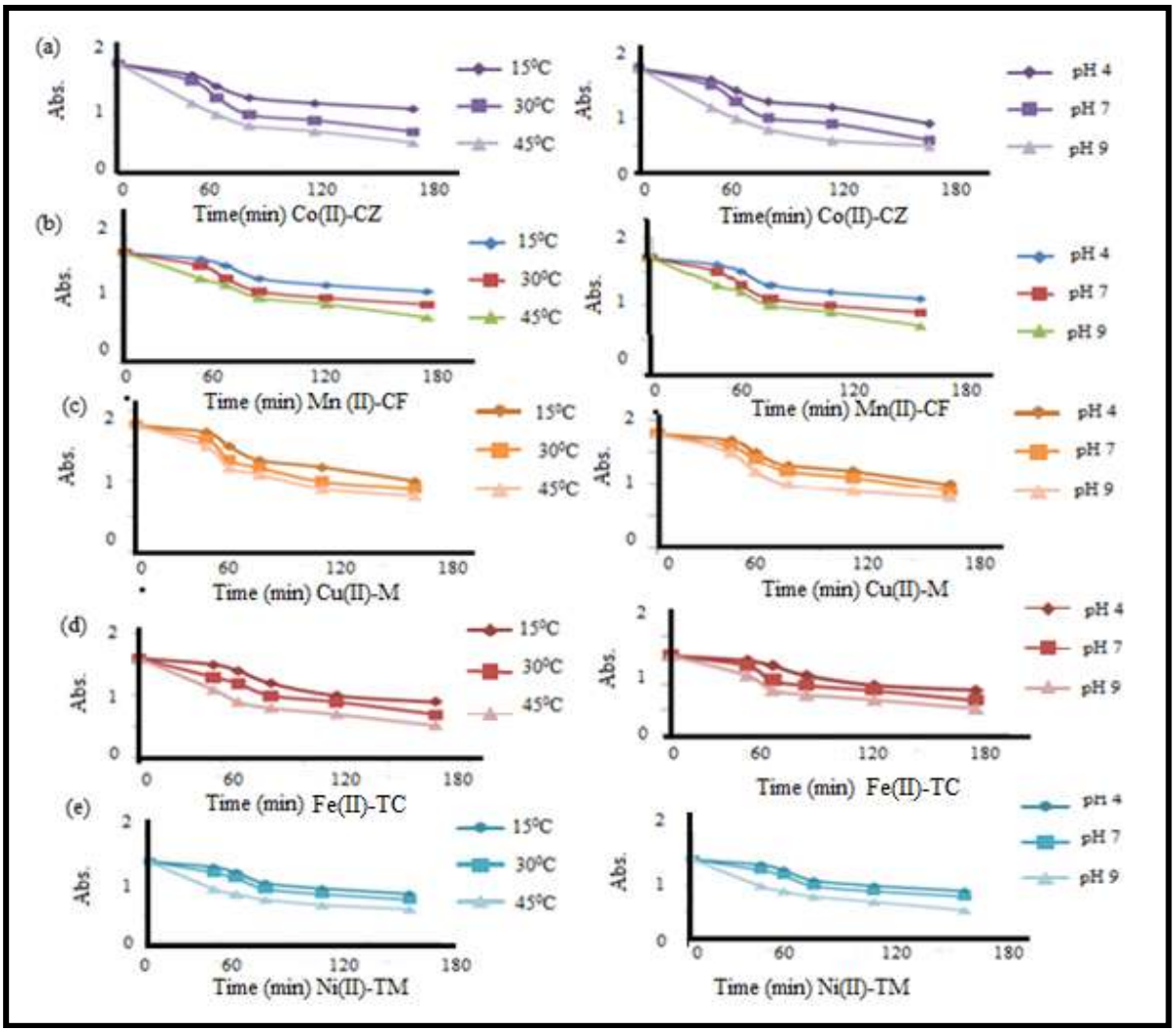


Figure 2.5: disappearace of carbamate with progress of reaction at three different temperature (15^0C, 30^0C and 45^0C at neutral pH) and three different pH at 25^0C. Figure a) shows disappearance of carbendazim on reaction with Co(II), at 240nm and 278nm b) shows disappearnce of carbofuran on interaction with Mn(II) at 273nm, c) shows disappearance of methomyl on interaction with Cu(II) at 229nm, d) shows disappearance of thiodicarb on interaction with Fe(II) at 230nm and e) shows disappearance of thiophanate methyl on interaction of Ni(II) at 269nm.

2.3.4. Site of interaction of carbamates with essential trace metal ions.

All carbamates (Carbofuran, Carbendazim, Thoidicarb , Thiophanate methyl and Methomyl) selected for our analysis had different geometry and therefore may have different chelation strategy with different metal ion in accordance with hard- soft acid base principle. Therefore, hereby we are discussing mode of interaction of individual carbamate with metal ion separately.

Characterization of all the formed complexes are difficult, because of insolubility of most of the formed complexes, therefore we only attempted to determine mode of interaction of pesticide with essential trace metal ions.

2.3.4.1. Carbofuran(CF) carbamate

2.3.4.1.1. UV-visible analysis of CF metal complexes.

Most of the formed metal complexes of carbamates (and therefore of carbofuran) are insoluble in almost all known laboratory solvents and solid state UV-vis spectra is too broad to analyze. The information (reaction progress stoichiometry etc.) about such interaction was therefore obtained by using UV-vis spectrum of carbofuran itself. Progress of reaction of carbofuran interaction with metal ions were observed by determining the percentage of the carbofuran consumed in the reaction after every half an hour of the reaction in progress and concentration was calculated using the Beer-Lambert's law at 273nm. The calculated values were subtracted from the initial value to get the amount of the ligand used in the reaction (see Figure 2.6). With the progress of the reaction the absorbance value of the ligand decreased. The amount of the concentration (in moles) consumed of the ligand is calculated with different interval of time. It is observed that maximum amount of the ligand is consumed within 5.0h of the reaction. In a similar manner, the consumed concentration of the CF ligand is deduced in percentage and tabulated in Table 2.2.

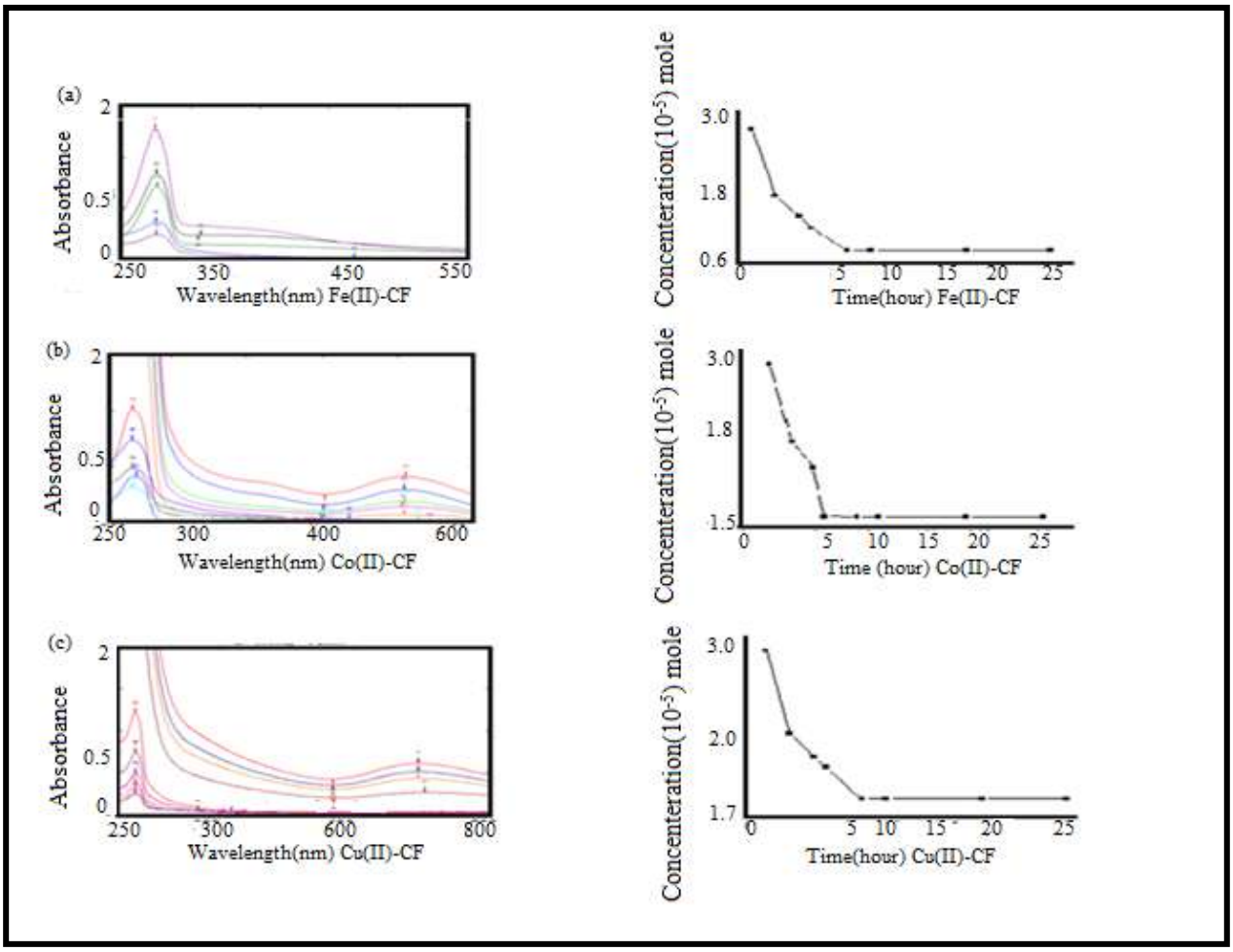


Figure 2.6: (a) Reaction progress during the Fe(II)-CF, Co(II)-CF and Cu(II)-CF interaction by UV-vis overlay curve (b) Plot depicting the concentration of the decrease in concentration of CF ligand with time.

Table 2.2: The percentage of the Carbofuran consumed after the interaction

S.NO	% of the carbofuran consumed with the time(h)						
	CF metal complexes	1	2	3	5	8	24
1	Mn(II)-CF	37	44	47	53	63	81
2	Fe(II)-CF	24	37	39	59	68	76
3	Co(II)-CF	26	35	40	52	51	49
4	Ni(II)-CF	24	31	35	43	42	40
5	Cu(II)-CF	22	28	31	39	38	39
6	Zn(II)-CF	20	26	30	33	35	37

* CF = carbofuran.

2.3.4.1.2. FTIR analysis of the CF metal complexes.

FTIR analysis of the CF metal complexes with respect to the CF ligand depicted that after the complex formation the shift and the broadening of the N-H stretching and bending peak was

observed towards the lower wavenumberwith respect to the N-H peak of the carbofuran ligand. The significant shifts observed in the range of 5-40$cm^{-1}$for N-H stretching band and the shift of 2-15cm^{-1}in case of the N-H bending, indicate the participation of the N-H bond in the complex formation. Very minor changes are observed in case of the C=O stretching peak. On contrary in case of the C-O and C-N stretching, the decrease in the peak intensity with respect to the peak of the carbofuran ligand was found with in the range of 20-35cm^{-1} for C-O and 5-10cm^{-1} for C-N. Small vibrational frequencies in the range of the 550-400cm^{-1} were also observed for metal-oxygen and metal-nitrogen bond. The deduced I.R frequencies for the CF ligand and the complexes are tabulated below in Table 2.3.

For the better understanding of the changes observed, IR spectra of carbofuran (CF) and some of its metal complexes are shown in Figure 2.7. It depicted the broadening of the N-H stretching, N-H bending, C-S stretching and C-N stretching in case of the metal-CF complex with respect to the CF ligand. N-H stretching peak was found to be shifted to lower wavenumber in case of metal-CF complex. At around 1724cm^{-1}, C=O stretching frequency were in general found not to be shifted from its place, but its intensity has decreased showing nitrogen may be a site of interaction, which is in hyperconjugation with C=O. Also, C-O stretching peak of carbofuran were also found to be shifted towards lower wavenumber indicating ring oxygen may be another mode of interaction with metal ions. The formed products were also characterized by M-O and M-N band in case of metal complexes.

Table 2.3: I.R absorption in cm^{-1} of the Carbofuran and its metal complexes

Type of IR-frequency	CF	Mn(II)-CF	Fe(II)-CF	Co(II)-CF	Ni(II)-CF	Cu(II)-CF	Zn(II)-CF
N-H stretching	3363m	3352m	3327m	3350m	3342m	3362m	3367m
C-H stretching	2980w	2987w	2982w	2998w	2990w	2992w	2993w
C=O Stretching	1718s	1724m	1723m	1724m	1730m	1721m	1724s
N-H bending	1525s	1532m	1593.2s	1556s	1542s	1548s	1530m
C-O stretching	1377s	1338m	1344.4s	1338m	1342m	1344m	1352m
C-N stretching	1236m	1235s	1228m	1234m	1230m	1235m	1239.3m
C-C Stretching	1197w	1135s	1149w	1161w	1154w	1151w	1158.3m
M-O	NA	546s	541.5s	521s	518s	428m	435w

stretching							
M-N stretching	NA	446s	422.3s	477s	464s	Not Obtained	Not Obtained

*s= strong, m = medium, w = weak and represent intensity of the IR absorption peaks. M-O and M-N represent metal- oxygen and metal- nitrogen stretching frequencies.

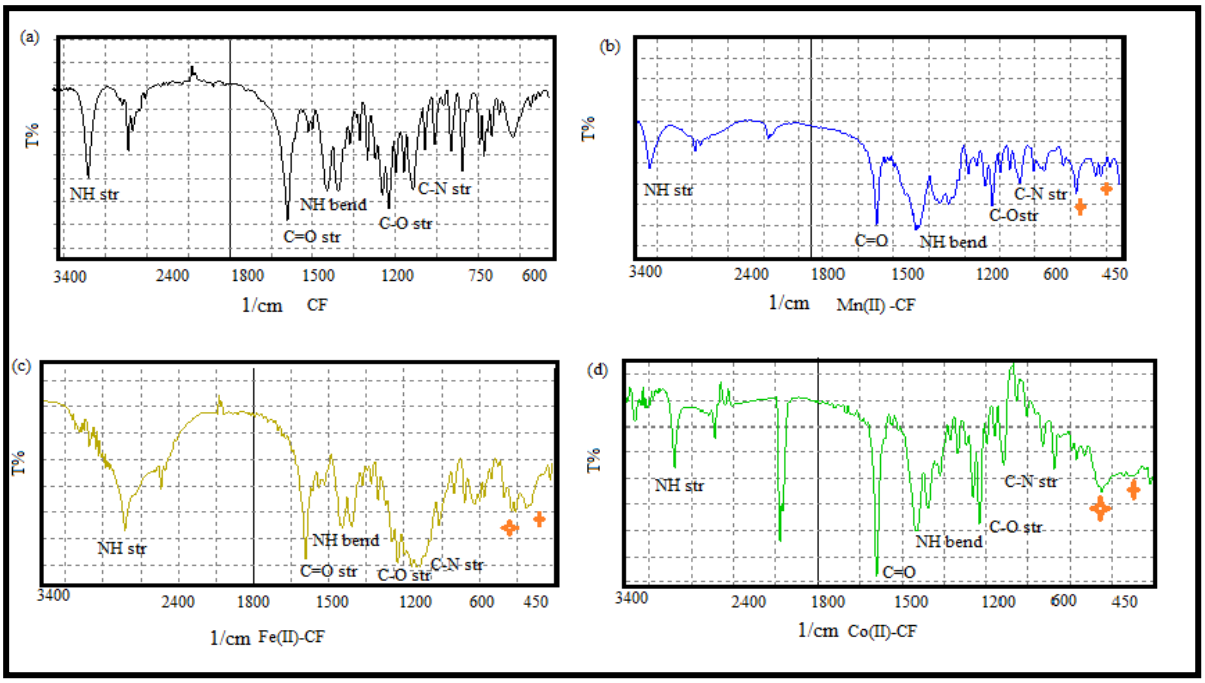


Figure 2.7: IR spectra of a) CF, b) Mn(II)-CF, c) Fe(II)-CF and d) Co(II)-CF in KBr.

2.3.4.1.3. ^{1}H-NMR of CF metal complex.

^{1}H-NMR spectrum of carbofuran shows absorption peaks for six different type of protons (as indicated in Figure 2.8 as a-f below). A singlet is observed for two methyl protons at 1.42 ppm, N-CH_3 absorbs at 2.53 ppm, CH_3 proton show singlet at 2.69 ppm, multiples of aromatic proton were observed in between 6.13 to 6.95 ppm and N-H proton peak is observed at 7.41ppm.

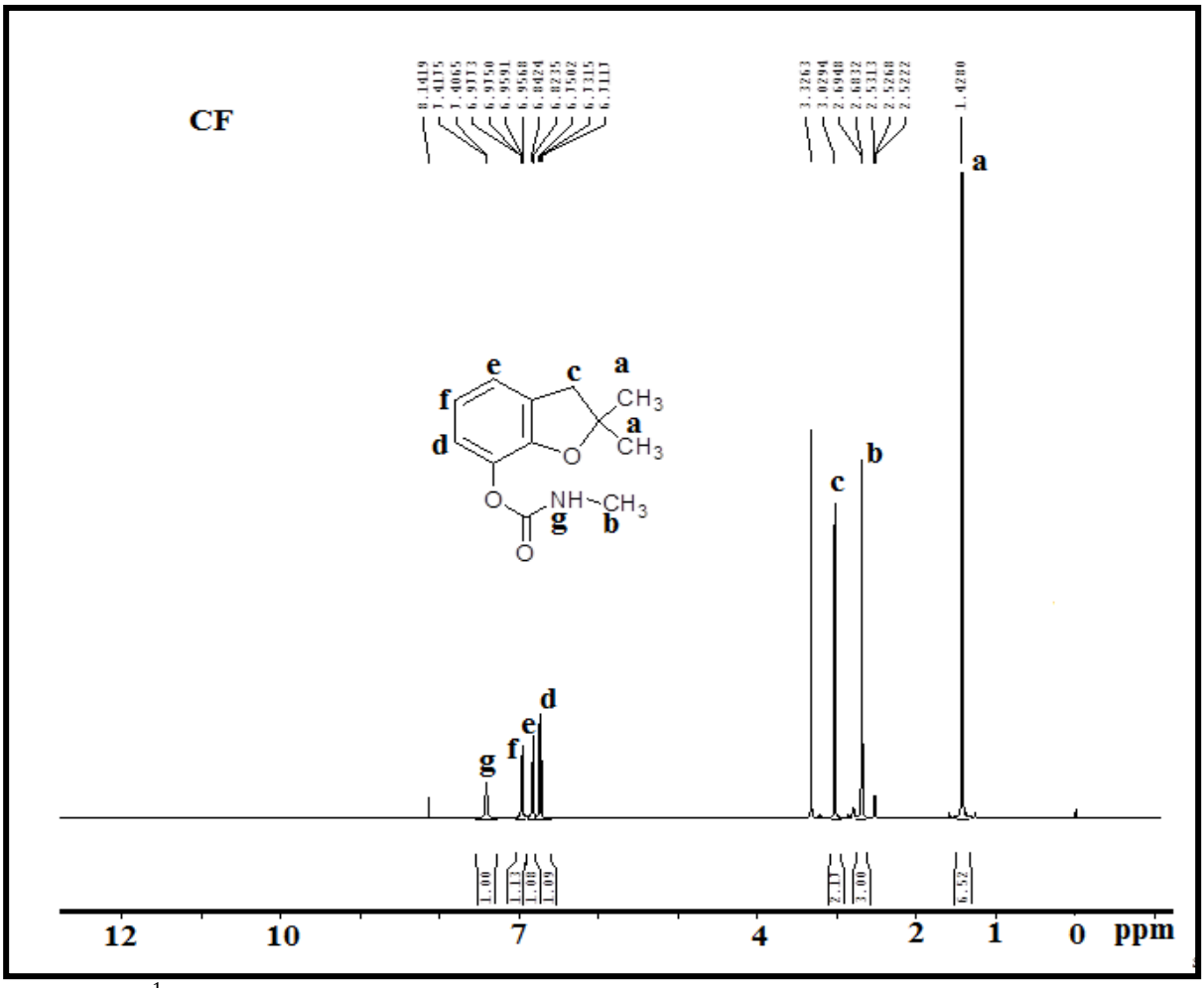


Figure 2.8: ^{1}HNMR spectra of CF in d_6-DMSO.

Most of the metal complexes of carbofuran were insoluble in almost all laboratory solvents, only Fe(II)-CF complex has shown very slight solubility in DMSO and therefore NMR was taken in d_6-DMSO. Because of very very-very less solubility of the product in DMSO, peak intensity was too small to be characterized. Still some peaks can be assigned. In case of Fe(II) complex of CF (as shown in Figure 2.9), methyl protons C-CH_3 absorbs at 1.23ppm.N-CH_3protons found to be shifted downfield to produce singlet at 8.21ppm showing its direct involvement in complex formation. As, ethanol was used as solvent its CH_3 proton peak is observed at 1.43ppm and methylene proton peak is observed at 3.3ppm. All aromatic protons and a N-H proton are broadened between 6.55 to 6.9ppm. Proton wise spectral analysis is shown in Figure 2.9.

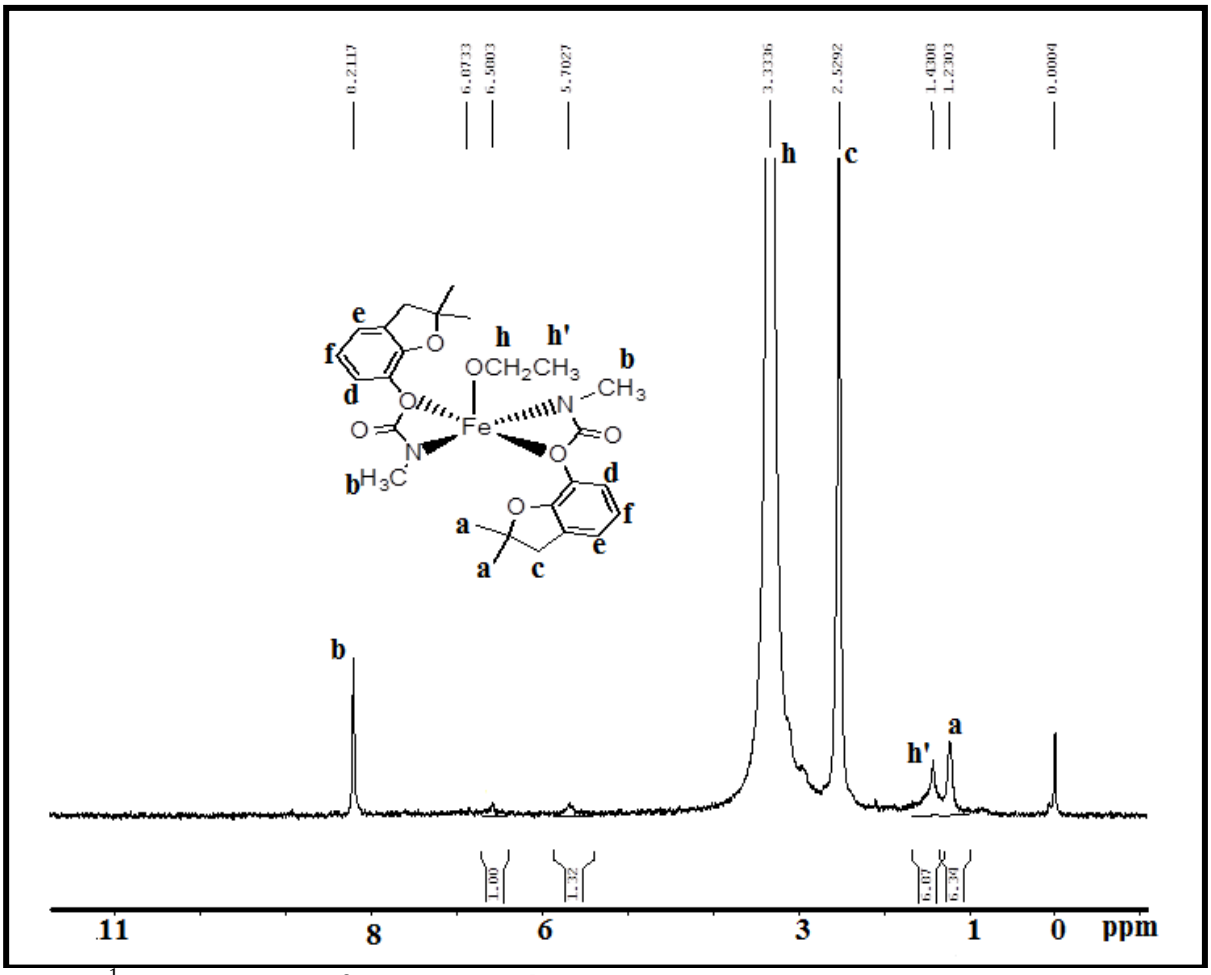


Fig 2.9: ^{1}HNMR spectra of Fe(II)-CF in d_6-DMSO.

2.3.4.1.4. Mass analyses of the CF metal complex

Mass spectrum of the Fe(II)-CF complex is exhibited in the Figure 2.10. It depicted that Fe(II)-CF has value 542.25 with the molecular formula [Fe(II)-$(C_{12}H_{13}NO_3)_2OC_2H_6$]. The fragmentation of the complex is proceeded with removal of two methyl group leads to the m/Z value of the 512. The removal of the two molecules of the ($C_{10}H_{12}O$) group, leads to the m/z value of the 216. It further fragmented to give the m/z value of171 after the removal of the (OC_2H_6) group. Important fragments and their masses are shown in Figure 2.11 and were found to be a part of mass spectrum of Fe(II)-CF.

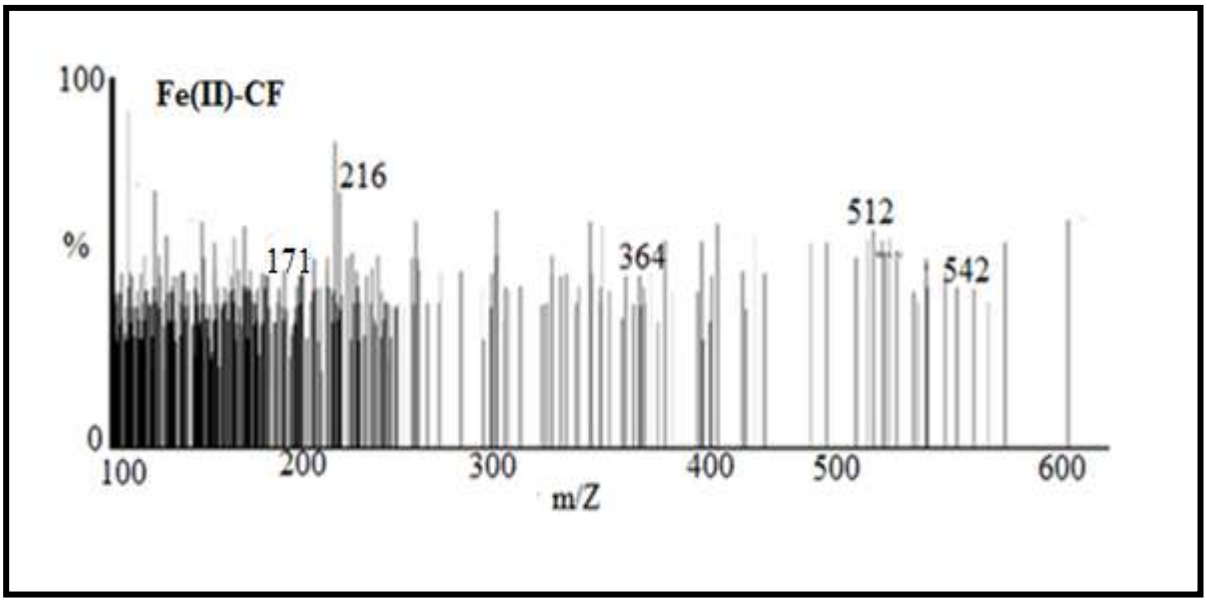


Figure 2.10: Mass spectrum of the Fe(II)-CF.

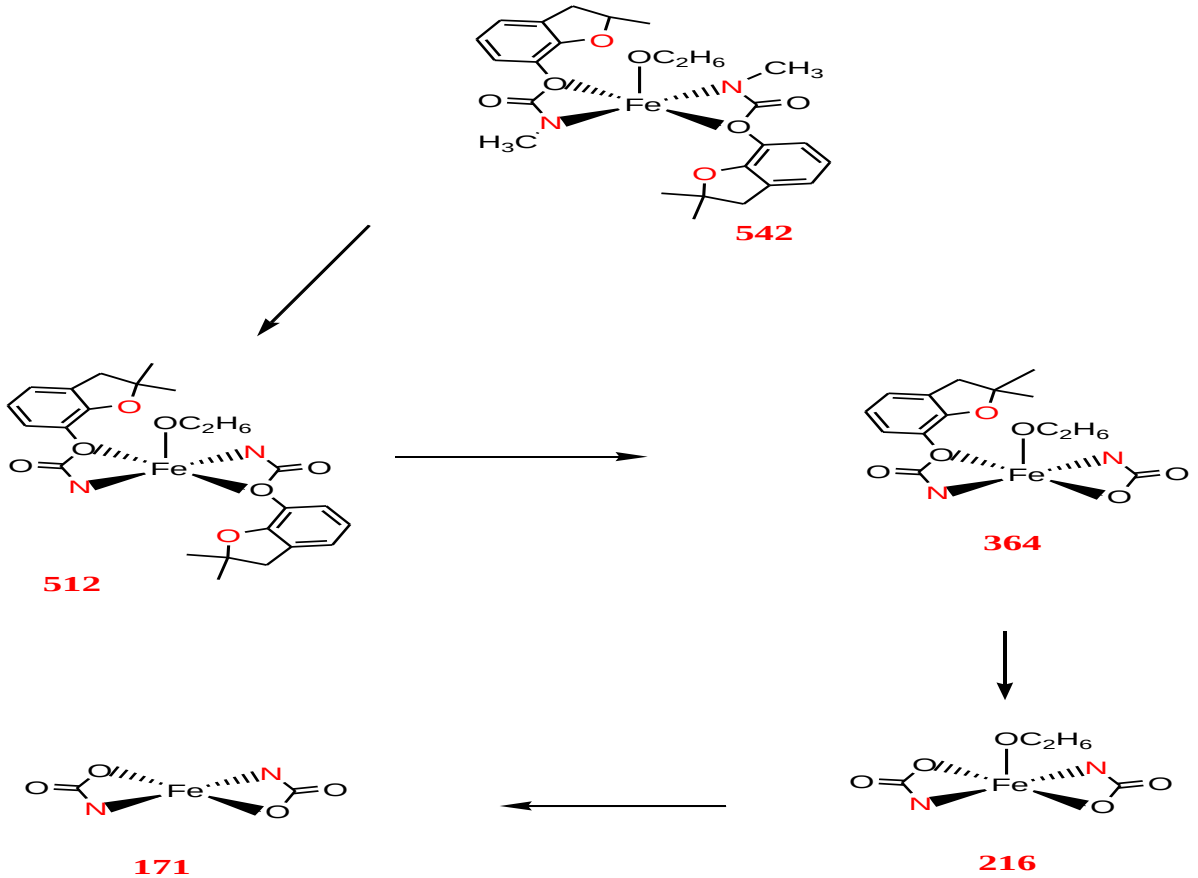


Figure 2.11: The mass spectra depicting the m/z values of the Fe(II)-CF complex.

Key points– From the interpreted result it was found that carbofuran reacted faster with Mn(II) and Fe(II) metal ions than that of the Co(II), Ni(II), Cu(II) and Zn (II) metal ions. In presence of the basic medium, the reaction process was fast. The IR spectral study exhibited

thatformation of the bond of the carbofuran with the metal ion took place through nitrogen and oxygen atom. The formed coordinated complex with Fe(II) metal ion through the donor oxygen and nitrogen atom was confirmed through m/z value of the complex.

2.3.4.2. Carbendazim (CZ) carbamate

2.3.4.2.1 UV-visible analysis of CZ metal complexes

For the determination of the interaction of the ligand with the metal ion, the concentration of the ligand was monitored in the reaction. For that purpose UV-vis spectrophotometric technique was used.

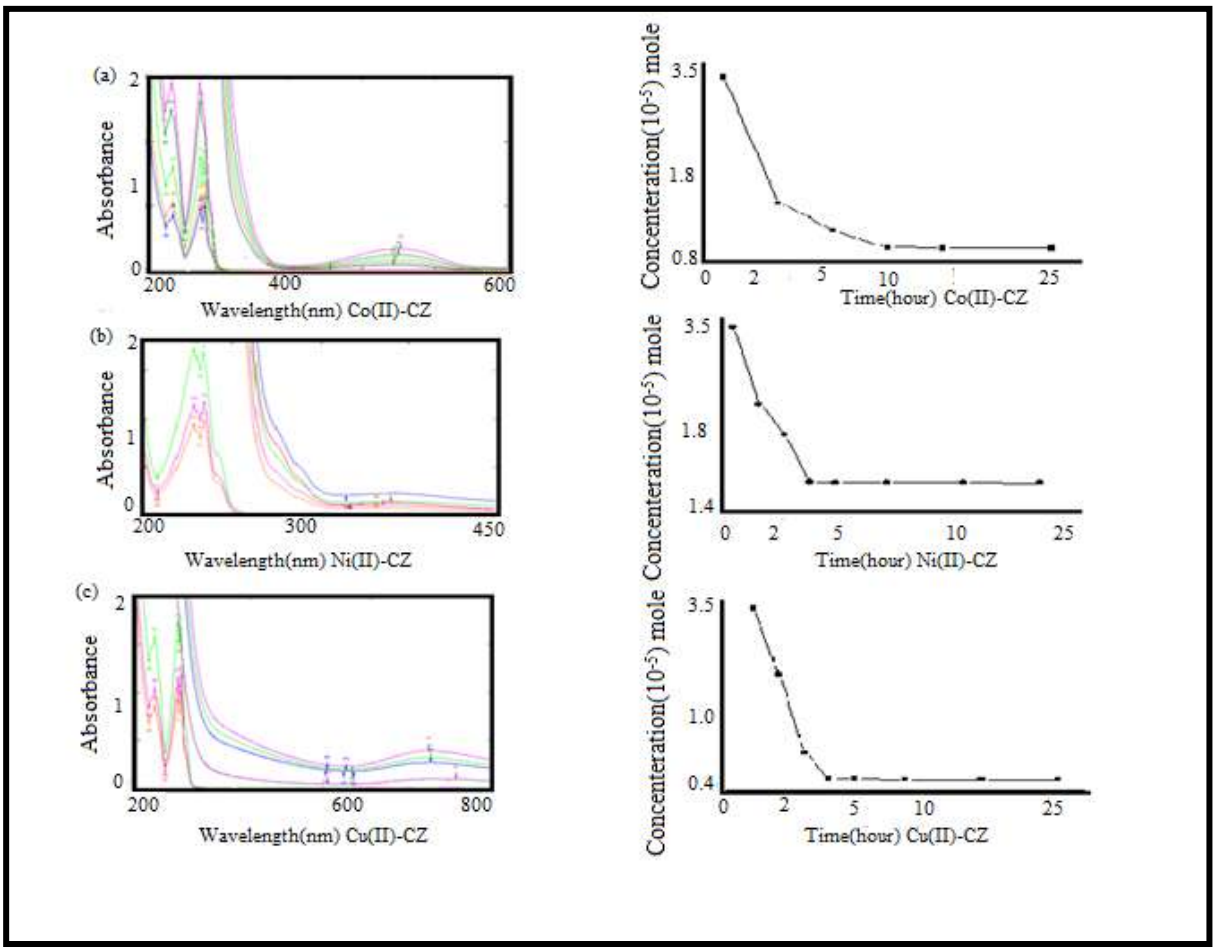


Figure 2.12: (a) Reaction progress during the Co(II)-CZ, Ni(II)-CZ and Cu(II)-CZ interaction by UV overlay curve (b) Plot depicting the concentration of the decrease in concentration of CZ ligand with time.

The concentration of carbendazim remained after different interval of time was determined by using the Beer-Lambert's law at wavelength 278nm. The percentage of the ligand consumed was deduced by subtracting the values from the initial concentration and the UV overlay curvedepicted that with the progress in the reaction, absorbance value of the carbendazim

ligand decreases (as shown in Figure 2.12). The concentration of the consumed carbendazim was calculated and plotted against time. It was found that the maximum amount of the carbendazim was consumed with in the 6h of the reaction. In a similar manner, the amount of the carbendazim consumed during the interaction with different metal ions is tabulated below in Table 2.4.

Table 2.4:-Percentage of carbendazim consumed in the reaction

S.NO	% of the carbendazim consumed with the time (h)						
	CZ metal complexes	1	2	3	5	8	24
1	Mn(II)-CZ	25	47	58	65	64	64
2	Fe(II)-CZ	23	58	80	81	81	92
3	Co(II)-CZ	21	39	49	58	59	58
4	Ni(II)-CZ	20	37	48	57	58	57
5	Cu(II)-CZ	25	43	72	74	76	88
6.	Zn(II)-CZ	18	34	42	53	53	52

2.3.4.2.2. FTIR analysis of the CZ metal complexes

IR analysis of the Carbendazim (CZ) complexes with respect to the CZ ligand depicted the broadening of the N-H, C=O and C=N stretching peak and shifting of the N-H stretching peak, N-H bending peak, C=O stretching peak, C=N stretching peak and C-O stretching peak. In case of the N-H stretching, N-H bending, C-H stretching and C-N stretching, peak shifting was found towards the higher wavenumber. The higher shift of wavenumber in case of the N-H stretching was found in the range of the 5-58cm^{-1}, 2-7cm^{-1} for N-H bending,C-H stretching band shifted 33-127cm^{-1} and C-N stretching 5-28cm^{-1}.In case of C=O stretching, C=N stretching and C-O stretching peaks, shifting was found towards the lower wavenumber. The broadening and the lower wavenumber shift occurred mainly due to the chelation, which leads to shift the electron density of the complex. However, in case of the C-N bond, peak shift to higher wavenumber due to the involvement of the nitrogen atom in the bond formation. It resulted in the stretching C-N bond length and shifting of the frequency towards the higher wavenumber. The vibrational frequency observed in the range of the 570-400cm^{-1} is due to the formation of the metal-oxygen bond. The deduced IR frequencies for the CZ ligand and

the complexes are tabulated below in Table 2.5 and some of the IR spectra (CZ, Fe(II)-CZ, Co(II)-CZ and Cu(II)-CZ) are clubbed in Figure 2.13.

Table 2.5: I.R frequencies of the Carbendazim and its metal complexes

Type of IR-frequency	CZ	Mn(II)-CZ	Fe(II)-CZ	Co(II)-CZ	Ni(II)-CZ	Cu(II)-CZ	Zn(II)-CZ
N-H stretching	3321.6s	3328b,m	3336b,m	3333.3b	3321b	3088b	3379.8m
C-H stretching	2949s	3066b, w	2982b,w	3105b	3042s	2824b	2950.8s
C=O stretching	1712s	1708s	1636m	1682m	1678s	1608s	1644s
C=N stretching	1643s	1629m	1631m	1640s	1635s	1545s	1600s
N-H bending	1593m	1600m	1597m	1603m	1599m	1473m	1542m
C-O stretching	1272s	1233s	1243s	1263s	1238s	1207m	1207m
C-N stretching	1095s	1100m	1011m	1103m	1101m	1123.4s	1108.2s
Mn-O stretching	NA	552w	514.5w	508.4w	492w	444.4w	468.3w
Mn-N stretching	NA	478w	422.3s	432 w	Not obtained	Not obtained	Not obtained

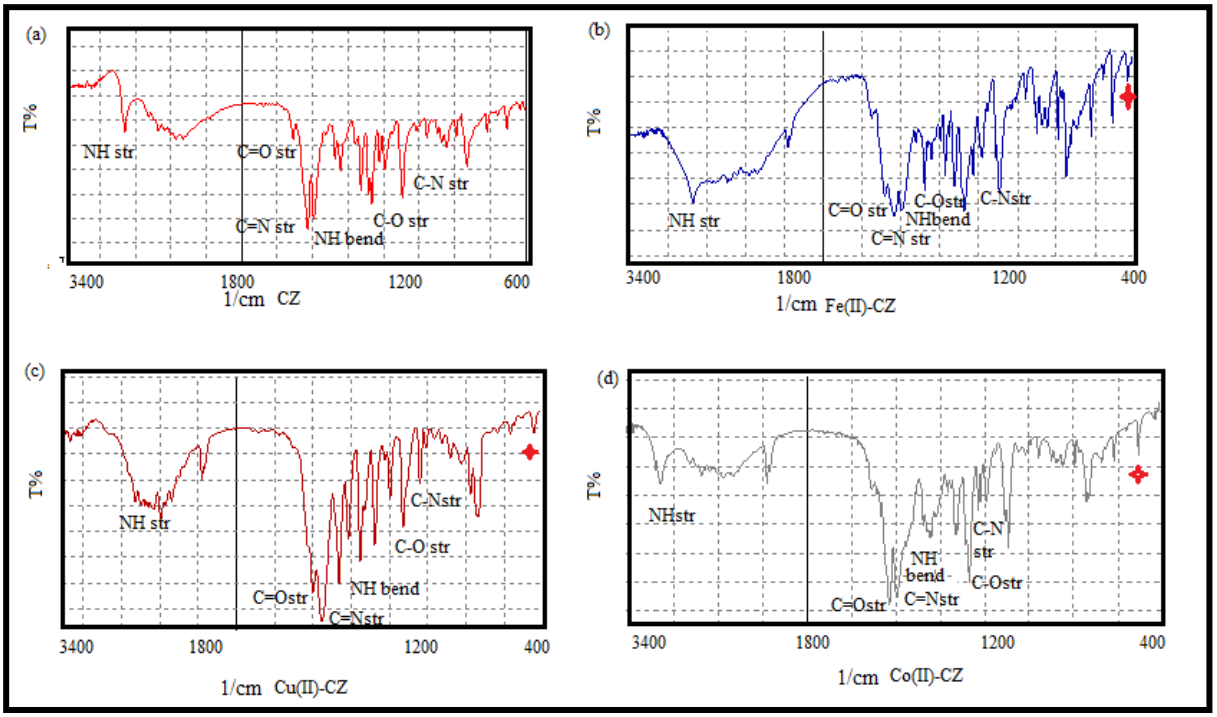


Figure 2.13: IR spectra of a) CZ, b) Fe(II)-CZ, c) Cu(II)-CZ and d) Co(II)-CZ in KBr.

2.3.4.2.3 ^{1}H NMR analysis of CZ metal complex.

In ^{1}H NMR of CZ four different proton type were observed (as shown in Figure 2.14). The CH_3 proton gave signal at 3.75ppm, the aromatic proton signals were observed at 7.07 ppm and 7.40ppm and the NH proton peak was observed at 11.63ppm. All type of protons of carbendazim is symbolized as proton type a, b, c and d in the Figure 2.14.

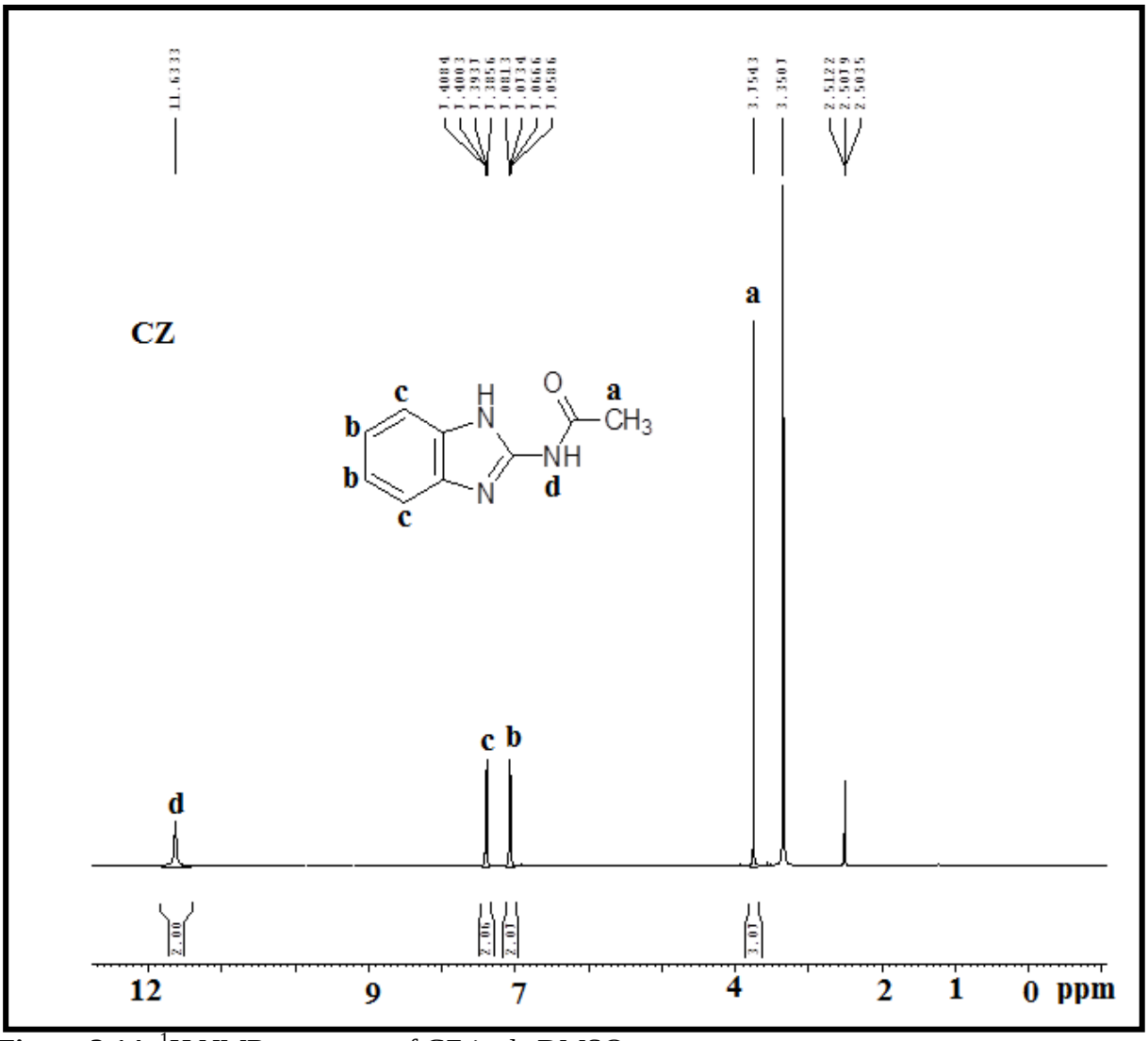


Figure 2.14: ^{1}H NMR spectrum of CZ in d_6-DMSO.

In case of the metal complex of carbendazim, O-CH_3CH_2peak was observed at 3.3ppm for CH_2 methylene proton and 1.24ppm for CH_3 proton of axially attached ethanol.The CH_3proton signal was shifted downward and observed at 3.7ppm. The broadening was observed for aromatic proton after complex formation and peaks were observed at 7.16ppm

and 7.38ppm. The N-H proton peak was shifted downfield and observed at 8.2ppm (as shown in Figure 2.15).

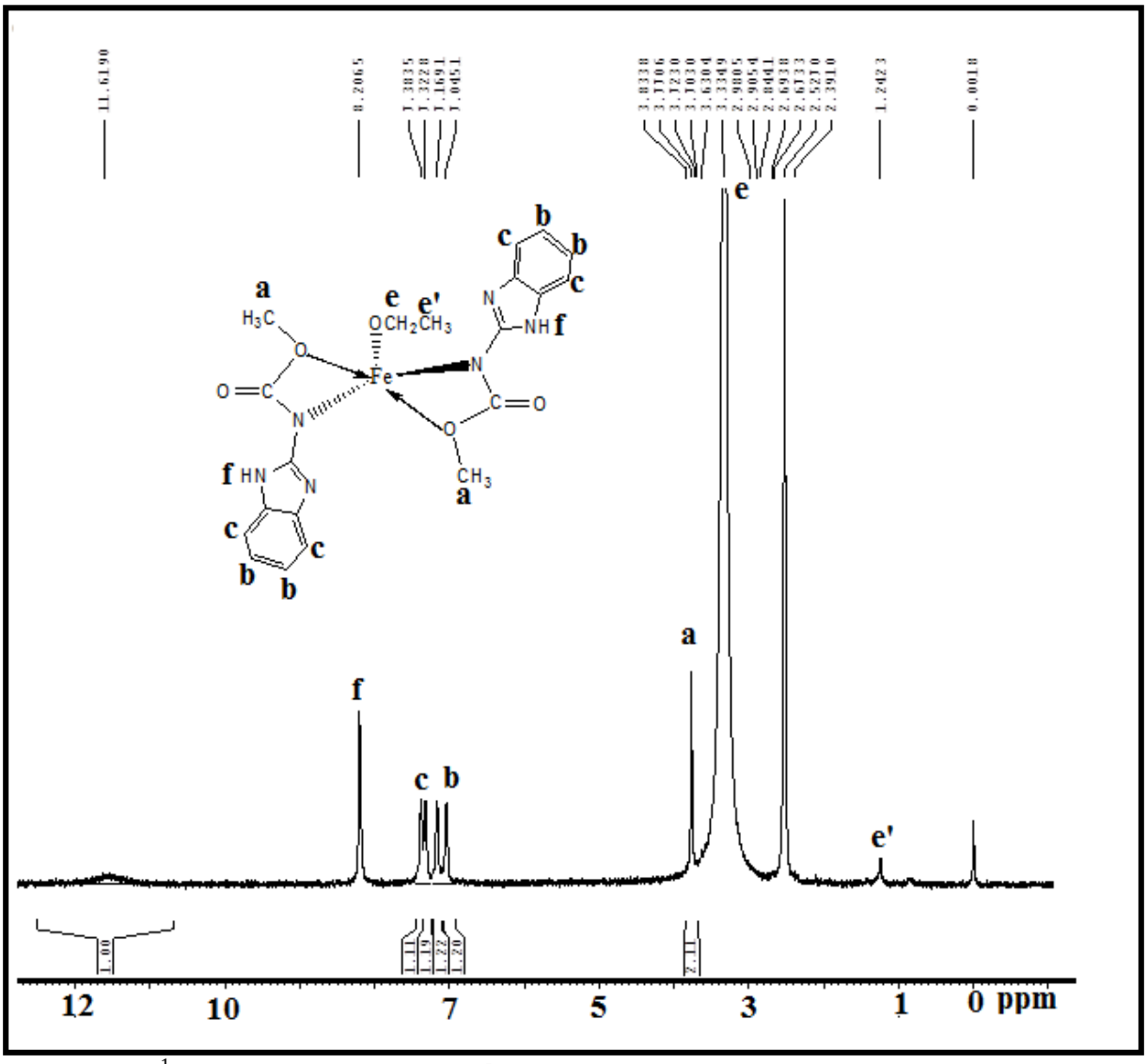


Figure 2.15: ^{1}H NMR OF Fe(II)-CZ in d_6-DMSO.

2.3.4.2.4 Mass analyses of the CZ metal complex

Mass fragmentation of the Fe(II)-CZ complex is shown in the Figure 2.16. It depicted that the Fe(II)-CZ has value 484 with the molecular formula [Fe(II)-$(C_9H_8N_3O_2)_2OC_2H_6$]. The fragmentation started with the removal of the two ($C_7H_5N_2$) group resulting into the m/Z value of m/z at 367 and then 250, further removal of the (C_2H_6) group took place forming the m/Z value observed at 220, afterwards (OC_2H_6) group was removed forming the m/Z value of the 174 and at the end fragmentation leads to the formation of the Fe, $2NH_2$, $2CO_2$ and OH group.

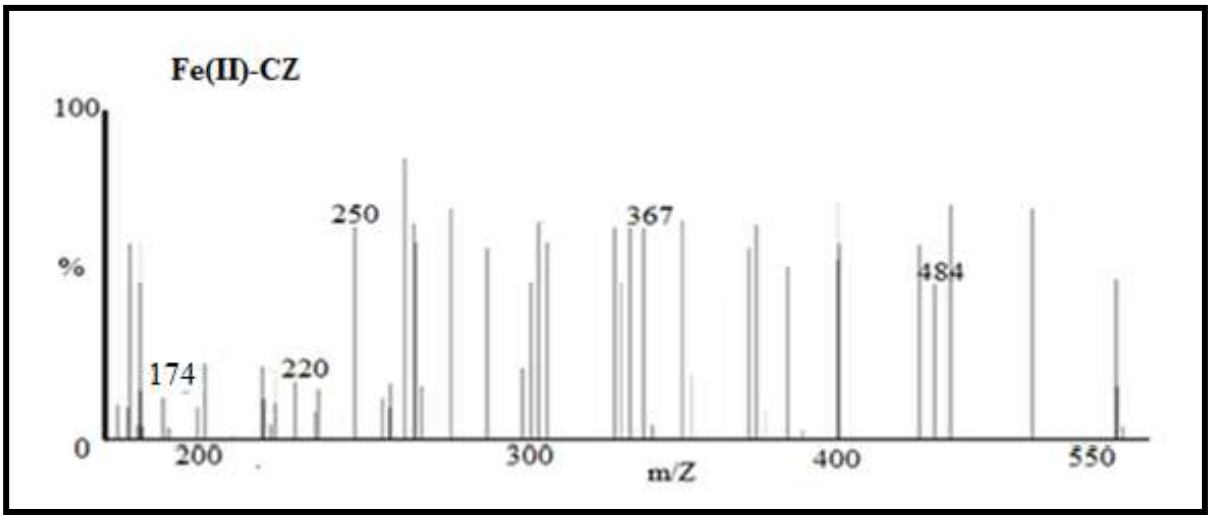


Figure 2.16: Mass spectrum for Fe(II)-CZ complex.

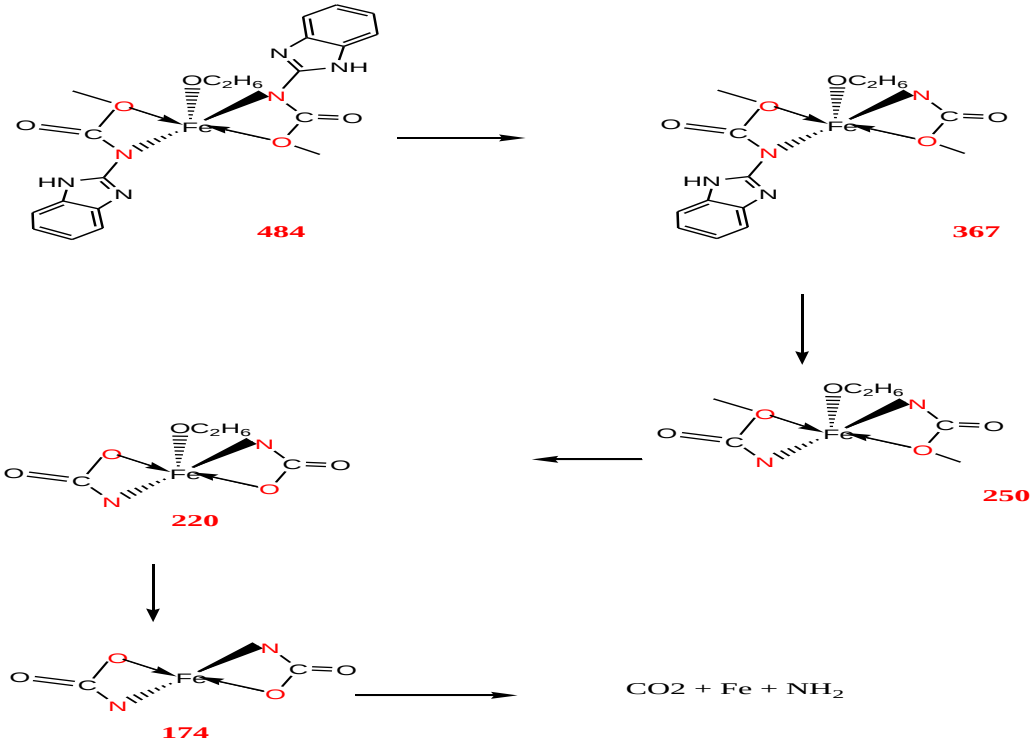


Figure 2.17: Depicting Mass fragmentation of the Fe(II)-CZ.

Keypoints -From the interpreted result it was found that carbendazim reacted faster with Mn(II) Fe(II) and Cu(II) metal ions than that of the Ni(II), Co(II), and Zn (II) metal ions. In presence of the basic media the reaction progress was fast. The ir spectral study exhibited the carbendazim bonded with the metal ion through nitrogen and the oxygen atom. The formed coordinated complex with Fe(II) metal ion through the donor oxygen and nitrogen atom was confirmed through m/z value of the complex.

2.3.4.3 Thoidicarb (TC)

2.3.4.3.1 UV-visible analysis of TC metal complexes.

The progress of reaction of thoidicarb with metal ion is deduced by calculating its amount left on interaction with the metal ion during the progress of reaction. For this purpose, concentration of the TC was monitored in the reaction by UV-vis spectrophotometric technique. The concentration was determined by using Beer-Lambert's law at 230nm. The percentage of the ligand consumed was deduced by subtracting the values from the initial concentration (as shown in Figure 2.18 clubbed UV-vis spectrophotometric analysis of TC, Fe(II)-TC, Co(II)-TC and Cu(II)-TC). It represented that the absorbance value of the thoidicarb pesticide decreased with the passage of time. The concentration of the consumed thoidicarb was calculated and plotted against time. It was found that the maximum amount of the thoidicarb was consumed with in the 8 hour of the reaction. In a similar manner, the amount of the thoidicarb consumed during the interaction with different metal ions is tabulated below in Table 2.6.

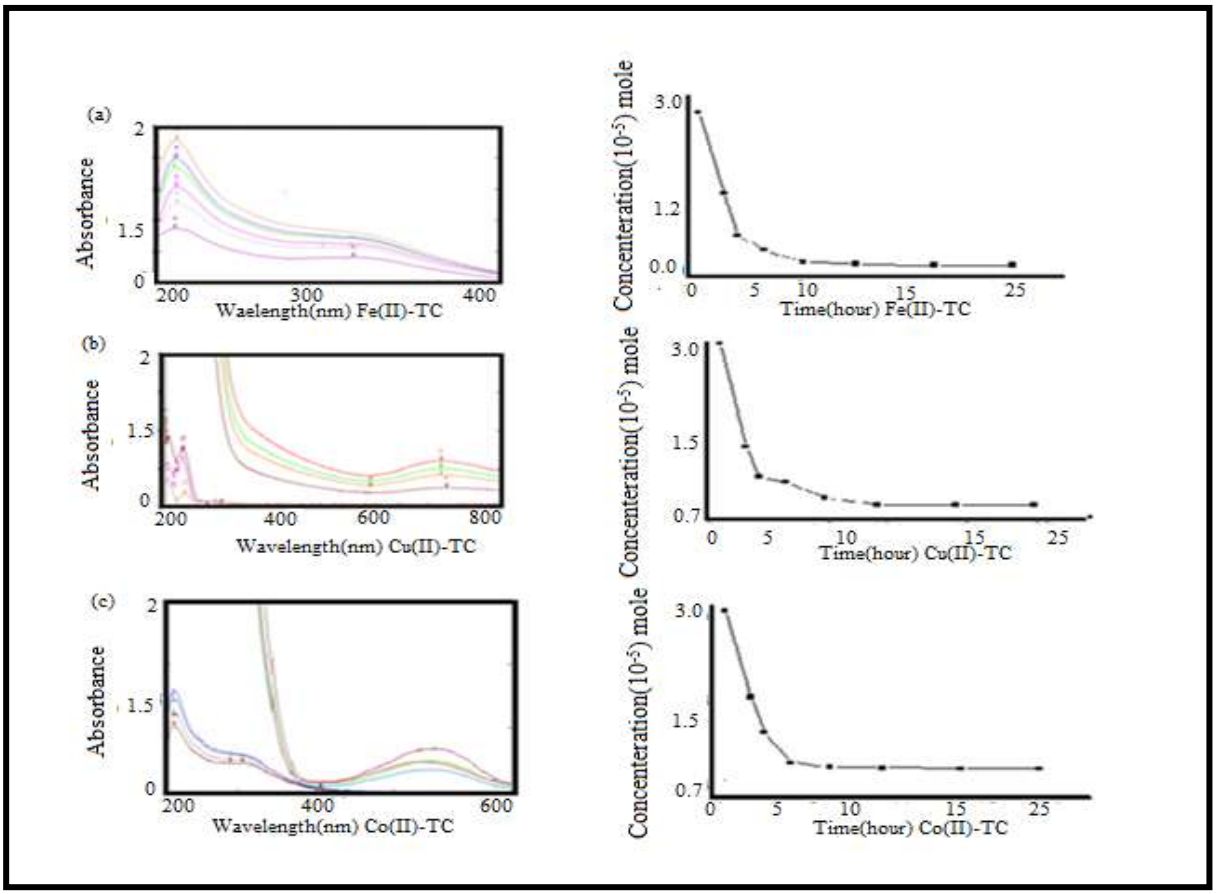


Figure 2.18: (a) Reaction progress during the Fe(II)-TC, Co(II)-TC and Cu(II)-TC interaction by UV overlay curve (b) Plot depicting the concentration of the decrease in concentration of TC ligand with time.

Table 2.6: Percentage of thoidicarb consumed during the reaction at different interval of time.

S.NO	% of the thoidicarb/ consumed with the time(h)						
	TC metal complexes	1	2	3	5	8	24
1	Mn(II)-TC	23	31	43	52	52	50
2	Fe(II)-TC	20	37	66	70	75	94
3	Co(II)-TC	28	40	56	68	72	71
4	Ni(II)-TC	30	43	47	59	68	60
5	Cu(II)-TC	32	49	54	61	70	70
6	Zn(II)-TC	21	39	67	71	76	95

2.3.4.3.2 FTIR analysis of the TC-metal complexes.

IR analysis of the TC complexes with respect to the TC ligand depicted the broadening of the C=O and C=N stretching peakafter the complex formation. Following peaks were shifted towards lower wavenumber: C=O stretching peak, C=N stretching peak, C-O stretching peak, C-N stretching peak and S-N stretching peak, because of the shift in the electron density occurred due to the complex formation. On contrary C-H stretching band was shifted to higher wave number, because of the stretching caused by sulphur after the bond formation with metal ion. The deduced IR frequencies for the TC ligand and the complexes are tabulated below in Table 2.7.

Table 2.7: IR frequencies of the Carbendazim and its metal complexes

Type of IR-stretching frequency	TC	Mn(II)-TC	Fe(II)-TC	Co(II)-TC	Ni(II)-TC	Cu(II)-TC	Zn(II)-TC
C-H	2999.2w	3365-3470w	3329-3032m	3570-3100m	3341-2937w	3390-3300w	3426-3400w
C=O	1720s	1651m	1629m	1635m	1632m	1628m	1626s
C=N	1724m	1633m	1593m	1601m	1602m	1590m	1575m
C-O	1377s	1350m	1329m	1320m	1273m	1302m	1278m
C-N	1276m	1250m	1267m	1262m	1250m	1223m	1247m
C-C	1178w	1106w	1093m	1143w	1100w	1113b	1102s
C-S	783.5m	795.2w	790.1m	736.2m	733.5m	739.4m	734.2m
S-N	883m	820m	846m	881m	733m	884m	848m
M-O	NA	549w	510w	513w	519w	468w	470w

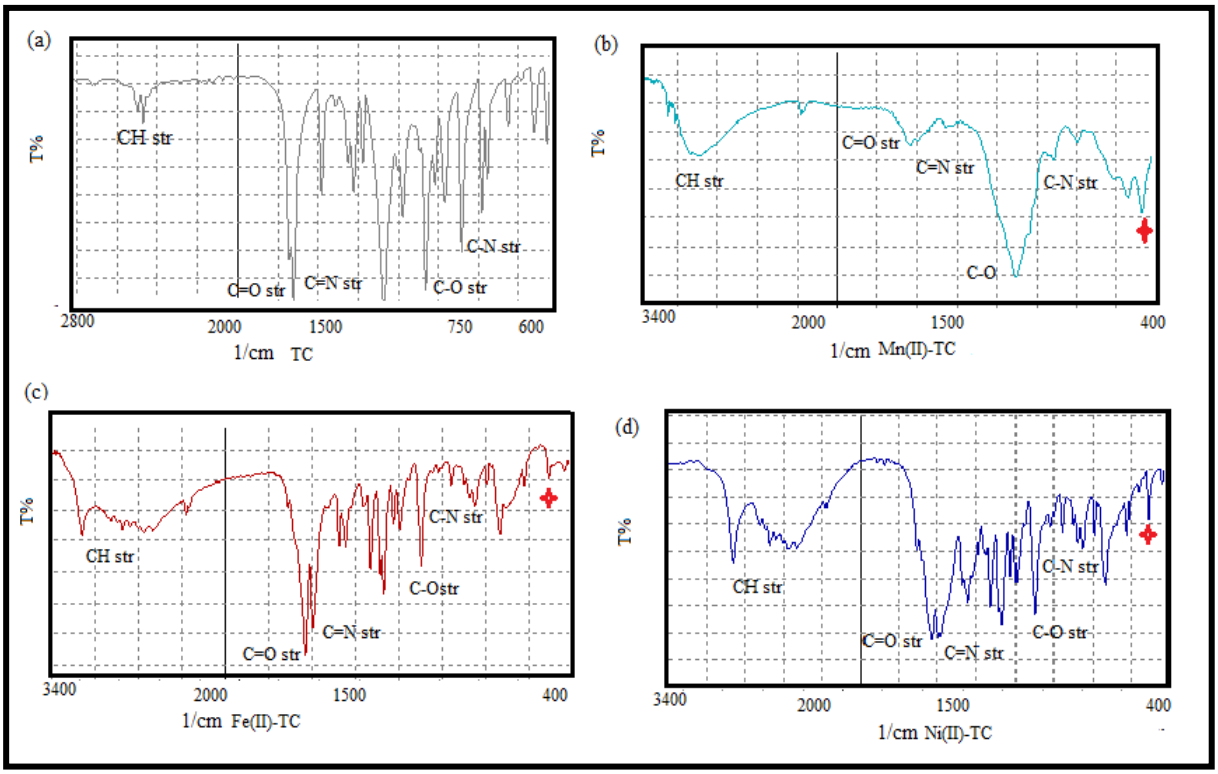


Figure 2.19: IR spectra of a) TC, b) Mn(II)-TC, c) Fe(II)-TC and d) Ni(II)-TC in KBr.

The broadening and the lowering in C=O, C=N, C-O, C-N and S-N stretching frequencies were observed in metal -TC complex. For example, Ni(II)-TC complex showed broad peaks for C=O stretching at 1632cm^{-1}, for C=N stretching at1602 cm^{-1}, for C-O stretching 1273 cm^{-1}, for C-N stretching 1250cm^{-1}, for C-S stretching at 733cm^{-1} and S-N at stretching vibration 850cm^{-1} compared to the TC ligand, where the frequencies were observed at 1720cm^{-1}for C=O stretching,1724cm^{-1}for C=N stretching, 1377cm^{-1}forC-O stretching, 1276cm^{-1}for C-N stretching, 783cm^{-1} for C-S stretching and 733cm^{-1} for S-N stretching(as shown in Figure 2.19 with the Mn(II)-TC and Fe(II)-TC spectra). The decrease in the intensity occurred due to the shift in electron density mainly occurred because of the formation of the complex. However, C-Hstretching band of the complex was found at 3341cm^{-1} which is higher than the C-H stretching frequency of the ligand found at 2999.2cm^{-1}. The main cause of the increase in the frequency is stretching which is caused by sulphur after the bond formation with metal ion.

2.3.4.3.3.^{1}H NMR analysis of TC metal complex.

^{1}H NMR spectrum of TC depicted three different type of protons (as shown in Figure 2.20) The S-CH_3 proton signal is absorbed at 2.1ppm, C-CH_3 proton signal absorb at 3.5 ppm and

N-CH_3proton signal absorb at 8.1ppm. These different types of protons are symbolized by a, b and c in the Figure 2.20 for better understanding.

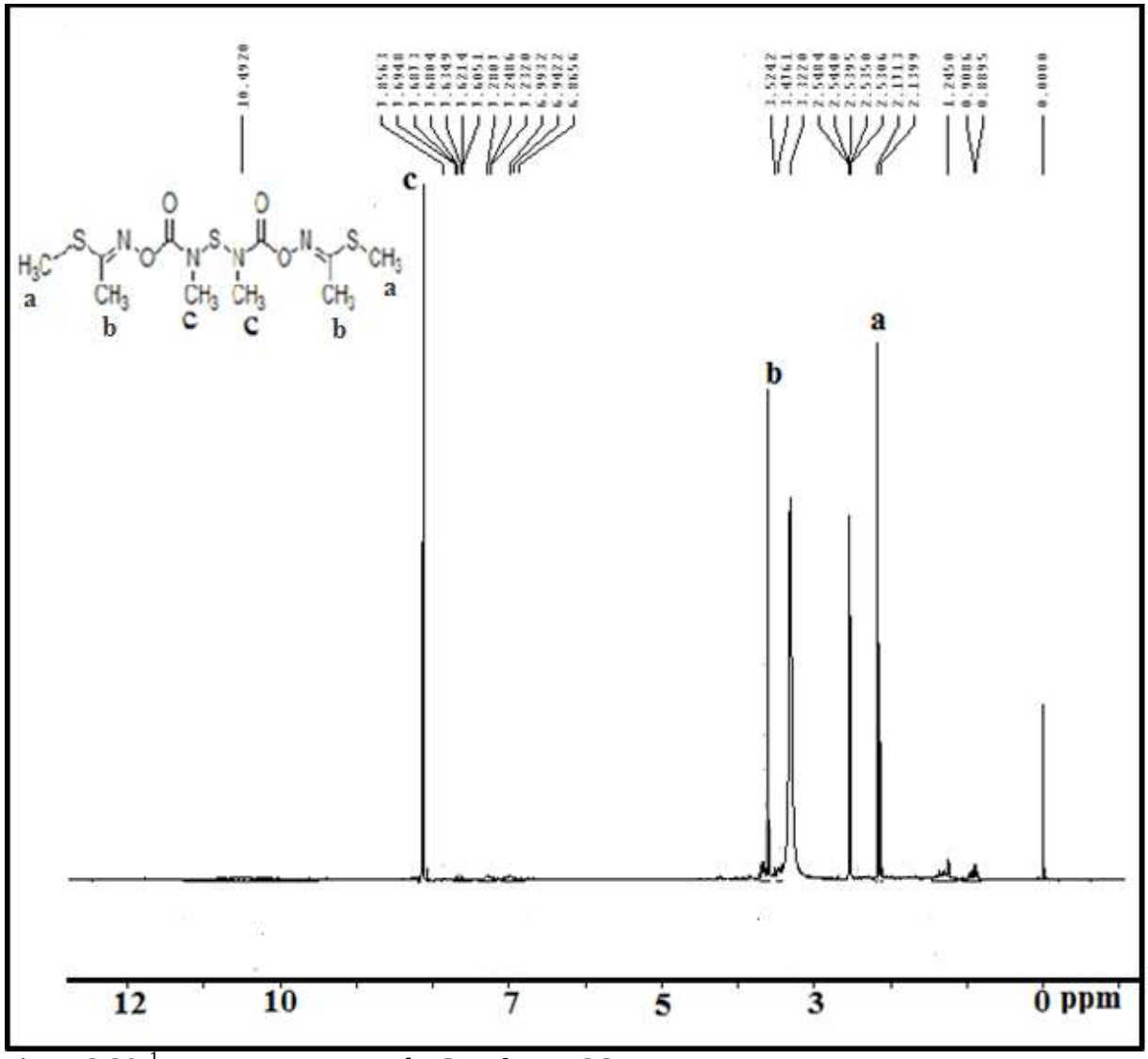


Figure2.20: ^{1}H-NMR spectrum of TC in d_6-DMSO.

In case of metal complexes of thiodicarb (as shown in Figure 2.21), peak of C-C**H**$_3$proton overlaps with the peak of the protonated DMSO and shifted to 2.5 ppm. In case of S-CH_3 proton, peak is observed at 1.9 ppm, while the N-CH_3 peak is shifted to higher frequency and resonate at 8.2 ppm. Axial ethanol is possibly replaced by DMSO and therefore, additional unassigned small peaks are observed in the NMR spectrum shown in Figure 2.21.

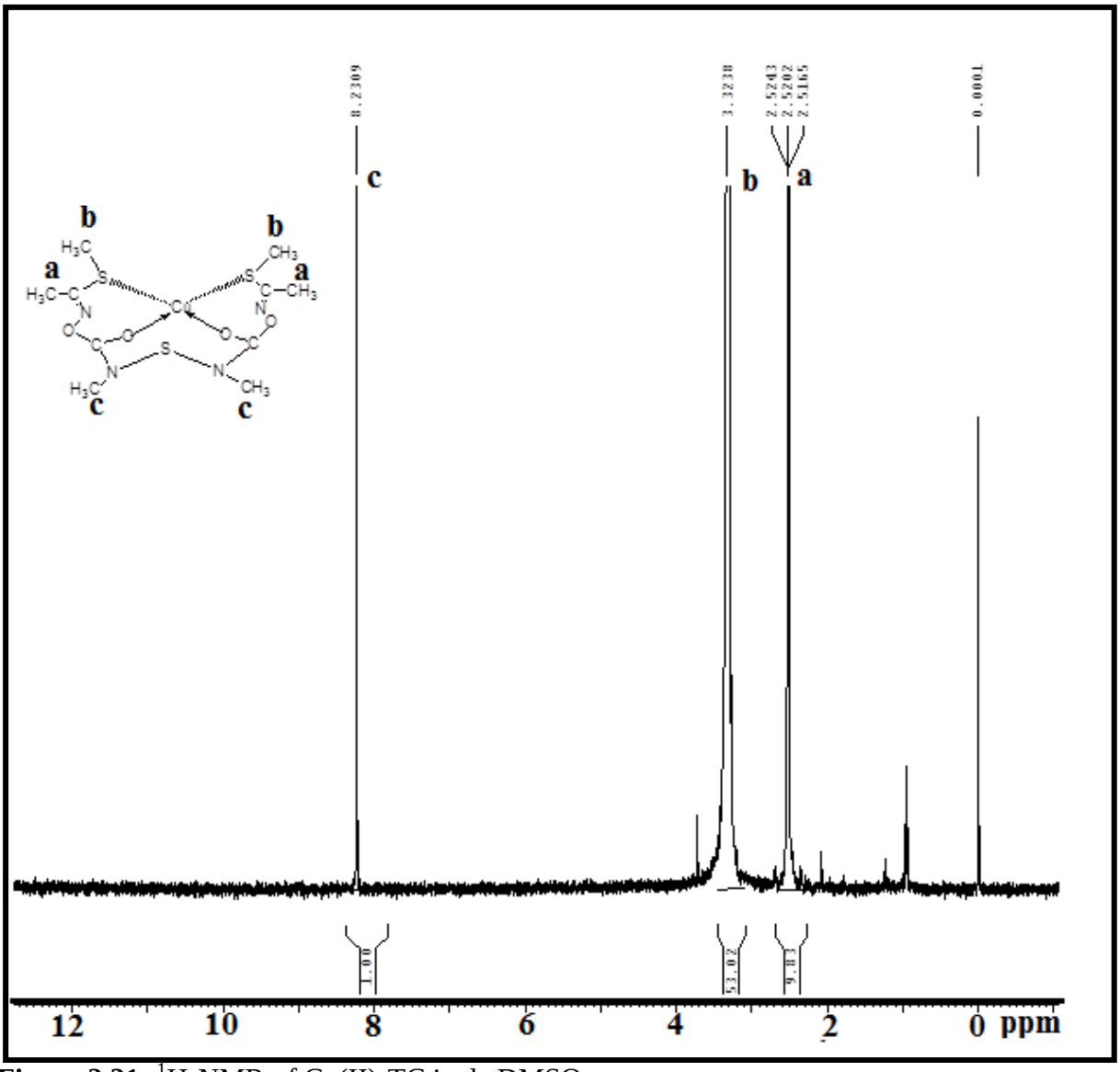


Figure 2.21: ^{1}H-NMR of Cu(II)-TC in d_6-DMSO.

2.3.4.3.4 Mass analyses of the TC metal complex

Mass fragmentation of the Fe(II)-TC complex is shown in the Figure 2.22. It depicted that Fe(II)-TC has 410 m/z value with the molecular formula [Fe(II)-($C_{10}H_{18}N_4O_4S_3$)].The mass fragmentation pattern is summarized in Figure2.23, which is proceeded by the removal of the (SH) group leading to the m/Z value of 380. It is advanced by the removal of the (C_2H_8N) forming the m/Z value of 324. In next step, the two methyl group were removed and the m/Z value was found as 293. Afterwards on removal of two more methyl group it was observed at m/z 265. At the removal of 2(CHN) group leads to the m/Z value of 156.

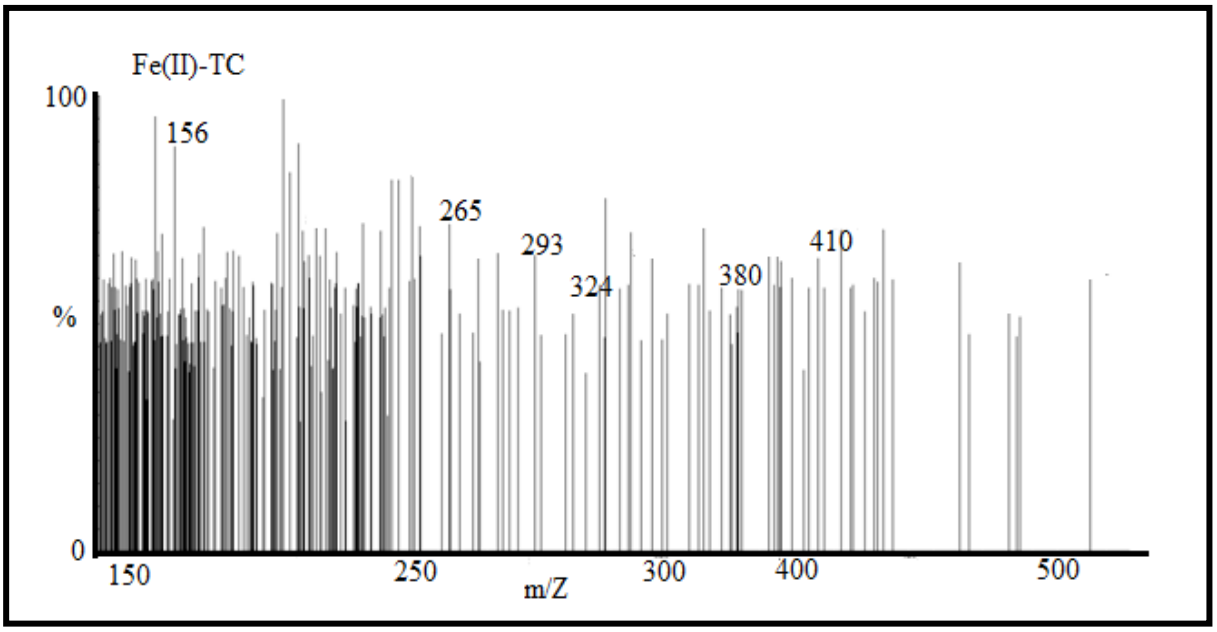


Figure 2.22: Mass spectrum of Fe(II)-TC complex.

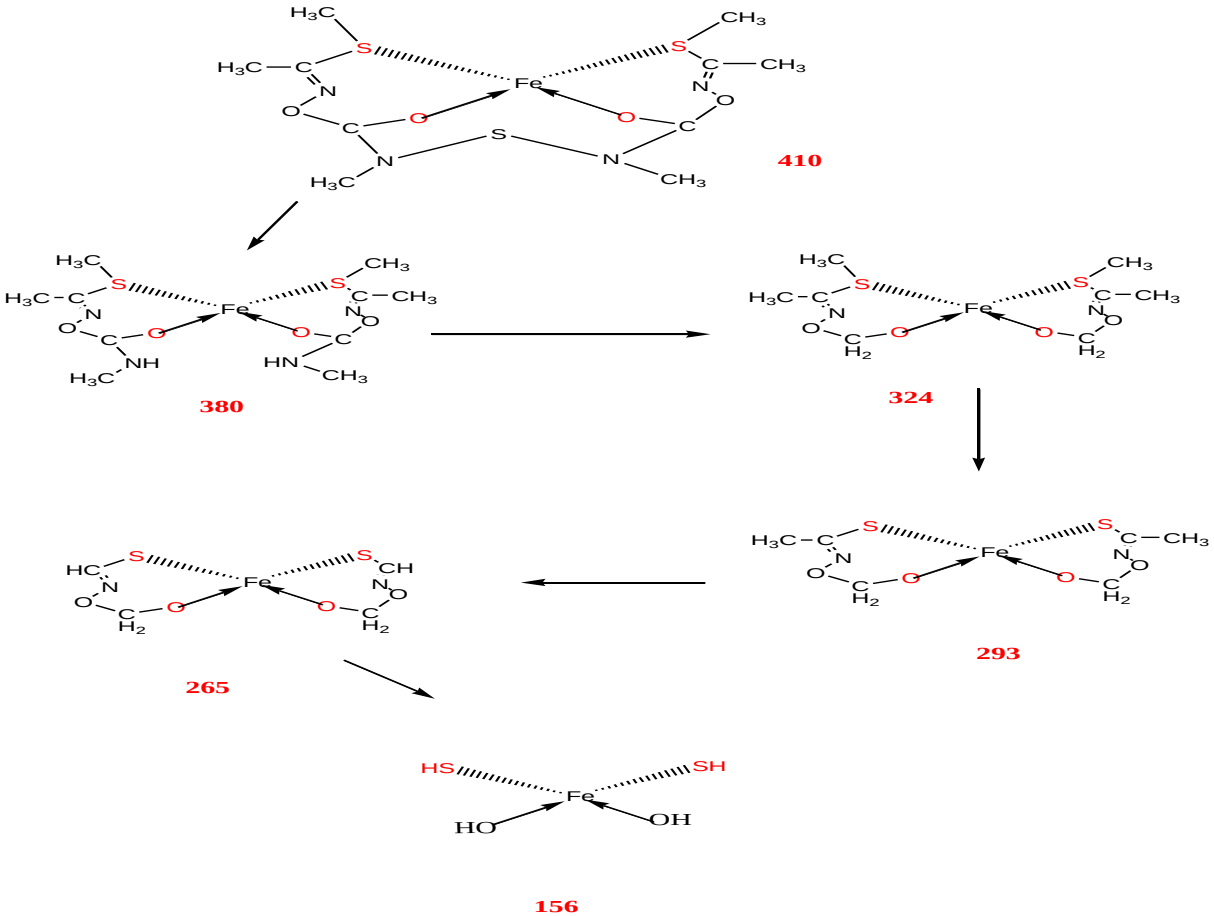


Figure 2.23: Mass fragmentation of Fe(II)-TC complex.

Key points: From the interpreted result, it was found that thoidicarb reacted faster with Fe(II), Co(II) and Zn (II) metal ions than that of the Mn(II), Ni(II) and Cu(II) metal ions. In presence

of the basic medium, the reaction process was fast, indicating such complex formation will quickly take place in basic medium. IR and H^1NMR spectral study exhibited formation of metal complexes took place through bonding of oxygen and sulphur atom of thoidicarb. The formed Fe metal ion complex with thoidicarb depicted the formation of bond took place through sulphur and oxygen atom and showing the m/z value of the 410.

2.3.4.4 Methomyl (M)

2.3.4.4.1 UV-visible analysis of M- metal complexes.

UV-vis spectrophotometric method was employed for determination of progress of reaction between methomyl and essential metal ions. For that purpose, concentration of methomyl at different interval of time was monitored using Beer-Lambert's law at 229nm. The calculated

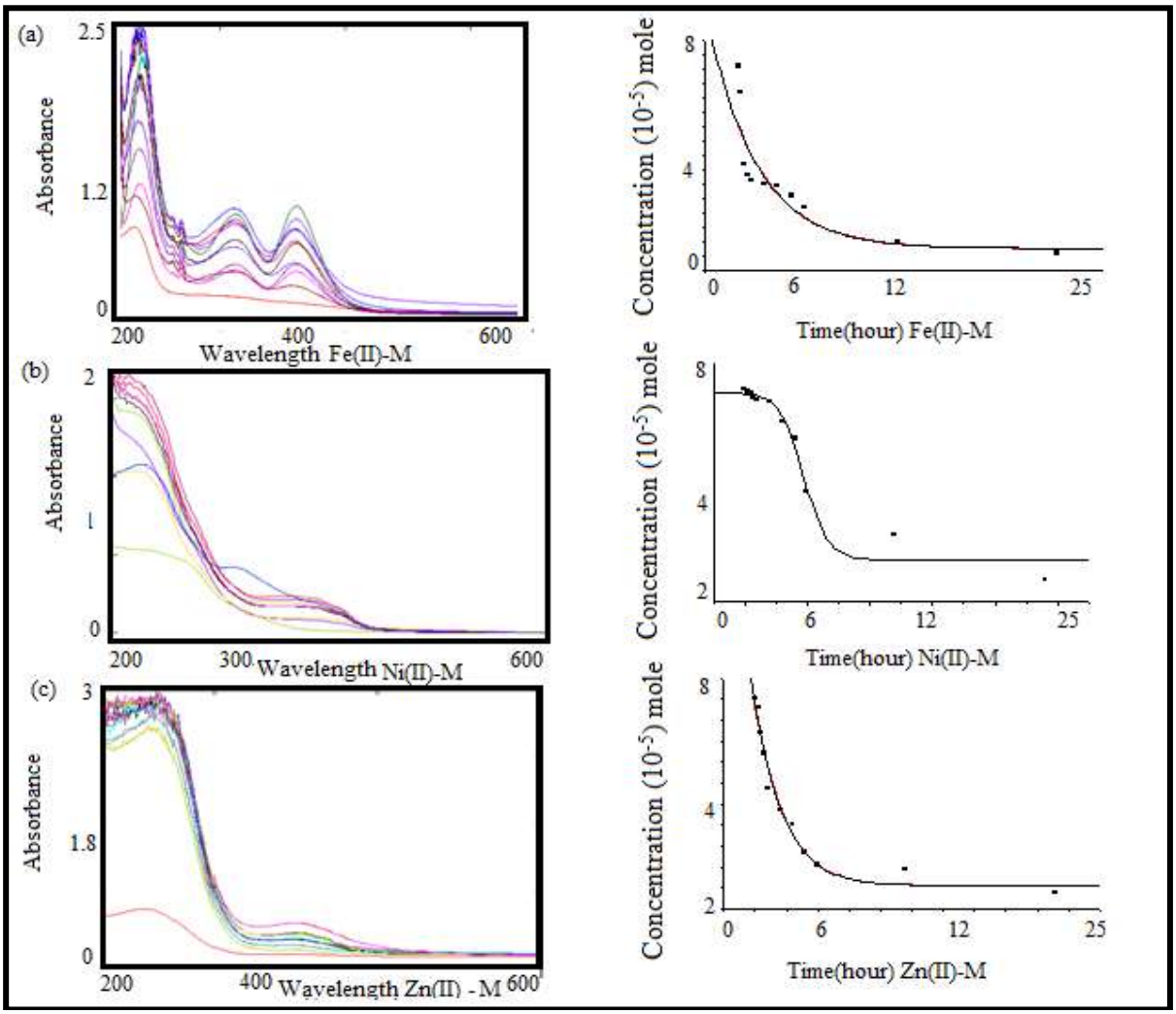


Figure 2.24: (a) Reaction progress during the Fe(II)-M, Ni(II)-M and Zn(II)-M interaction by UV overlay curve (b) Plot depicting the concentration of the decrease in concentration of CZ ligand with time.

values of absorbance (proportional to concentration of methomyl) were subtracted from the initial value of absorbance to get the amount of the methomyl used in the reaction. The progress of reaction is illustrated below in Figure 2.24. The interaction depicted the decreased absorbance value of the methomyl ligand with increased interaction with the metal ion. The observed changes in the concentration were calculated and plotted against the time. The maximum decrease in the concentration was observed within 6 hour of progress of reaction.
The changes observed in the concentration for in percentage after evaluating the reaction for 24 hour for the different metal ions are compiled in Table 2.8.

Table 2.8: Percentage of methomyl consumed at different interval of time during progress of reaction with essential metal ions

S.NO	% of the methomyl consumed with the time (h)						
	M metal complexes	1	2	3	5	8	24
1	Mn(II)-M	45	56	59	68	68	67
2	Fe(II)-M	43	55	57	62	72	87
3	Co(II)-M	29	38	46	55	55	55
4	Ni(II)-M	26	35	43	48	47	47
5	Cu(II)-M	20	27	28	32	45	58
6	Zn(II)-M	17	23	25	37	37	42

2.3.4.4.2 FTIR analysis of the M- metal complexes.

The IR spectrum of M-metal complex undergoes broadening of peaks, because of formation of complex of methomyl with metal ions. Due to broadening of spectra, IR spectra (as shown in Figure 2.25) of the complexes are not very useful in predicting the structure of the compounds, even some important peaks got merged and therefore important conclusions couldn't be drawn. At one side, when C-H band was shifted to lower wave number, C-O and C-S stretching bands are shifted to higher wavenumber. A medium intensity peak was observed in the interval of 1100-1050cm^{-1} and the strong band of carbonyl was found disappeared due to the formation of the imine group. The medium intensity band at the range of 1100-1050cm^{-1} were also observed after complex formation, seems to arise due to presence of C-N stretching. The observed IR frequency is tabulated below in Table 2.9.

Table 2.9: IR stretching frequencies of methomyl and its metal complexes

Type of IR-frequency	M	Mn(II)-M	Fe(II)-M	Co(II)-M	Ni(II)-M	Cu(II)-M	Zn(II)-M
N-H	3300.2m	3394.3m	3342.6m	3298.5m	3300.3m	3477.7m	3298.3m

stretching							
C-H stretching	3000.2w	2859.2w	2750.4w	2928.4w	2920.4w	2854.3w	2928.5w
C=O Stretching	1712.2s	----	----	----	----	----	----
C=N stretching	----	1618.3w	1623.5w	1710.6w	1708.2w	1600.9w	1712.3w
C-O Stretching	1247.2s	1253.6s	12505.6s	1247.9s	1252.3s	1248.2s	1250.2s
C-S Stretching	1021w	1051.3w	1090.6w	1095.6w	1098.4w	1033.4w	1098.5w

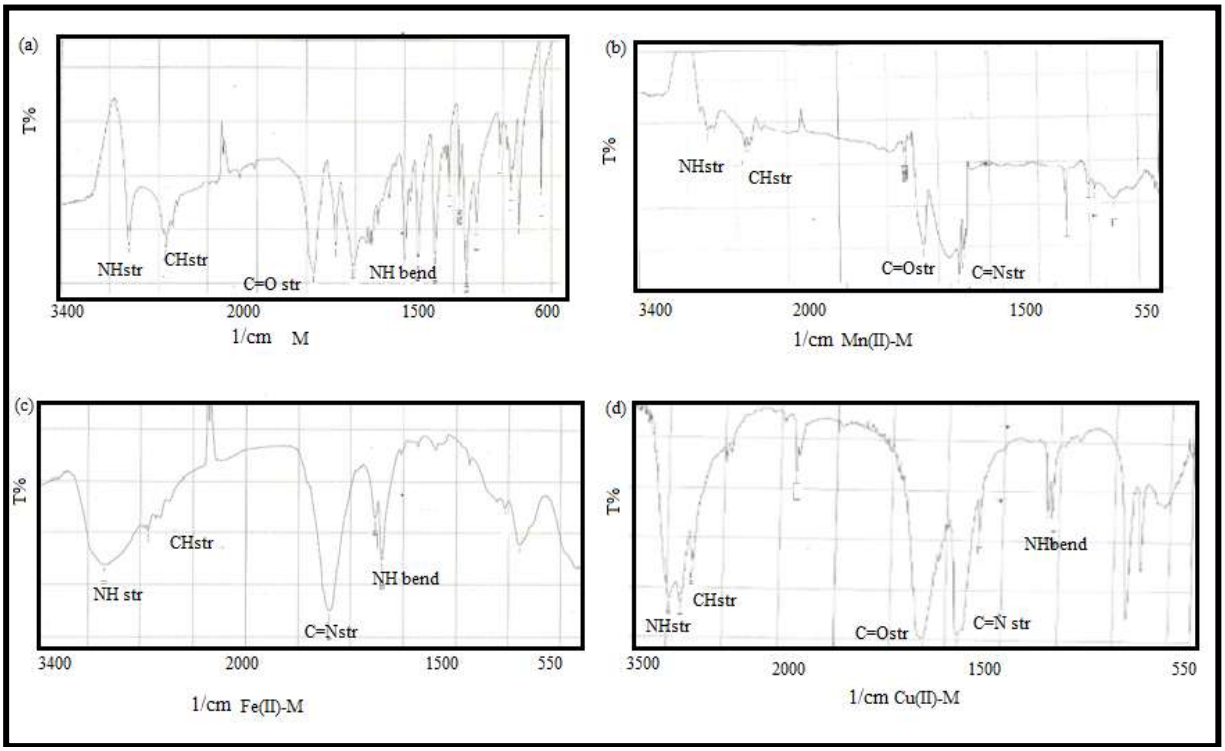


Figure 2.25: IR spectra of the M , Fe(II)-M , Mn(II)-M, Fe(II)-M and Cu(II)-M in KBr.

2.3.4.4.3.^{1}H NMR analysis of M complex.

The ^{1}HNMR of methomyl exhibited four different proton signal (as shown in Figure 2.26). The C-CH_3 proton signal was observed at 2.16 ppm, N-CH_3 was observed at 2.39ppm, S-CH_3 proton signal and NH proton signal depicted at 2.9ppm and 6.09ppm respectively. However, on complexation N-H peak disappears, because of its removal after complex formation and the peak of S-CH_3 overlapped with the peak of DMSO-d_6 at 3.2ppm, while N-CH_3 remain in the plane of the complex and undergo anisotropic shift to 7.8ppm (C-CH_3 and S-CH_3

probably goes out of the plane). For more clarity, different types of protons are symbolized as a, b, c d in Figure 2.25 for methomyl and Cu-complex of methomyl in Figure 2.27.

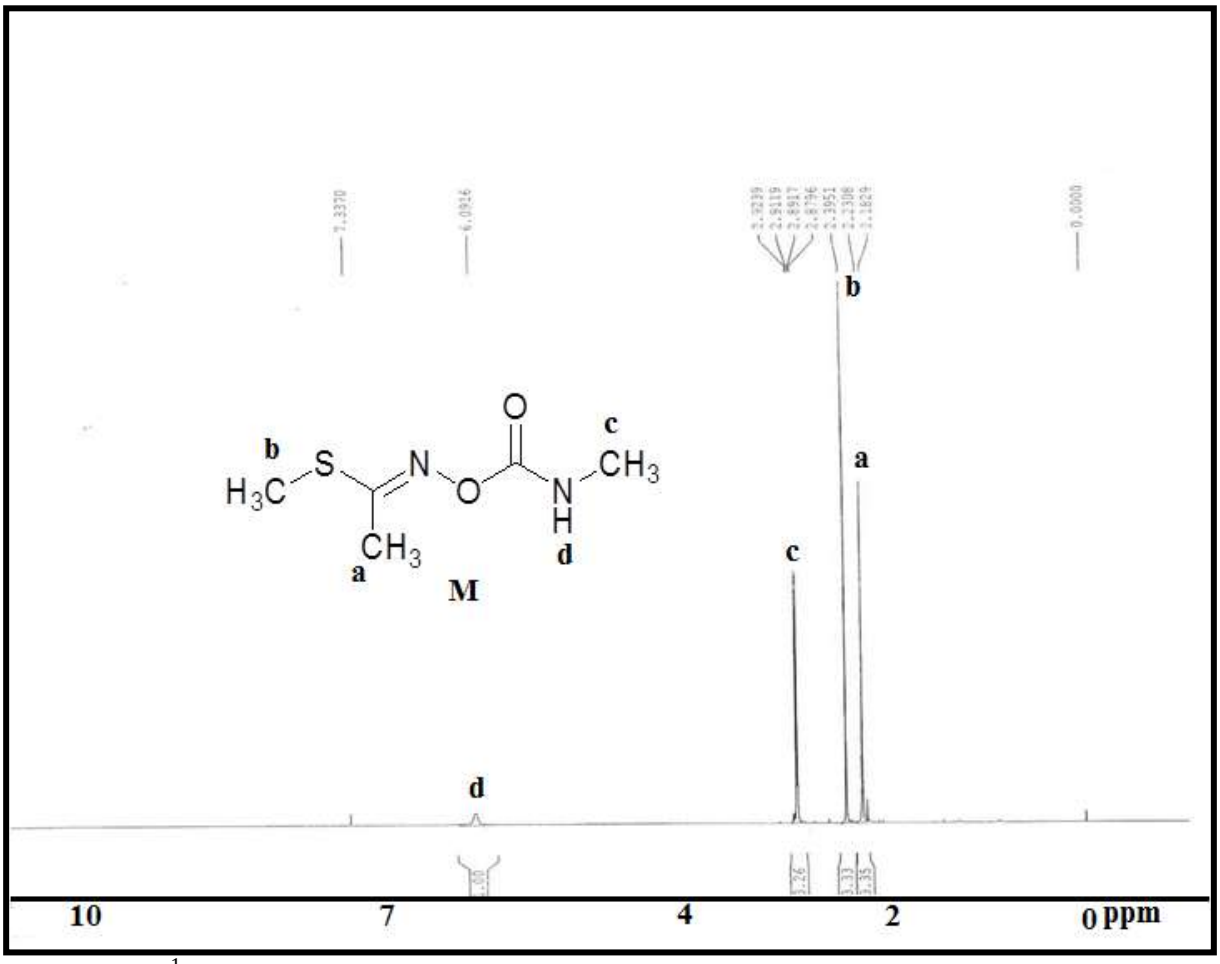


Figure 2.26: ^{1}H NMR spectrum of methomyl in $CDCl_3$.

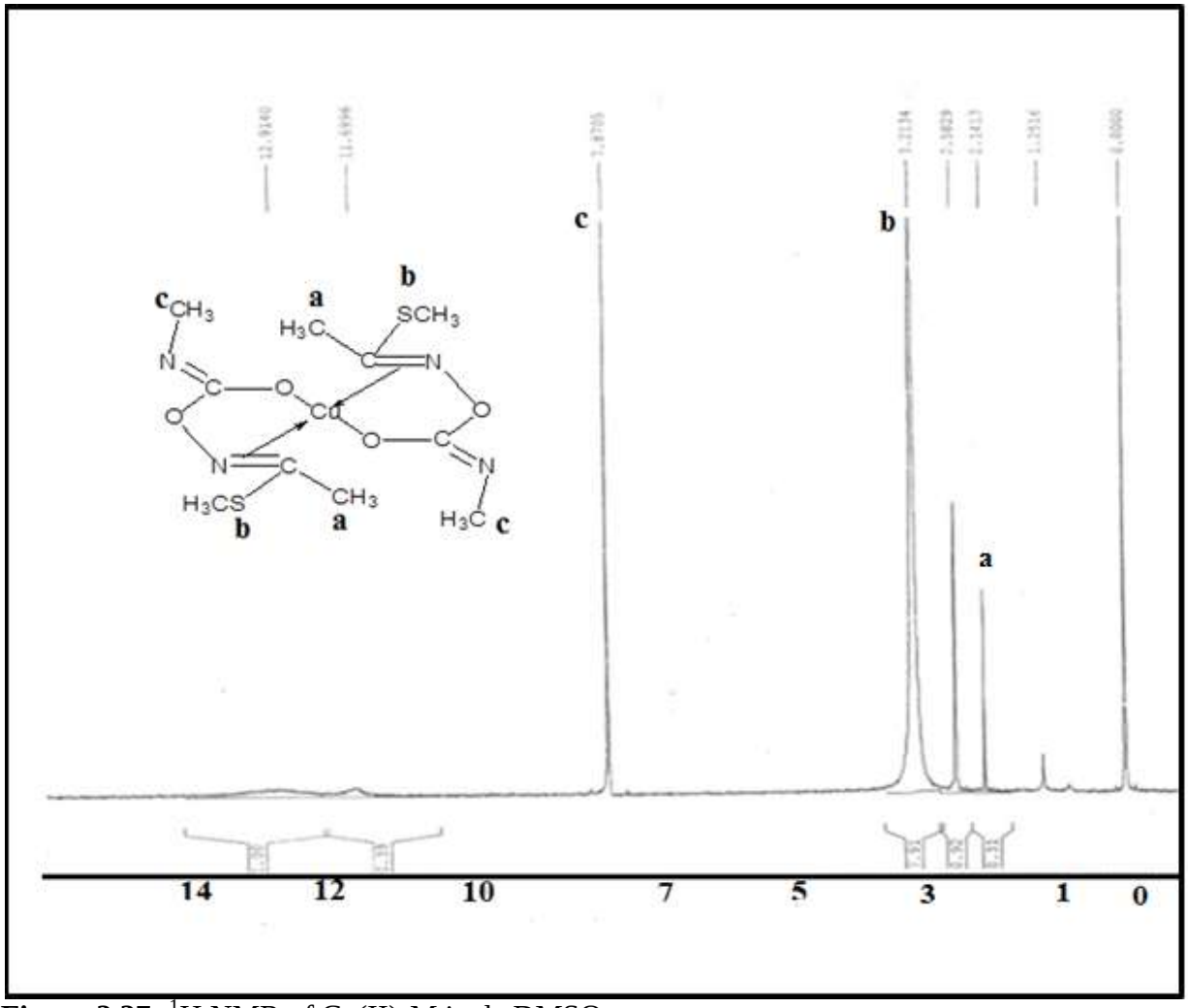


Figure 2.27: ^{1}H NMR of Cu(II)-M in d_6-DMSO.

Keypoints - From the interpreted result, it was found that methomyl reacted faster with Mn(II) and Fe(II) metal ions than that of Ni(II), Co(II), Cu(II) and Zn (II) metal ions. In presence of the basic media the reaction progress was fast, means in basic soil, metal depletion will be higher. The IR and H^1 NMR spectral study, suggest interaction of methomylwith the metal ion took place through oxygen (of carbonyl group) and carbon atom (of imine group) of methomyl.

2.3.4.5 Thiophanate methyl (TM)

2.3.4.5.1 UV-visible analysis of TM complexes

To determine progress of the reaction, the concentration of thiophanate methyl used in the reaction was determined using the UV-vis spectrophotometer. Since, almost all metal complexes of thiophanate methyl were insoluble in almost all laboratory solvents, the value of concentration of TM remained in methanol after different interval of time was calculated by

Beer-Lambert's law at 269nm. The calculated values were subtracted from the initial value to get the amount of the thiophanate methyl used in the reaction.

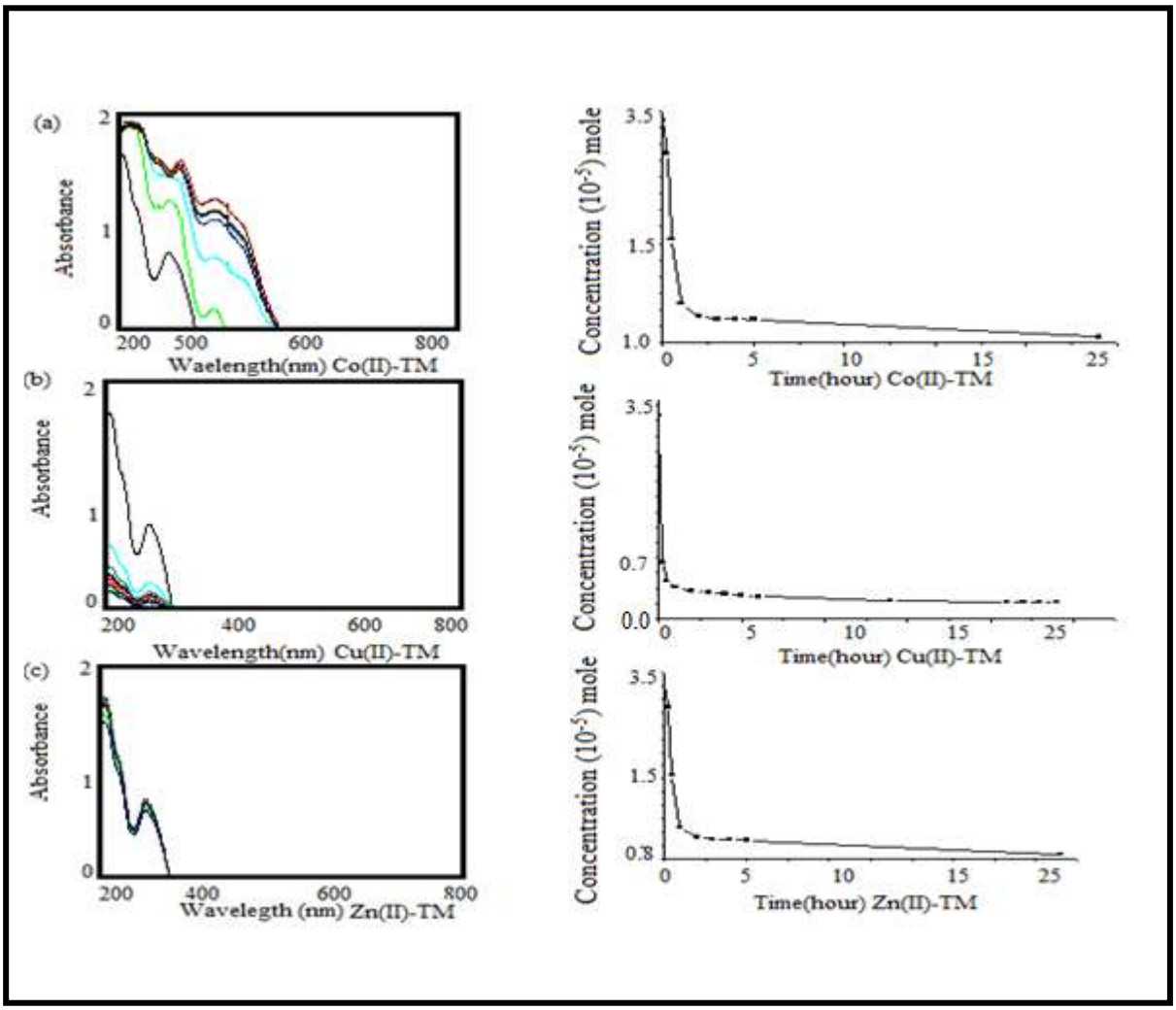


Figure 2.28: (a) Reaction progress during the Co(II)-TM, Cu(II)-M and Zn(II)-TM interaction by UV overlay curve (b) Plot depicting the decrease in concentration of TM ligand with time.

During the complex formation, decrease in absorbance of the thiophanate methyl was observed. The observed changes in the concentration were calculated and plotted against time. The maximum decrease in the concentration was observed within 3rd hour of the reaction (as shown in Figure 2.28). Likewise, the changes observed in the concentration were calculated in percentage after evaluating the reaction for 24 hour for the different metal ions are compiled in Table 2.10.

Table 2.10 - Stoichiometric ratio for the interaction of the metal ions with thiophanate methyl

S.NO	% of the thiophanate methyl consumed with the time (h)						
	Thiophanate methyl metal complexes	1	2	3	5	8	24
1	Mn(II)-TM	5	10	14	15	17	21
2	Fe(II)-TM	8	14	18	23	32	42
3	Co(II)-TM	30	38	46	56	54	54
4	Ni(II)-TM	43	49	53	57	57	57
5	Cu(II)-TM	83	84	85	85	91	95
6	Zn(II)-TM	47	52	63	69	72	72

2.3.4.5.2. FTIR analysis of the TM- metal complexes

On comparing the IR spectra of TM complexes with respect to the TM, broadening in the N-H and the C-H stretching band was observed. The broadening was accompanied by the shifting of the peak towards the lower wave number in case of NH stretching peak. The shift in the peak was observed in the range of 50-10cm^{-1} for NH stretching bandand 60-20 cm^{-1} for C=S stretching band. In case of CH stretching band shift was found toward higher wavenumber in the range of 197-50cm^{-1}.Moreover, C=O stretching band which was observed at 1710cm^{-1}in case of TM was not observed after complex formation. In replacement, medium intensity peaks were observed within the range of 1550-1630cm^{-1}. It could be due to the formation of the C=N bond after the complex formation. The deduced IR frequencies for the TM ligand and the complexes are tabulated below in table 2.11.

Table 2.11: IR frequencies of the thiophanate methyl and its metal complexes

Type of IR-frequency	TM	Mn(II)-TM	Fe(II)-TM	Co(II)-TM	Ni(II)-TM	Cu(II)-TM	Zn(II)-TM
N-H stretching	3350m	3300m	3322m	3324 m	3326m	3330 m	3340.2m
C-H stretching	2953w	3050w	3150w	3000 w	3100w	3070 w	3050w
C=O Stretching	1710s	--------	--------	---------	---------	----------	----------
C=N Stretching	-------	1623w	1555w	1561w	1560w	1562w	1561w
C=S Stretching	1255w	1215w	1188w	1188w	1200w	1206w	1180w
C=C	1451, 1338,	1450, 1402,	1455, 1408,	1446, 1380,	1468, 1437,	1466.2, 1442.2,	1488, 1397,

Stretching	1378m	1382m	1378m	1356m	1378m	1360m	1382m
N-H bending	1521m	1595m	1565m	1555m	1552m	1597m	1554m
C-N stretching	1170w	1174w	1178w	1183w	1180w	1175w	1178w
M-O stretching	NA	546w	523w	502w	518w	475w	418w

*s= strong, m = medium, w = weak and represent intensity of the IR absorption peaks. M-O and M-N represent metal- oxygen and metal- nitrogen stretching frequencies.

The observed shift in the frequencies can be explained by taking example of Cu-TM (as shown in Figure 2.29 with Co(II)-TM andNi(II)-TM spectra) with respect to TM. The broadening of the N-H and the C-H stretching band was observed, accompanied by lowering of N-H, and C=S stretching band in the Cu-TM complex with respect to the TM ligand. The shift in the lower frequency was observed as 20cm^{-1} for NH stretching band, and 61 cm^{-1} for C=S stretching band. The lower shift in the frequency is observed due to the shift in the electron density after the complex formation.Moreover, broad peak was observed at 1562cm^{1}. The observed peak is due to the C=N stretching frequency which is formed after the complex formation. The new medium intensity peak was observed at 475 cm^{-1} which is due to the formation of the Cu-O bond and marked by red asterisk in the spectra.

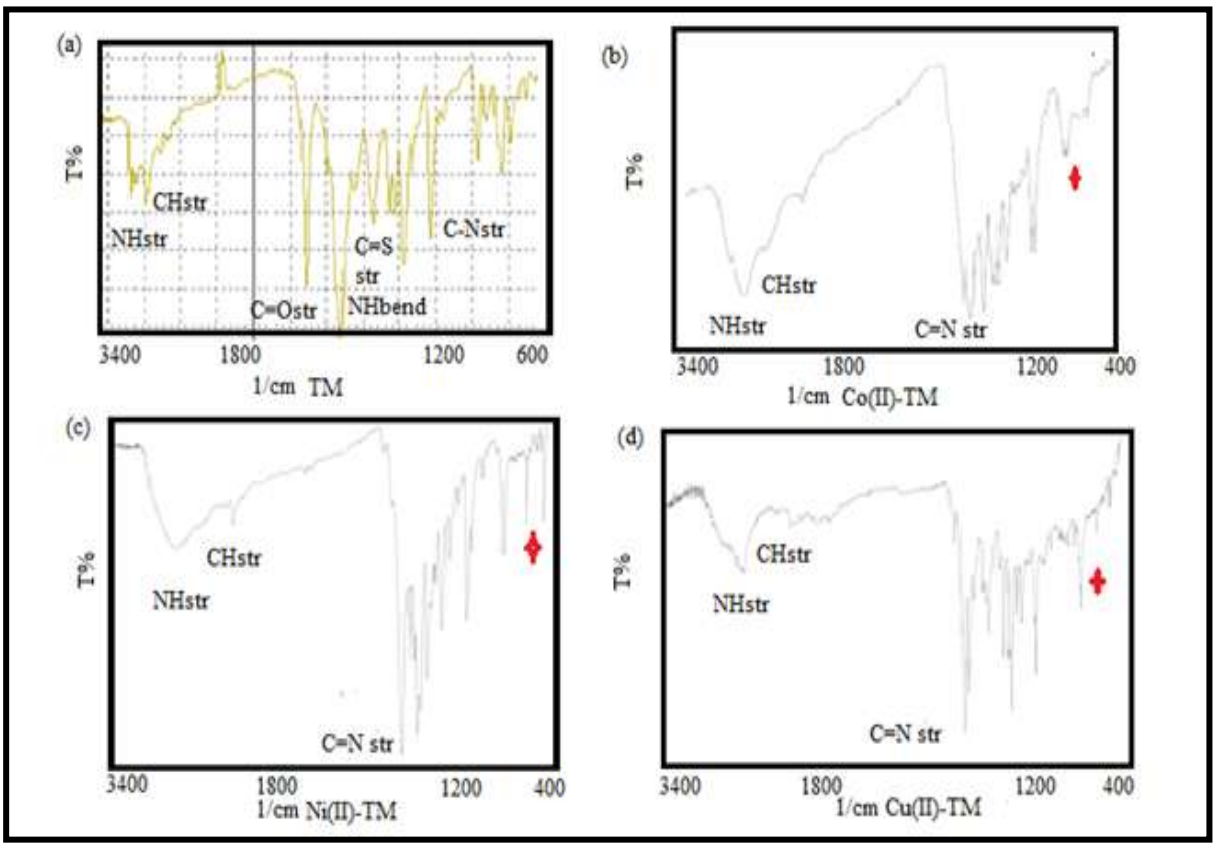


Figure 2.29: IR of TM, Co(II)-TM, Ni(II)-TM and Cu(II)-TM.

2.3.4.5.3 ^{1}H-NMR spectral analysis of TM complex.

Because of poor solubility of TM- metal complexes in any of the laboratory solvent, ^{1}H-NMR spectra of metal complexes of thiophanate methyl were not achieved.

2.3.4.5.4 Mass spectral analysis of the TM metal complex.

Mass fragmentation of the Cu(II)-TM complex is shown in the Figure 2.30. It depicted that (Cu(II)-TM) has 403 m/z value with the molecular formula [Cu(II)-$C_{12}H_{14}N_4O_4S_2$].

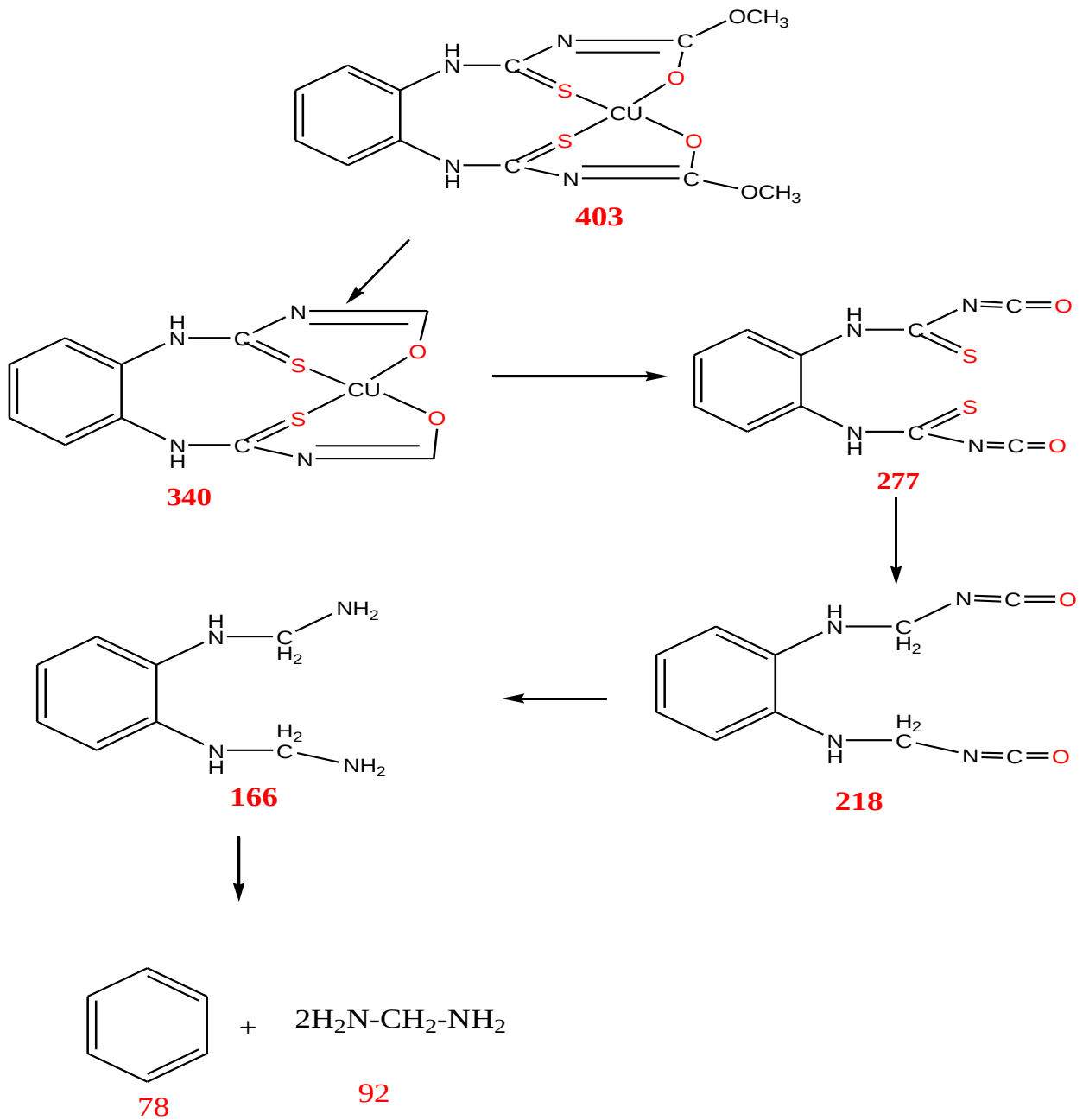


Figure 2.30: Mass fragmentation of the Cu(II)-TM.

The mass fragmentation is proceeded by the removal of the two (OCH_3) group leading to the m/Z value 340. It is advanced by the removal of the Cu metal ion and the m/Z value was found as 277. Further, two (SH) group were removed and the m/Z value was found as 218. After the removal of the two (CO) groups the m/Z value was found as 166. At the end, it is fragmented to form the (C_6H_6) and two NH_2CHNH_2 groups.

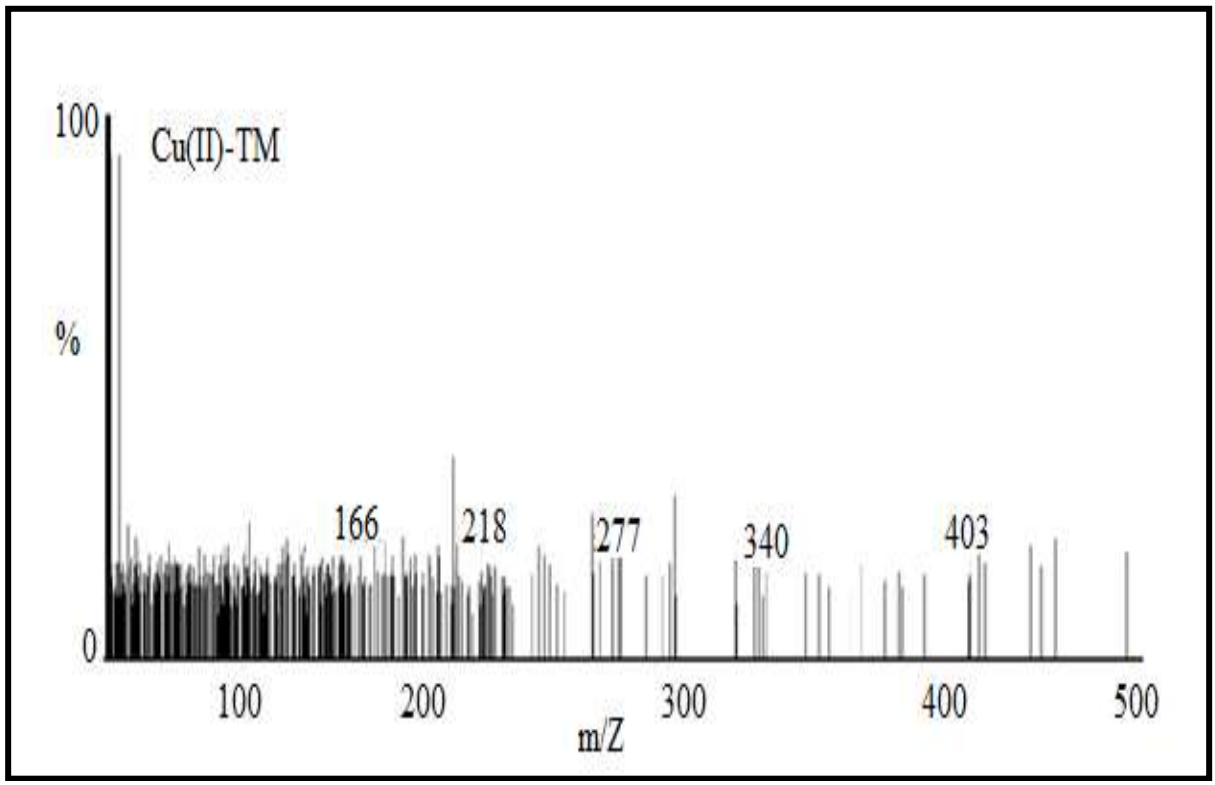


Figure2.31: Mass spectrum of the Cu(II)-TM complex.

Key points-From the interpreted result it was found that thiophanate methyl reacted faster with Ni(II), Cu(II) and Zn (II) metal ions than that of the Mn(II) and Fe(II) metal ions. In presence of the basic mediumand higher temperature the reaction process was fast. The IR spectral study exhibited possibility of complexation of thiophanate methyl with metal ion took place through oxygen and sulphur atom. The formed complex of thiophanate methyl with copper metal ion also depicted the formation of bond took place through donor oxygen and sulphur atom and depicted m/z value of 403.

2.3.5 FESEM analysis of Carbamates metal complexes.

FESEM (Field-Emission Scanning Electron Microscopy) images of carbamate- metal complexes are shown in Figure 2.32. Images revealed that most of the metal complexes are nano sized and can be trapped in the soil and could retain in it for longer duration.

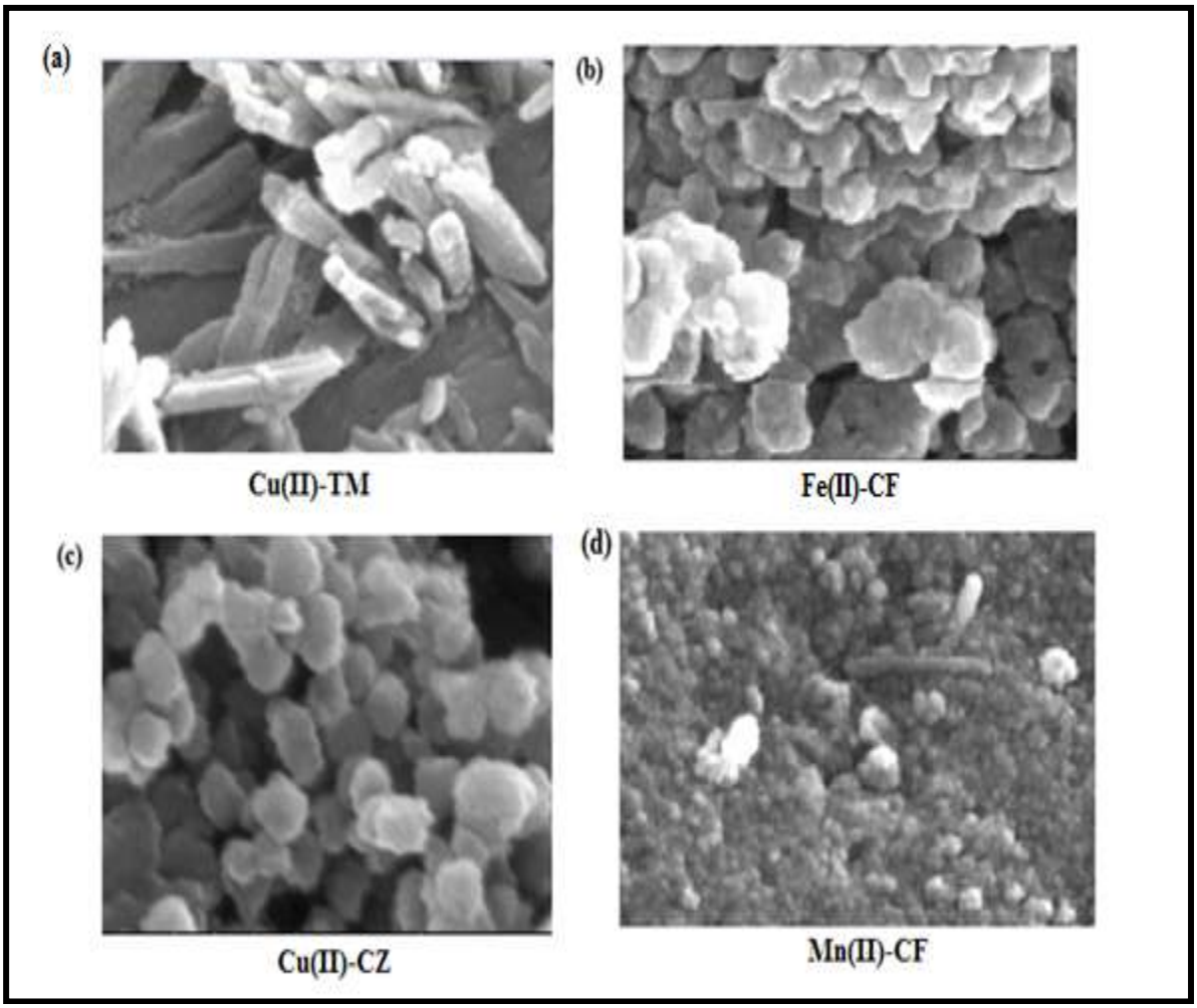


Figure 2.32: Representing FESEM analysis of the (a) and (c)Thiophanate methyl and Carbendazim with Cu(II) metal ion (b) and (d) representing interaction of Mn(II) and Fe(II) metal ion with carbofuran.

2.3.6 TGA analysis of CF/CZ/TM/TC/M carbamates and metal complexes.

TGA analysis was performed to examine the thermal stability of the formed metal complexes. It was observed that the thermal stability of the complexes directly links with their chelating ability. The interpreted results for each of the carbamates are as followed:

2.3.6.1 TGA analyses of the CF metal complex.

For the determination of the thermal stability of the Fe(II)-CF complex with respect to the CF ligand, thermal analyses was performed. It was observed that decomposition in case of the CF ligand occurred in a single step that too in between 201- 250^{0}C. On the other hand Fe(II)-CF decomposes in four steps, which is summarized in Table 2.12.

Table 2.12: Thermal decomposition of the CF and Fe(II)-CF complex

Ligand

Sample	Stage	*T_i(°C)	Tp (DTGmax) (°C)	T_f (°C)	Mass loss Calcd (%)	Assignment	Metallic residue	DTA (^oC)
CF	1^{st}stage	201	232.06	250	58	Loss of $C_{10}H_{12}$	-	232.2 (+)

Ligand-Metal

Fe(II)-CF	1^{st} stage	39	126.07	200	5	Loss of C_2H_6	-	-
	2^{nd} stage	201	248.03	350	14	Loss of C_2H_6 and OC_2H_6	-	384.91(+)
	3^{rd} stage	401	432.83	450	32	Loss of $C_{10}H_{22}O_2$	-	-
	4^{th} stage	680	758.35	790	38	Loss of $C_{12}H_{12}$ and 2CO	FeO_2N_2	

*Here, Ti, Tp and Tf are initial, peak and final temperatures.

TGA plot of Fe(II)-CF complex indicate that the decomposition started with loss of C_2H_6 molecule with mass loss of 5%. In second step loss of the C_2H_6 and the OC_2H_6 molecular fragment was found with mass loss of the 14%.The third step advanced by the loss of the $C_{10}H_{22}O_2$ molecule with the mass loss of the 32%. In the last step, the loss of the two CO and $C_{12}H_{12}$ molecule caused the 38% of the mass loss. The total mass loss was found 89% and leaving FeO_2N_2 as metallic residue. The comparative mass loss is tabulated above as Table 2.12.and depicted in Figure 2.33.

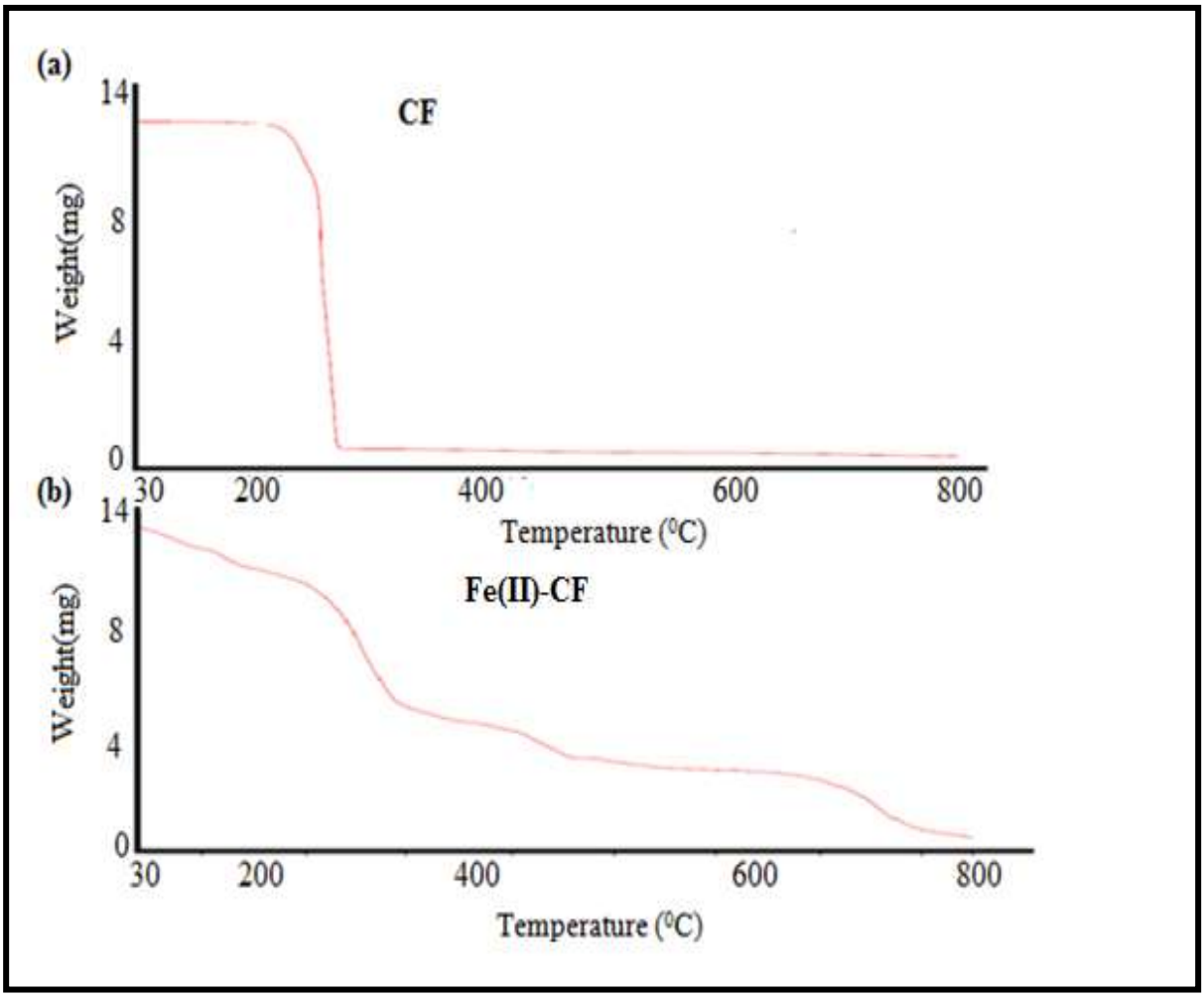


Figure 2.33: TGA curve of CF ligand and Fe(II)-CF complex.

From the data it is depicted that Fe(II)-CF complex is thermally stable within the temperature range of the 39-400^{0}C. As, the total mass loss in the range was found is 19% whereas in case of the CF ligand the loss was >90%.

2.3.6.2 TGA analyses of the CZ metal complex.

Carbendazim form strong complex with Cu(II), but weak with Fe(II). For the determination of the thermal stability of the Fe(II)-CZ complex with respect to the CZ ligand thermal analyses was performed. Decomposition of the CZ ligand and Fe(II)-CZ complex was found in four steps within the temperature range of 39-850^{0}C. In case of the CZ ligand, the decomposition initiated by the loss of the C_4H_4 molecular fragment with mass loss of 28% (as shown in Table 2.13). In the second step, mass loss was found 30% with loss of $C_2H_3O_2$. The third step resulted by the loss of the CNO molecule with mass loss of 22%. In the fourth step, the mass loss was found 9% for NH group. The total mass loss found was 89%. In case of Fe(II)-CZ

complex, decomposition started with the loss of two CH_3group with mass loss of 6%. In the second step, loss of the C_2H_6O and $C_7H_5N_2$ molecule was observed with mass loss of 9% and 24%. The third step correspond to the loss of $C_7H_5N_2$ with mass loss of 24%.In the fourth step mass loss was found as 18% with the loss of the two CO_2 molecule. The total mass loss was found as 81% and leaving FeN_2 as the metallic residue.

Table 2.13: Thermal decomposition of the CZ and Fe(II)-CZ complex

Ligand

Sample	Stage	T_i(°C)	T_p(DTGmax) (°C)	T_f (°C)	Mass loss Calcd (%)	Assignment	Metallic residue	DTA (^{o}C)
CZ	1st stage	39	203.50	210	28%	Loss of C_4H_4	-	178.94(+), 209.64(-)
	2nd stage	210	303.36	400	30%	Loss of $C_2H_3O_2$	-	
	3rd stage	401	-	600	22%	Loss of CNO	-	
	4th stage	601	706.12	850	10%	Loss of NH	-	

Ligand-Metal

Fe(II)-CZ	1st stage	39	-	200	5%	Loss of 2 CH_3	-	-
	2nd stage	201	239.04, 342.55	400	9%, 23%	Loss of C_2H_6O and $C_7H_5N_2$	-	243.68 (+)
	3rd stage	401	475.55	600	23%	Loss of $C_7H_5N_2$	-	-
	4th stage	601	-	850	18%	Loss of $2CO_2$	FeN_2	-

Here, Ti, Tp and Tf are initial, peak and final temperatures.

From the data it is depicted that in case of the CZ, mass loss occurred in the first stage is 28% within the temperature range of the 39-200°C. In the second stage, mass loss percentage was found 30% within the temperature range of the 200-400°C. The total mass loss was 58% up to the temperature of the 400°C. On contrary in case of the Fe(II)-CZ complex mass loss occurred in the first stage was 5% within the temperature range of the 39-200^{0}C. And in the second stage mass loss was found 32% in the temperature range of 200-400^{0}C. The total mass loss of the 37% is found up to 400^{0}C. On comparison, we could deduce that the formed complex is found thermally stable than the ligand with in the temperature of 400^{0}C. As, the

rise in the temperature increased the loss of the mass percentage, leading to the 78% of the mass loss on heating upto the 850^0C for the CZ complex and 90% in case of the CZ ligand (as shown in Fig 2.34).

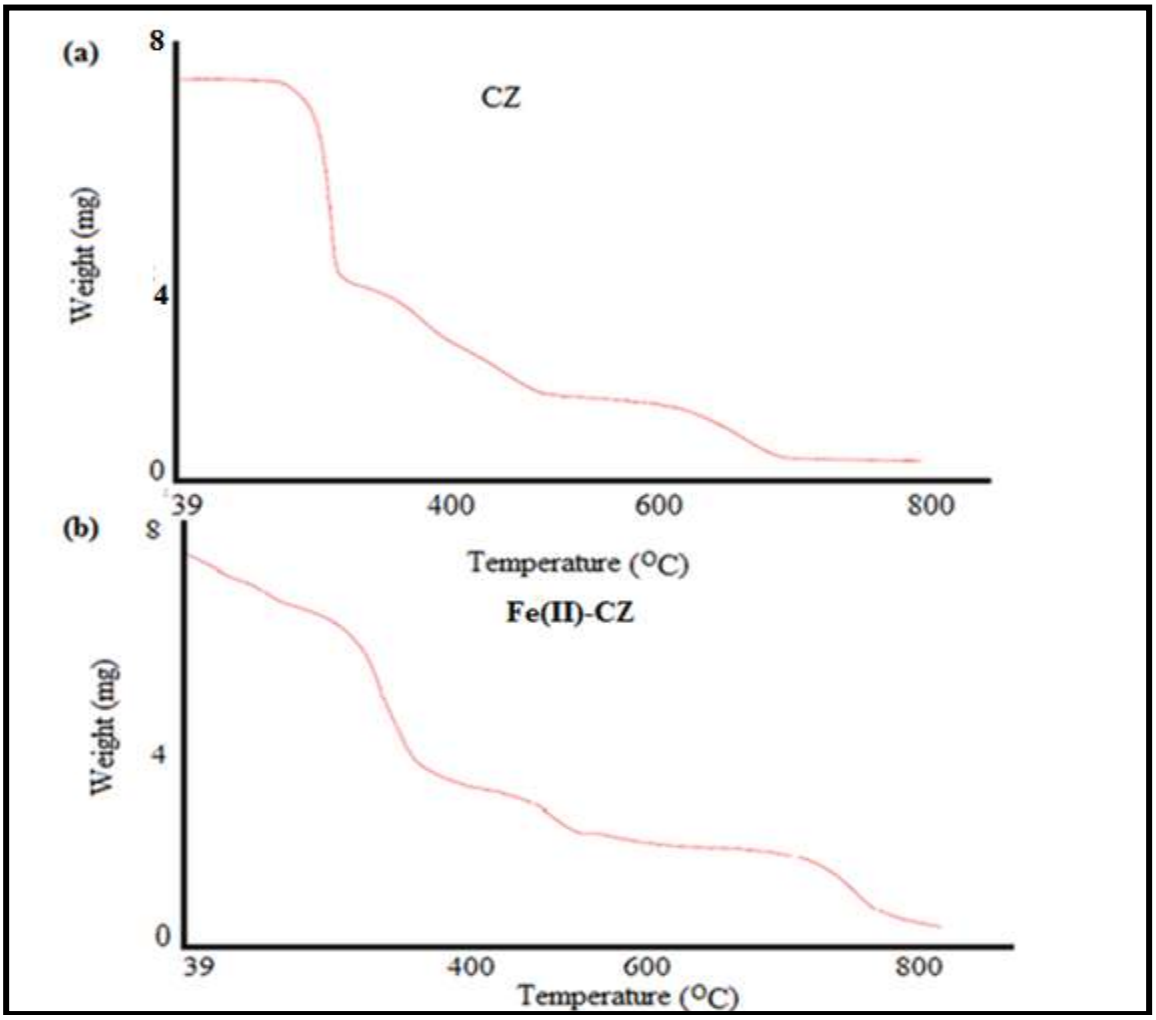


Figure 2.34: TGA curve of CZ ligand and Fe(II)-CZ ligand.

2.3.6.3. TGA analyses of the TC metal complex.

For the determination of the thermal stability of the Cu(II)-TC complex with respect to the TC ligand thermal analyses was performed. It was found that stability of formed metal complex is much more in comparison to the stability of the TC. Thermal decomposition of the TC and the Cu(II)-TC complex is tabulated in Table 2.14.

From the data, it is depicted that in case of the TC, decomposition took place in two steps. The first step involved decomposition of TC with loss of $C_9H_{15}N_4O_4S_2$ group equivalent to mass loss of 86% within the temperature range of the 39-200^0C. Second step of thermal decomposition of TC is resulted due to loss of CH_3S group, equivalent to mass loss of 13% in the temperature range of 200-400^0C. The total mass loss was found 99%. Whereas, in case

Cu(II)-TC complex, thermal decomposition involved four steps. The mass loss of 7% took place with loss of two CH_3 molecule within the temperature range of the 39-200^{0}C. The second step corresponds to the loss of $C_4N_2O_2$ equivalent to 26% mass loss, third stage of decomposition represent loss of $C_2H_6N_2S$ (equivalent to 22%) while the last stage of decomposition involve the loss of 19% for the loss of CH_3S_2 group (as shown in Fig 2.35).

Table 2.14: Thermal decomposition of the TC and Cu(II)-TC complex

Ligand

Sample	Stage	T_i(°C)	T_p(DTGmax) (°C)	T_f (°C)	Mass loss Calcd (%)	Assignment	Metallic residue	DTA (^{o}C)
TC	1st stage	39	206.83	210	86%	Loss of $C_9H_{15}N_4O_4S_2$	-	107.6 (+), 210(+)
	2nd stage	201	-	400	13%	Loss of CH_3S	-	

Ligand-Metal

Cu(II)-TC	1st stage	39	82.20	200	7%	Loss of 2 CH_3		
	2nd stage	201	298.86	400	26%	Loss of $C_4N_2O_2$		
	3rd stage	401	478.17	600	22%	Loss of $C_2H_6N_2S$		420.12 (+)
	4th stage	601		850	19%	Loss of CH_3S_2	FeO_2	

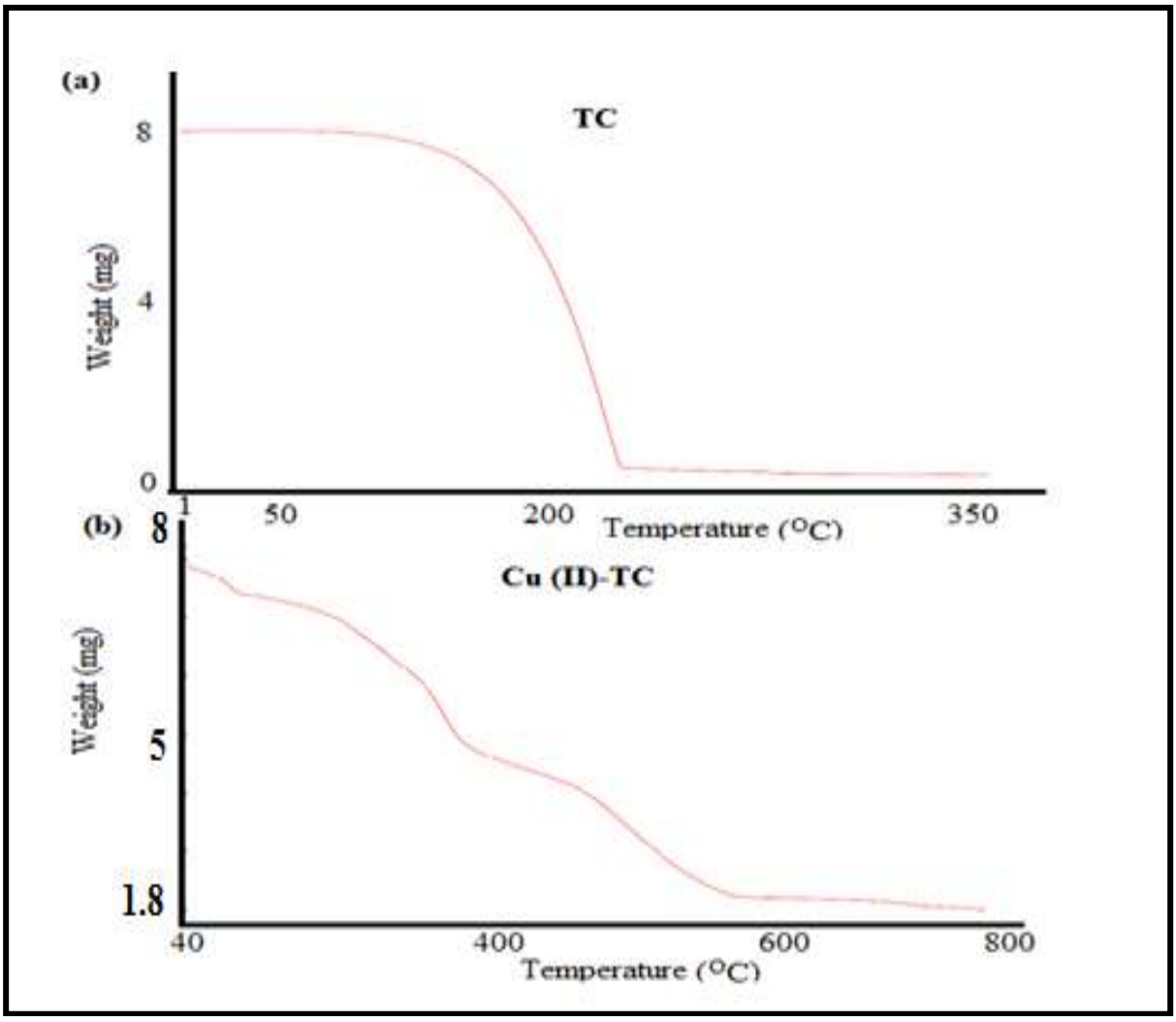


Figure 2.35: TGA curve of TC ligand and Cu(II)-TC ligand.

2.3.6.4 TGA analyses of the M- metal complex.

Thermal stability of the Fe(II)-M complex with respect to methomyl was observed by comparing both the TGA spectra . It was found that, methomyl undergone a single step decomposition between 160- 220^{0}C. Whereas, in case Fe(II)-M complex decomposition took place in four steps, within the temperature range of the 39-800^{0}C. Loss of different kind of molecular fractions is tabulated in the Table 2.15.

Table 2.15: Thermal decomposition of the M and Fe(II)-M complex

Ligand

Sample	Stage	T_i(°C)	T_p(DTGmax) (°C)	T_f (°C)	Mass loss Calcd (%)	Assignment	Metallic residue	DTA (^{o}C)
M	1st stage	39	200	200	98	Loss of $C_5H_9N_2O_2S$	-	

	2nd stage	201	-	400	1			
Ligand-Metal								
Fe(II)-M	1st stage	39	100.03	200	3	Loss of CH_3	-	
	2nd stage	201	395.01	400	17	Loss of CH_3N	-	
	3rd stage	401	480	600	2	Loss of $3H_2$	-	
	4th stage	601	702	850	8	Loss of SH	$C_7H_4FeO_4N_4S_3$	

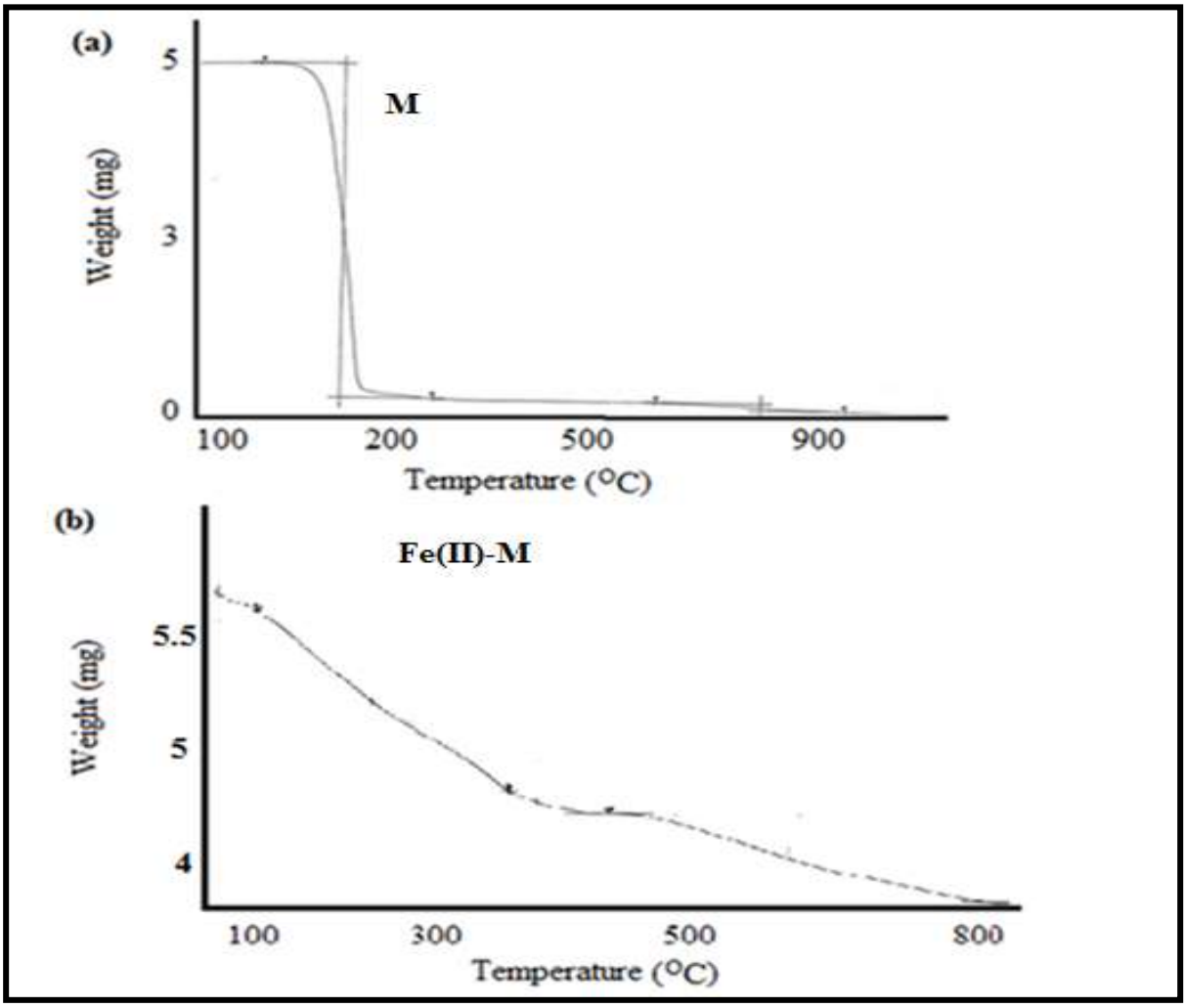


Figure 2.36: TGA curve of M ligand and Fe(II)-M ligand.

In case of the Fe(II)-M complex, decomposition step was advanced by loss of CH_3 group with mass loss of 3%. The second step corresponds to the loss of the CH_3N molecule with mass loss of 17%. In the third step, mass loss was found as 2% with loss of three H_2 molecules. In

the fourth step loss of SH molecule caused the mass loss of 8%. The total mass decomposed was found as 30% with left $C_7H_4FeO_4N_4S_3$ metallic residue (as shown in Figure 2.36).

2.3.6.5 TGA analysis of the TM metal complex

For the determination of the thermal stability of the Cu(II)-TM complex with respect to the TM ligand thermal analyses was performed. From the data it is depicted, the decomposition in case of the TM ligand took place in four steps whereas, in case of the Cu(II)-TM complex took place in three steps within the temperature range of the 39-800^{0}C. The decomposition started in case of the TM ligand with loss of two OCH_3 group with mass loss of the 18%. In second step, the mass loss was found as 40% with the loss of $C_6H_6N_2$and CO groups. In the third step, decomposition correspond to loss of two NH and CO molecule with mass loss of 18%. In the fourth step decomposition was found as 12% with loss of CS molecule. The total mass loss was found as 88%. In case of the Cu(II)-TM complex, decomposition advanced by loss of NH molecule with mass loss of 2%. The second step proceeded with loss of two OCH_3 molecules with total mass loss of the 16%. In the third step, mass loss was found as 23% with loss of the C_6H_6N molecule. The total mass loss was found as 41% till 800°C.

Table 2.16: Thermal decomposition pattern in case of the TM and Cu(II)-TM complex

Ligand

Sample	Stage	T_i(°C)	T_p(DTGmax) (°C)	T_f(°C)	Mass loss Calcd (%)	Assignment	Metallic residue	DTA (^{o}C)
TM	1st stage	39	189.40	200	18%	Loss of 2 OCH_3	-	188.35 (+)
	2nd stage	201	287.60	400	40%	Loss of $C_6H_6N_2$and CO	-	-
	3rd stage	401	-	600	18%	Loss of 2NH and CO	-	-
	4th stage	601		800	12%	Loss of CS	-	-

Ligand-Metal

Cu(II)-TM	1st stage	39	-	200	2	Loss of NH	-	144.57 (+)
	2nd stage	201	266 , 317	400	8.8	Loss of 2 OCH_3	-	363.41 (+)
	3rd stage	401	498.2	800	23%	Loss of C_6H_6N	$C_4N_2CuO_2S_2$	-

It is observed from the tabulated data 2.16, that Cu(II)-TM complex is highly stable than that of the ligand. As it is observed that, after heating of up to 800^0C, only 41% of the mass is decomposed. On contrary, TM ligand decomposed up to 58% within the temperature range of 400^0C and total decomposition found as 88%. The Figure 2.37 is depicting thermogravimetric curves of TM and Cu(II)-TM.

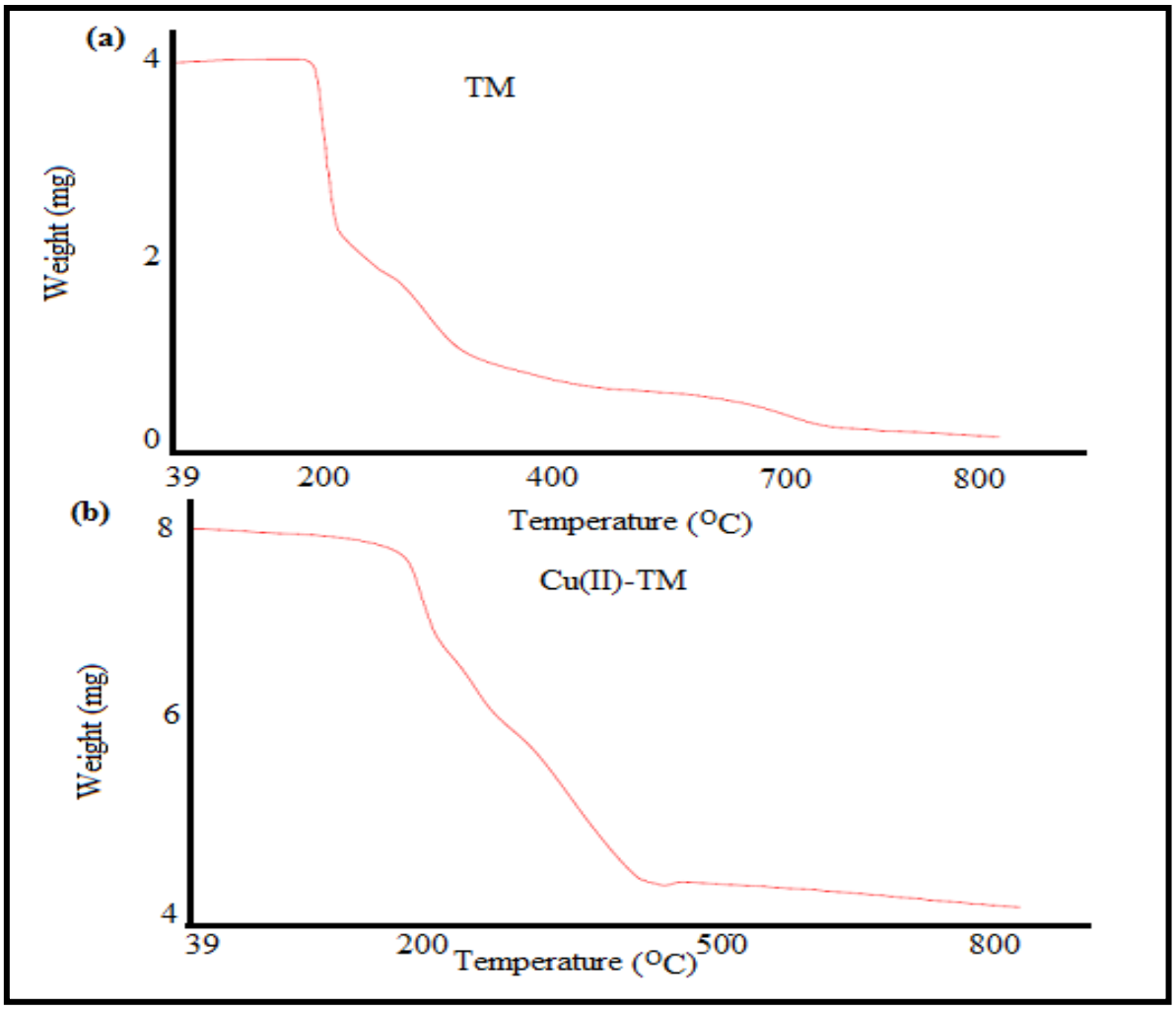
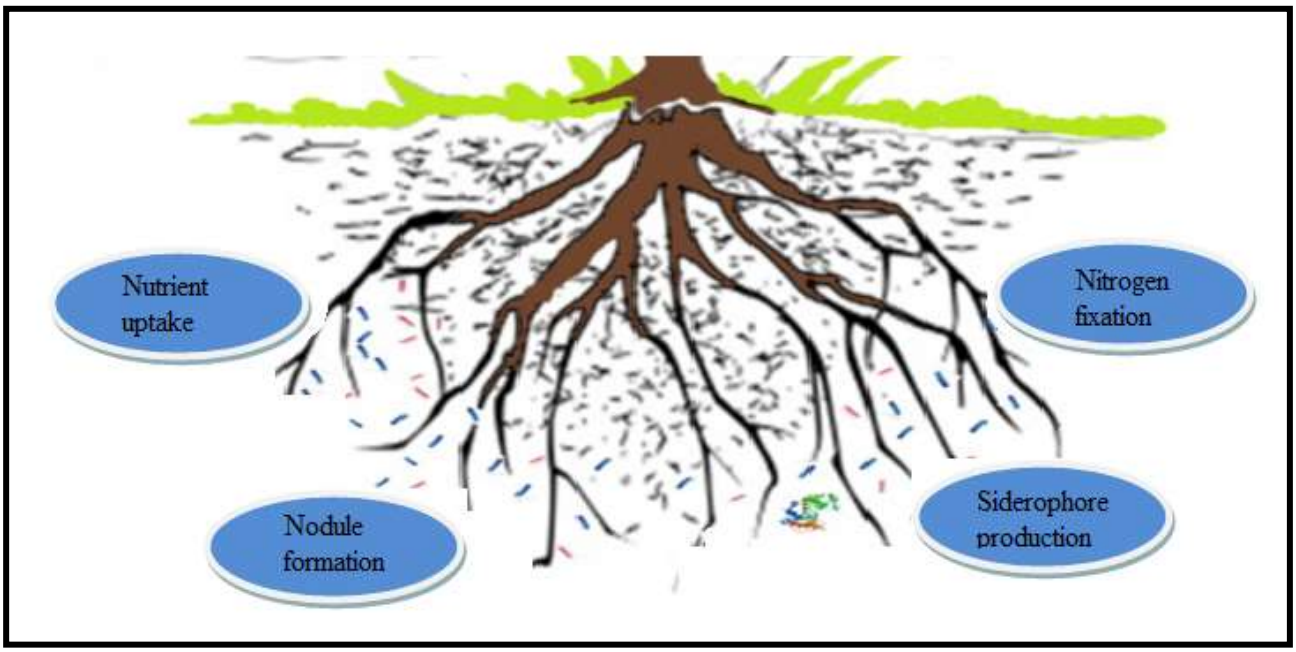


Figure 2.37: TGA curve of TM and Cu(II)-TM complex.

Chapter-3

Effect of the pesticides on the plant growth promoting bacteria



3.1 INTRODUCTION

In a natural soil environment, microbial communities portrayed the cooperative relationship with food crops through plant growth promoting rhizobacteria also known as PGPR. PGPR benefits food crop by producing plant growth regulators and inducing resistance against plant pathogens. Another important mechanism related to PGPR is siderophores production [106,107]. Siderophores are low molecular weight, high affinity iron chelating compounds evolved out through microorganisms highly specific pathways.[108]

Siderophores are employed by PGPR for scavenging the iron from the surrounding soil and make it available to the plants. Their role is indispensable, as they help to convert the Fe(III) oxidation state to Fe(II) oxidation state .The conversion of the oxidation state is essential, as in oxic conditions Fe exist in (+3) oxidation state and forms very insoluble mineral precipitates such as hematite, and goethite. It leads to make the Fe metal ion concentration below the requirement for adequate supply in plants. In contrast, Fe(II) oxidation is water soluble but found in anoxic condition.[109,110] To overcome the deficiency of Fe metal ion, siderophores get attached with mineral surface and facilitated the dissolution by coordinating with Fe (III) metal ion and reduce it to water soluble Fe (II) metal ion.[111](represented in Figure 3.1)

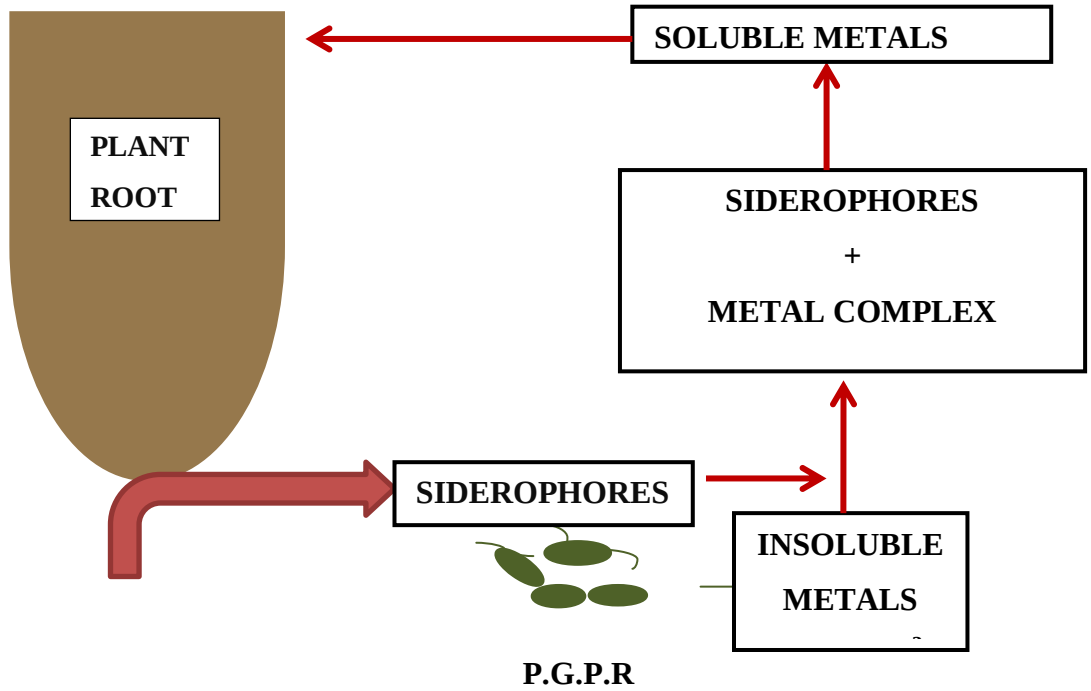


Figure 3.1 Schematic representation of the importance of the siderophores produced by PGPR for plant in metal uptake.

Siderophores act as ligand to bind with the metal ion and usually their structure for binding includes catecholate, hydroxamate and carboxylate groups.[112] Their coordination with metals ions is ruled by hard and soft (Lewis) acids bases concept, also known as Pearson acid- base concept. It could be simplified as, Fe^{3+} is a hard lewis acid and prefer to form strong coordinate bond with hard lewis base, which is provided to the metal ion by the anionic or the neutral oxygen atom present in the siderophores.[113] Just after the reduction of the iron from $Fe^{3+}$to Fe^{2+}state, the metal ion is released by the siderophores due to its less affinity with the ligands, as Fe^{2+} is not as good hard acid as Fe^{3+}.[114] Siderophores are found to form the stable bond with Fe^{3+} metal ion through the octahedral or hexadentate complex.[115] They are also known to coordinate with other essential metal ions such as Al, Cr, Mn, Cu and Zn.[116]

However, when the pesticides are applied on soil they intervene with the growth and the functioning of the PGPR.[99,100,102] As, pesticides have shown the ability to interact with metal ions and limited their availability for better plant growth, they might show the possibility to compete with natural metal ion carrier (which are produce by plant growth promoting rhizobacteria) for trapping the metal ion from the soil. To accentuate such facts relating to the pesticides, study is conducted to check the minimum amount of concentration which could adversely affect the population of the plant growth promoting bacteria as well as initial attempts are made to analyze the siderophores (qualitatively and quantitatively) in presence of the pesticides.

3.2 EXPERIMENTAL

3.2.1. Materials

For experimentation technical grade (98% pure) Carbofuran, Carbendazim ,Thiodicarb , Thiophanate methyl and Methomyl was provided by Gautmi Ltd, Hyderabad (India). The other laboratory chemicals of AR grade were purchased from Loba chemie which included NaOH, HCl, DMSO, acetic acid, and metal salts (iron).

Plant growth promoting bacterial strains were supplied by NCL Pune-India, with unique identity number mentioned as; *Rhizobium leguminosarum* (NCIM-2749); *Pseudomonas fluorescence* (NCIM-5096); Bacillus *brevis* (NCIM-2532); *Azotobacter vinelandii* (NCIM-2821); *Salmonella typhimurium* (NCIM-2501) and were cultured as per standard specifications obtained from NCL Pune.

3.2.1.1 Media Preparations.

To determine the effect of the Carbofuran, Carbendazim , Thiodicarb , Thiophanate methyl and Methomyl on tfhe population of the plant growth promoting bacteria , minimal inhibitory concentration test was conducted nutrient agar media. The media was prepared by adding 28 grams of nutrient agar into the 1 liter of double distilled water in a flask, where it was mixed thoroughly and pH was adjusted at 7.5 ± 0.2. The prepared mixtures were heated for obtaining the clear solution. The obtained solutions of media were autoclaved at 121°C for 45 minutes at 15 pbs pressure. The autoclaved media between 15-20 ml were separately poured into petri dish for the experimentation.

For understanding the effect of Carbofuran, Carbendazim, Thiodicarb, Thiophanate methyl and Methomyl on the siderophores production qualitative test was conducted. For qualitative test experimentation, King's B media was prepared using the following ingredients (g/L); Protease peptone 25.00, Dipotassium hydrogen phosphate 1.87, Magnesium sulphate heptahydrate 1.87, and Agar 25.00. After preparation of the King's B media 54 mg/L $FeCl_3$ was added in it and the mixture was adjusted to the final pH of 7.2±0.05 at 25°C.

In case of the quantitative analysis of siderophores, the media was prepared using the above written methodology except solidifying agent agar was not used in later case. Distilled water was used as solvent in both experiments. Moreover, to obtain the accurate results comparative study was conducted in which, both experiments were divided into two parts; in first part, the production of siderophore were observed without addition of Carbofuran, Carbendazim , Thiodicarb , Thiophanate methyl and Methomyl carbamates pesticides (referred as control), while in second part, growth of siderophores were evaluated after the addition of 25 mg/L and 200 mg/L of Carbofuran, Carbendazim , Thiodicarb , Thiophanate methyl and Methomyl carbamates. To avoid the contamination, all the experiments were performed under controlled conditions and triplicate analysis were done for checking the reproducibility.

3.2.1.2. Minimal Inhibition Concentration Test.

The minimal inhibition concentration test on selected plant growth promoting bacteria was conducted by using of disk-diffusion method. For that purpose, Whatman no. 1 filter paper was sterilized, as they were used for preparing the disks by autoclaving at 160°C for 1h. Then the sterile disks were impregnated with the chosen carbamates pesticides at different concentrations (0ppm, 25ppm, 100ppm and 200 ppm). Cultures having 10^5CFU/mL were used against each concentration levels. The impregnated disks were placed on the medium

suitably spaced apart, and the plates were incubated at 37°C for 24 h. DMSO was used as solvent control and as 0 ppm. Finally the zones of inhibition were measured in mm scale.[117]

3.2.1.3. Qualitative analysis of siderophores.

For qualitative analysis, King's B media was prepared (using following ingredients (g/L); Protease peptone 25.00, Dipotassium hydrogen phosphate 1.87, Magnesium sulphate heptahydrate 1.87, and Agar 25.00) and put into the 250 ml capacity of the separate flasks. The volume of the flask was made up to the 150mL by adding the distilled water in each flasks. Afterwards, 7.0mg $FeCl_3$ was added to the respective flasks. Each of the flasks was treated distinctively. As in case of the first flask, the prepared solution was left untreated with the pesticides and it is marked as control. The other flasks were treated with the three different concentrations each pesticides (Methomyl, Carbofuran, Carbendezim, Thiophanate methyl and Thiodicarb), to prepare 25mg/L, 100mg/L and 200mg/L solution of each pesticide. All the flasks were autoclaved for 1hr at 160°C and then autoclaved media of each flask (10mL/plate) was poured on the autoclaved petri plates in a laminar flow till solidification. After solidification, the cultures, of the above mentioned siderophore producing PGPR bacterial strains were spread in concentration 100 µL/petriplates. All the plates were capped gently and sealed using the paraffin film and sealed plates were incubated at 28±2°C for 72h. The fluorescence pigments of the bacterial colonies were assessed using an ultraviolet lamp. Fluorescence pigment formed were considered as an indication of siderophore production.[118]

3.2.1.4. Quantitative analysis of siderophore production.

In a similar manner, for quantitative analyses King's B media was prepared (using; Proteose peptone 3.00g, Dipotassium hydrogen phosphate 0.23g, and Magnesium sulphate heptahydrate 0.23g), and the solution of the prepared media was made up to 150mL using the distilled water in the 250mL flasks. In addition, 7.0mg $FeCl_3$ was added to each flask. First flask was left untreated and considered as control. The remaining flask were distinct from each other by adding, three different concentrations (25ppm, 100ppm and 200 ppm) of the each pesticides (Methomyl, Carbofuran, Carbendezim, Thiophanate methyl and Thiodicarb). The different concentrations were prepared by adding 25mg/L, and 30.00 mg for 200mg/L of the each pesticide in the flasks. All the flasks were autoclaved. The autoclaved media of each flask (10mL/test tube) was poured in autoclaved test tubes under a laminar flow and kept inside for cooling. After cooling, 100µL/test tube of cultures, of above mentioned siderophore

producing PGPR bacterial strains were added. All the test tubes were capped by using foil and then sealed by using the paraffin film. The sealed tubes were incubated at 28 ± 2 ℃ for 72 h. Afterwards, the centrifugation of the incubated material was done by transferring it into the sterilized centrifuged tubes under the laminar flow at 5,000 rpm for 15 min. At the end, clear supernatants were obtained and utilized for analysis. The absorbance spectra were recorded using a double beam spectrophotometer (Shimadzu 1800) in 1.0 cm cells, against distilled water blank.[118]

Percentage changes in siderophore production at 25.0 and 200.0mg/L of the pesticides (Methomyl, Carbofuran, Carbendezim, Thiophanate methyl and Thiodicarb) were determined by using formula, % change = (A-B)/A*100, where A = absorbance of different strains at 0 mg/L of the carbamates pesticides; B = absorbance of different strains at 25.0 and 200.0 mg/L of the above stated carbamate pesticides.

3.3. RESULTS AND DISCUSSIONS

Particular plant growth promoting bacterial strains were chosen on their ability to secrete the distinctive types of siderophores structure. For instance, *Rhizobium leguminosarum* secreted trihydroxamate siderophore; *Pseudomonas fluorescence* produced hydroxamate siderophore; *Bacillus brevis* are known for Bacillibactin siderophore; *Azotobacter vinelandii* for Azotobactin siderophore; *Salmonella typhimurium* secreted Enterobactin siderophore. The result has been incorporated in order of their adverse effect on plant growth promoting bacteria.

3.3.1. Outcome of minimal inhibition Concentration Test

Deduced result from the experimentation revealed that, each of the carbamate pesticides showed the ability to inhibit the selected plant growth promoting bacteria. The inhibition zone was started developing at 25ppm and clearly visible at 200 ppm concentration level of the pesticides as shown in Figure. 3.2.

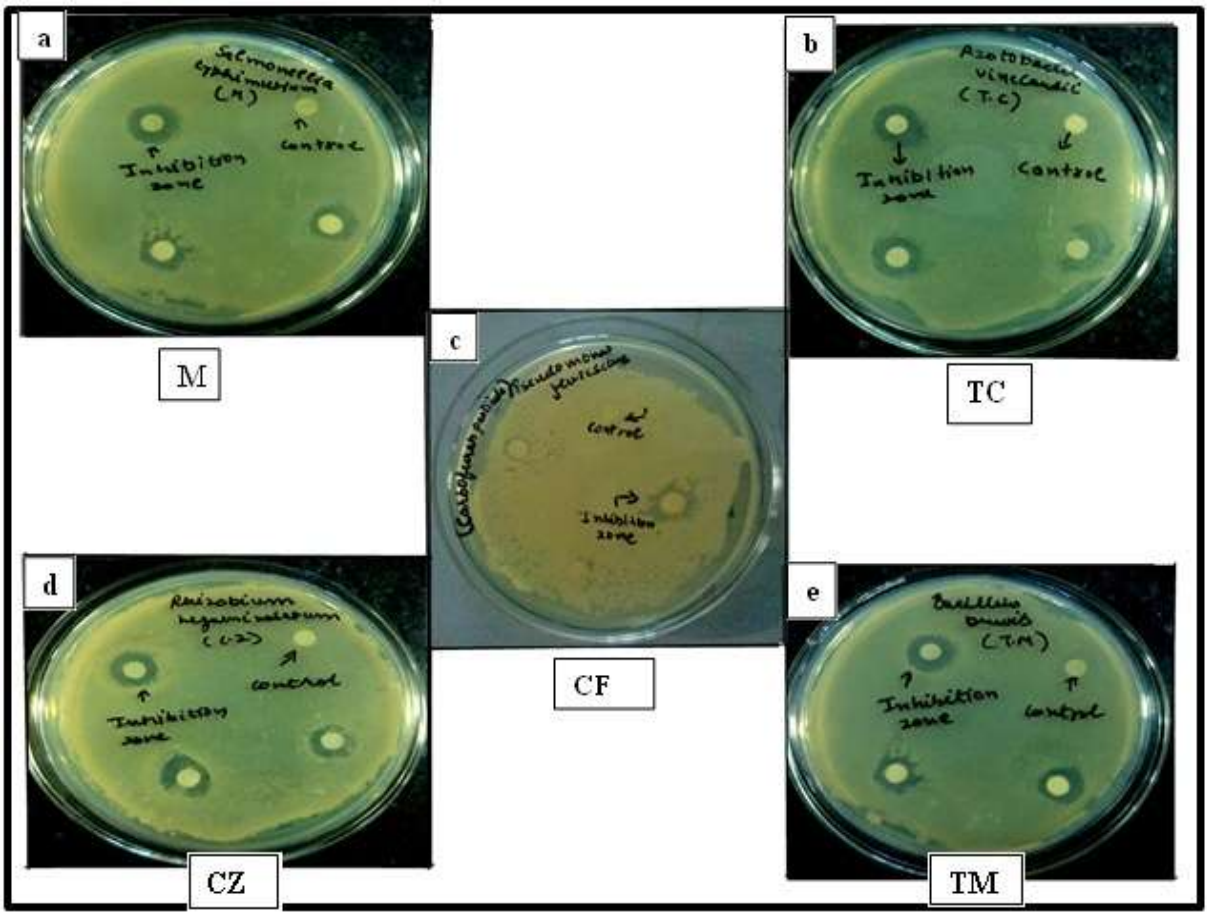


Figure 3.2 - Inhibition zone showcased by Methomyl (M) on *Salmonella typhimurium* (a), Thiodicarb (TC) on *Azotobacter vinelandii* (b), Carbofuran (CF) on *Pseudomonas fluorescence* (c), Carbendazim (CZ) on *Rhizobium leguminosarum* (d) and Thiophanate methyl (TM) on *Bacillus brevis.*

The measured inhibition zone for each carbamate pesticides (Methomyl, Carbofuran, Carbendezim, Thiophanate methyl and Thiodicarb) on selected plant growth promoting bacteria (*Azotobacter vinelandii*, *Pseudomonas fluorescence*, *Salmonella typhimurium*, *Rhizobium leguminosarum* and *Bacillus brevis*) was compiled and represented in tabular form in Table 3.1.

Table 3.1 Inhibition zone by the carbamates on *Azotobacter vinelandi*, *Pseudomonas fluorescence*, *Salmonella typhimurium*, *Rhizobium leguminosaru*m and *Bacillus brevis*

Concentration of carbamates	0ppm	15ppm	25ppm	75ppm	100ppm	150ppm	200ppm
Name of carbamates	Inhibition zone measured in (mm)						
	Rhizobium leguminosarum (NCIM-2749)						
Methomyl	×	2.0mm	3.0mm	5.0mm	7.0mm	8.0mm	10mm
Thiodicarb	×	1.2mm	2.1mm	3.2mm	5.7 mm	7.3 mm	9.2mm
Thiophanate methyl	×	1.0mm	2.1mm	3.2mm	5.7 mm	7.2 mm	9.2 mm
Carbendazim	×	1.4mm	2.3mm	3.5mm	5.4mm	6.9mm	8.3 mm
Carbofuran	×	×	1.0mm	1.3mm	2.1 mm	3.5mm	5.2 mm
Pseudomonas *fluorescence* (NCIM-5096)							
Methomyl	×	2.5mm	3.4mm	5.6mm	7.4 mm	9.2mm	12mm
Thiodicarb	×	1.6mm	3.2mm	5.1mm	7.1mm	8.3mm	11mm
Thiophante methyl	×	1.2mm	2.1mm	4.3mm	5.4mm	6.8mm	7.8mm
Carbendazim	×	×	1.6mm	2.3mm	3.8mm	4.9mm	6.3mm
Carbofuran	×	0.4mm	1.1mm	1.8mm	2.4mm	3.9mm	4.3mm
Bacillus *brevis* (NCIM-2532)							
Methomyl	×	1.8mm	2.4mm	3.7mm	5.5mm	6.8mm	8.1mm
Thiodicarb	×	1.4mm	2.2mm	3.1mm	5.2mm	6.0mm	7.2mm
Thoiphanate methyl	×	1.0mm	1.7mm	2.6 mm	3.8mm	5.3mm	6.3mm
Carbendazim	×	×	1.1mm	1.4mm	2.6 mm	4.3mm	5.2mm
Carbofuran	×	×	1.0 mm	1.3 mm	2.9mm	3.4mm	4.0mm
Azotobacter *vinelandii* (NCIM-2821)							
Methomyl	×	2.3mm	5.4 mm	7.9 mm	10.1mm	11.8mm	12.1mm
Thiodicarb	×	1.7mm	2.7mm	6.4mm	8.9mm	10.8mm	11.6mm
Thiophanate Methyl	×	1.0mm	2.3mm	5.9mm	7.4mm	9.5mm	11.5mm
Carbendazim	×	1.2mm	2.4mm	4.2mm	7.1mm	8.9mm	11.2mm
Carbofuran	×	1.0mm	1.6mm	2.4mm	5.5mm	6.8mm	7.4mm
*Salmonella typhimurium (*NCIM-2501)							
Methomyl	×	1.4mm	2.2mm	4.2mm	5.5mm	7.1mm	8.4mm
Thiodicarb	×	1.2mm	1.8mm	3.4mm	5.1mm	6.4mm	7.5mm
Thiophanate methyl	×	0.8mm	1.5mm	2.8mm	4.4mm	5.4mm	6.3mm
Carbendazim	×	0.6mm	1.2mm	2.2mm	3.8mm	4.2mm	5.4mm
Carbofuran	×	×	0.7mm	1.4mm	2.6mm	3.5mm	5.4mm

3.3.2 Outcome of qualitative and quantitative analyses of siderophores.

Siderophores are produced by the plant growth promoting bacteria and they played pivotal role of transporting metal ions from soil to plants. However, carbamates at the same time are showing interactive ability with these metal ions and therefore a competition between siderophores and carbamate is obvious. To chalk out outcome of this competition, qualitative and quantitative analyses have been done, which is summarized below.

3.3.2.1 Qualitative analyses of siderophores.

Qualitatively, the siderophores production was depicted by the yellow green florescence pigment produced by *Pseudomonas fluorescence* strain under the presence of UV light.

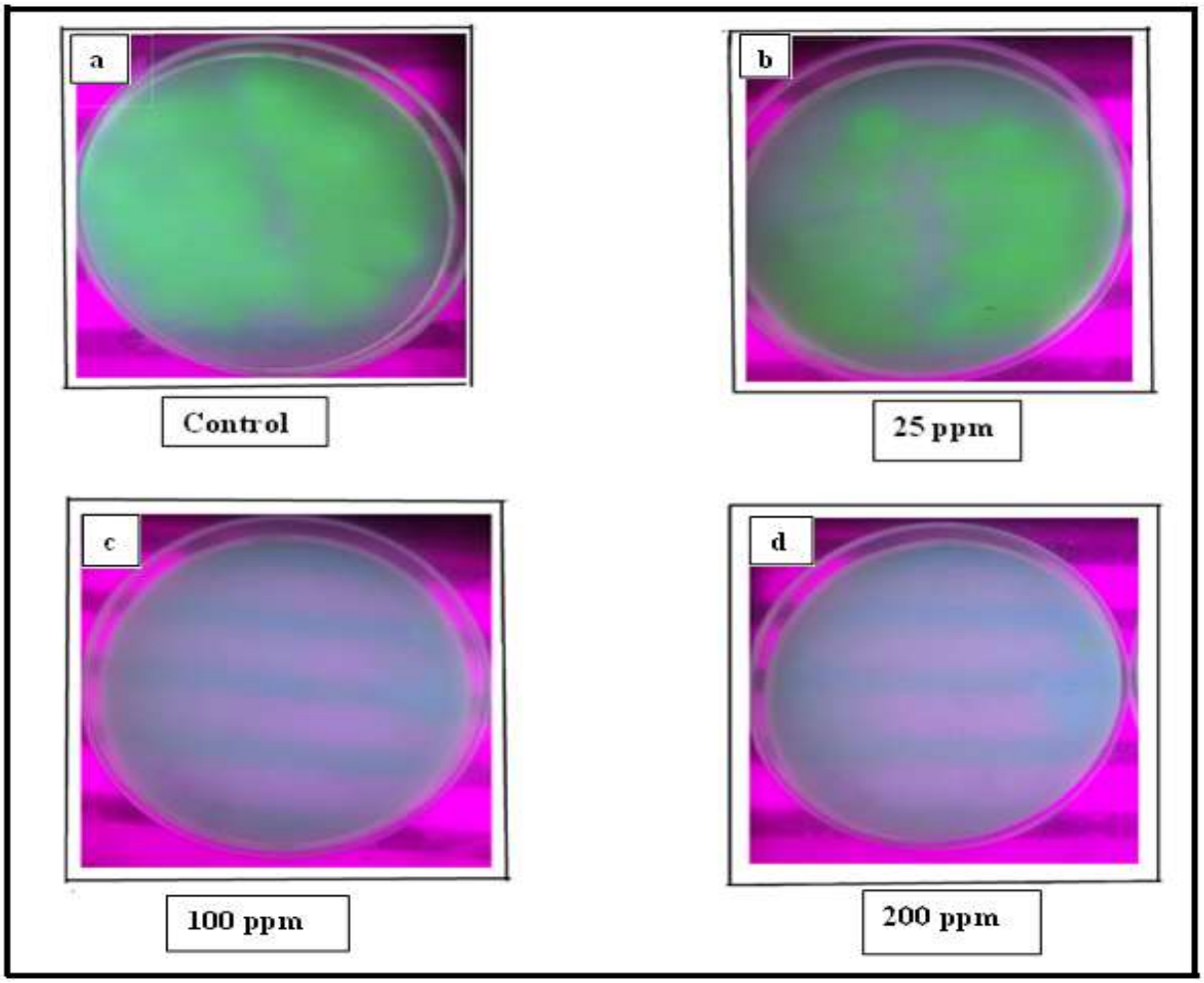


Figure 3.3: Siderophores production exhibited by the yellow green florescence pigment produced by *Pseudomonas fluorescence* strain under UV light as control (a), carbofuran at 25 ppm concentration level (b) and absence of florescence light in presence of the carbofuran at 100ppm (c) and 200ppm (d) depicted inhibition of the siderophores growth.

On contrary, when plant growth promoting bacteria were not able to produce siderophores, the yellow green florescence pigment was not produced by them. Through experimentation it is revealed that, all the carbamate pesticides (Methomyl, Carbendezim, Thiophanate methyl

and Thiodicarb) inhibited the siderophores production. Except for the carbofuran, which exhibited the siderophore production at 25 ppm concentration level and inhibited at the 100 and 200 ppm concentration level (as shown in Figure 3.3)

3.3.2.2. Quantitative analyses of siderophores.

For evaluating the effect of the carbamates pesticides,on siderophores, iron metal binding capacity was performed through quantitative analyses. For that purpose, the double beam UV-visible spectrophotometer was used. The absorption spectra for siderophores iron metal complexes (labeled as control) was measured at 370nm. The investigation was achieved by comparing the changes observed in the absorbance value found with/ without presence of the carbamates pesticides at (25ppm and 200ppm) concentration level in siderophores iron metal binding solutions. From the results, effect of the respective carbamates pesticides on siderophores iron metal binding capacity in percentage are deduced below

3.3.2.2.1. Quantitative analysis of siderophores in presence of Carbofuran (CF).

Carbofuran was found to hinder the siderophore iron binding capacity at doses (25ppm and 200ppm). The adverse effect of the carbofuran pesticide on PGPR, rose with higher concentration level which was depicted by the decrease in the absorbance value at higher concentration level with respect to control (as shown in Figure 3.4).

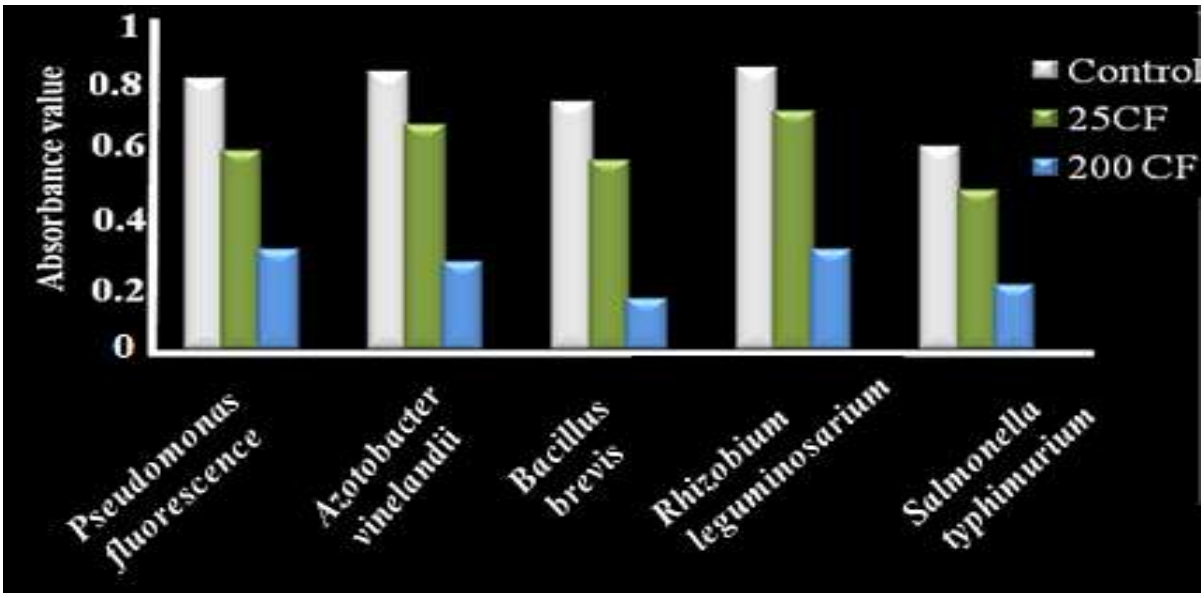


Figure 3.4: Change in the absorbance value of siderophores in presence of CF carbamate.

The deduced result in the percentage depicted, that at lower dose of 25 ppm siderophores iron metal binding capacity of each bacterial strain was adversely influenced in decreasing order as

: 26% (*Bacillius brevis*) > 24% (*Pseudomonas fluorescence*) > 21 % (*Salmonella typhimurium*) > 19% (*Azotobacter vinelandii*) and 15 % (*Rhizobium* leguminosarium) with respect to control. At the higher dose of 200ppm carbofuran results in decreasing order for above stated bacterial strains were found as : 80% (*Bacillius brevis*) >) 68 % (*Salmonella typhimurium*) > 66 % (*Azotobacter vinelandii*) > 64% (*Rhizobium leguminosarium*) and 63% (*Pseudomonas fluorescence*) with respect to control . The found results are graphically represented in Figure 3.5

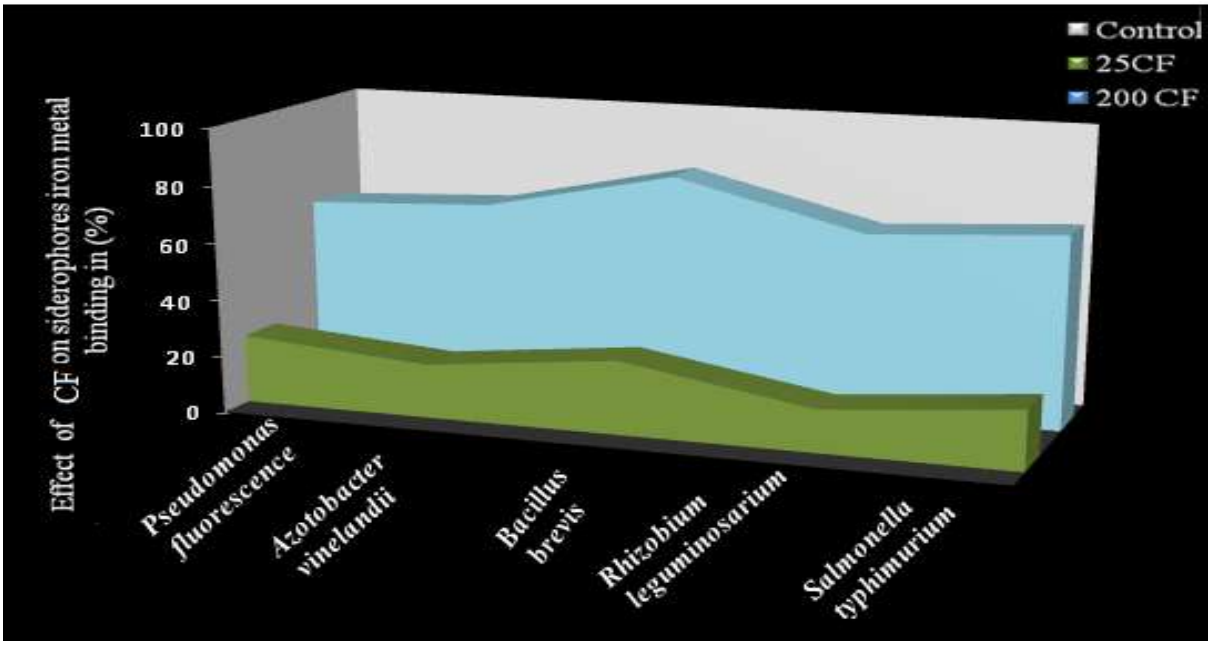


Figure 3.5: Harmful effect of CF on siderophores iron metal bimding in percentage.

3.3.2.2.2. Quantitative analysis of siderophores in presence of Thiophanate methyl (TM).

In a similar manner, thiophanate methyl carbamate was found negatively influencing the siderophores iron metal binding capacity. Moreover, they were found to be much more effective than that of carbofuran (selected for this study). The fact ascertaining the statement is depcted in the absorbance values of PGPR in presence of thiophanate methyl with respect to control as shown in Figure 3.6.

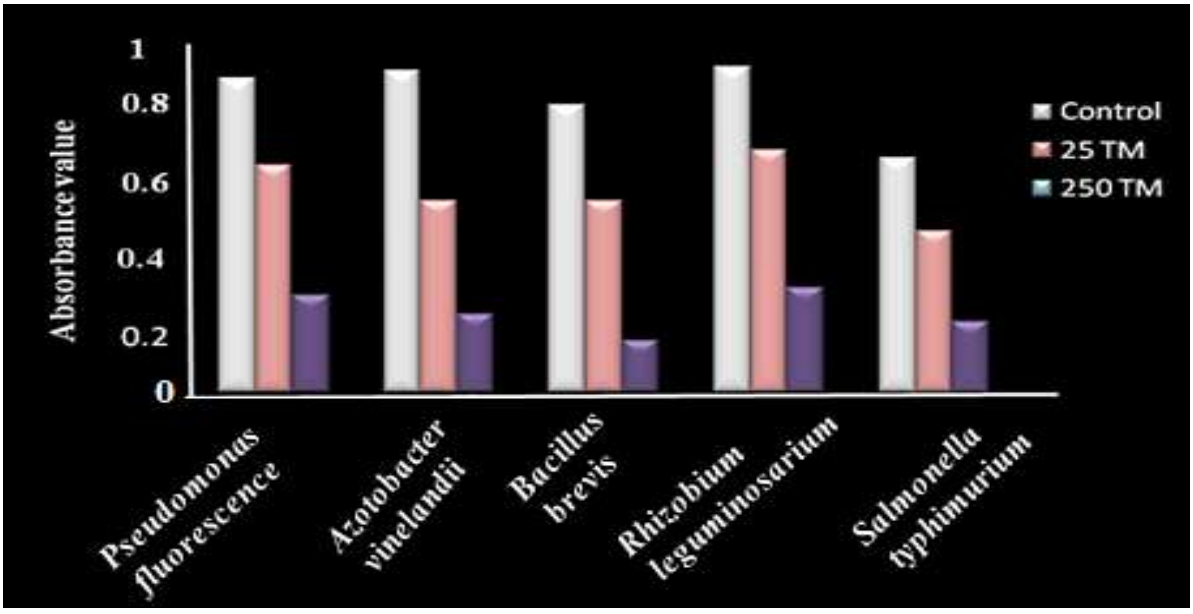


Figure 3.6: Change in the absorbance value of siderophores in presence of TM carbamate.

On deducing the effect in percentage, it was found that at lower concentration (25ppm), thiophanate methyl inhibited strains as follows: 40% (*Azotobacter vinelandii*)> 33% (*Bacillus brevis*)> 31% (*Salmonella typhimurium*) > 28% (*Pseudomonas fluorescence*) > and25% (*Rhizobium leguminosarium*) with respect to control. At higher concentration of thiophanate methyl(250 ppm) the adverse effect found was higher on them as: 82% (*Azotobacter vinelandii*) > 76% (Bacillius *brevis*)> 70% (*Salmonella typhimurium*)> 69% (*Pseudomonasfluorescence*) and 68% (*Rhizobium leguminosarium)* with respect to control.The graphical representation of the result is shown in Figure 3.7

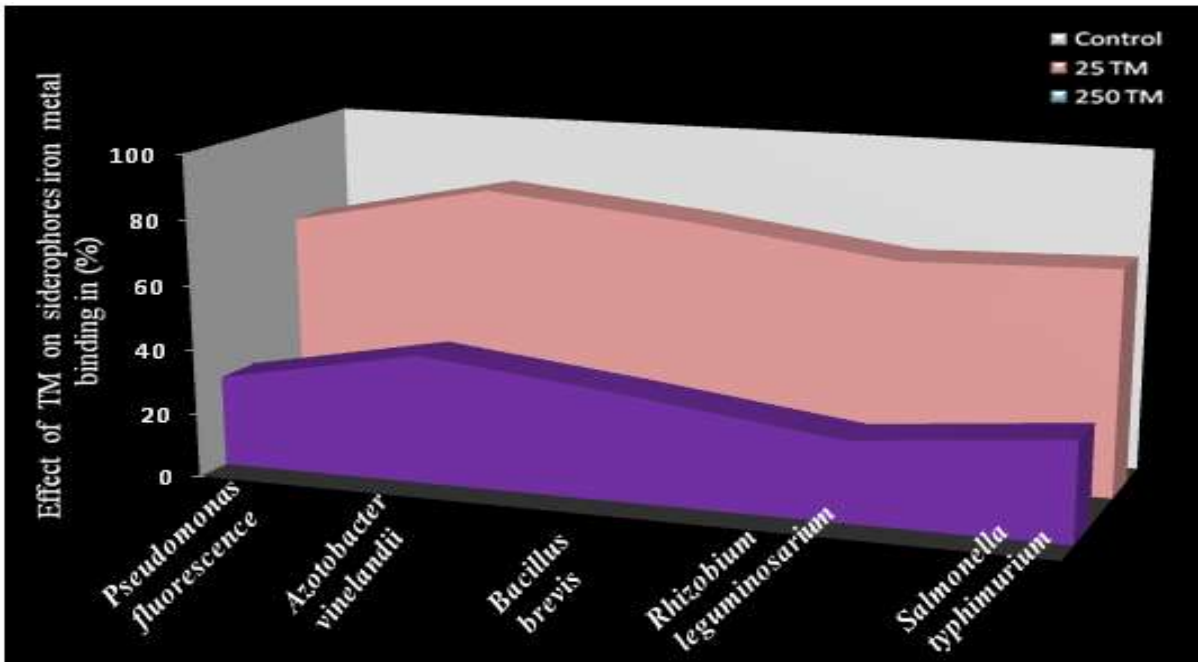


Figure 3.7: Harmful effect of TM on siderophores iron metal bimding in percentage.

3.3.2.2.3. Quantitative analysis of siderophores in presence of Thiodicarb (TC).

In case of thiodicarb, the effect on the siderophores metal binding was found to be much higher than that of the previously used carbamates. It is verified through the absorbance values obtained in presence of thiodicarb with respect to control as shown in Figure 3.8.

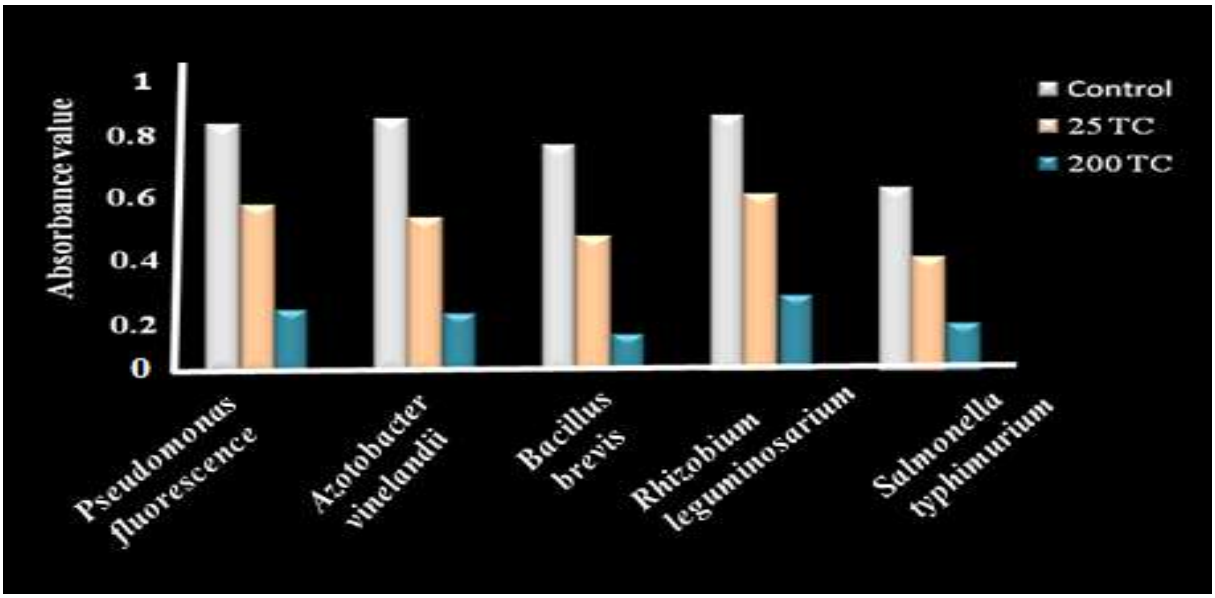


Figure 3.8: Change in the absorbance value of siderophores in presence of TC carbamate.

In percentage, the analyzed result for the harmful effect of the thiodicarb on the siderophores metal binding capacity of the bacterial strain is as follows for 25 ppm: 40% (Bacillius *brevis)*> 39% (Azotobacter *vinelandii*)> 37% (*Salmonellatyphimurium*)> 32% (*Pseudomonasfluorescene*) and 30% (*Rhizobium leguminosarium)* with respect to control. At 250 ppm concentration level the deduced harmful effect was 84% (Bacillius *brevis*)> 77% (Azotobacter *vinelandii*)> 75% (*Pseudomonasfluorescene*) > 73% (*Salmonellatyphimurium*) and 70% (*Rhizobium leguminosarium*) with respect to control as shown in Figure 3.9.

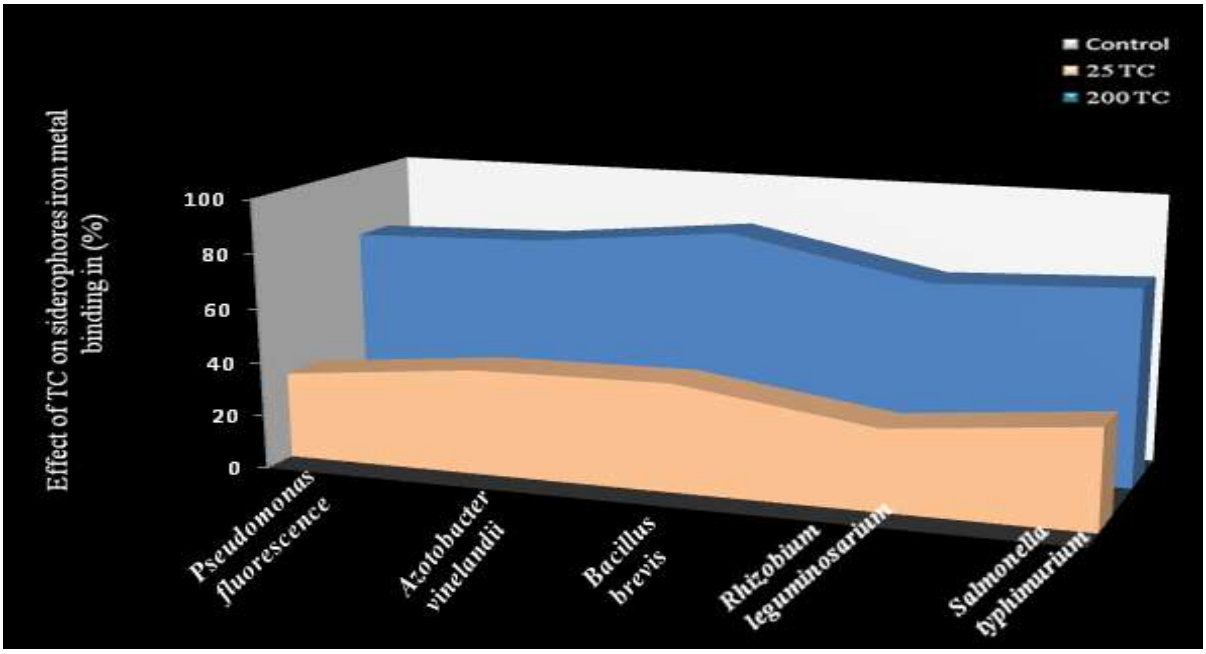


Figure 3.9: Harmful effect of TC on siderophores iron metal bimding in percentage.

3.3.2.2.4. Quantitative analysis of siderophores in presence of Carbendazim (CZ).

Carbendazim has also shown adverse effect on the siderophores-iron metal binding ability. The quantitative analyses of the impact of carbendazim on five different siderophore producing bacteria are represented in Figure 3.10 in a graphical form.

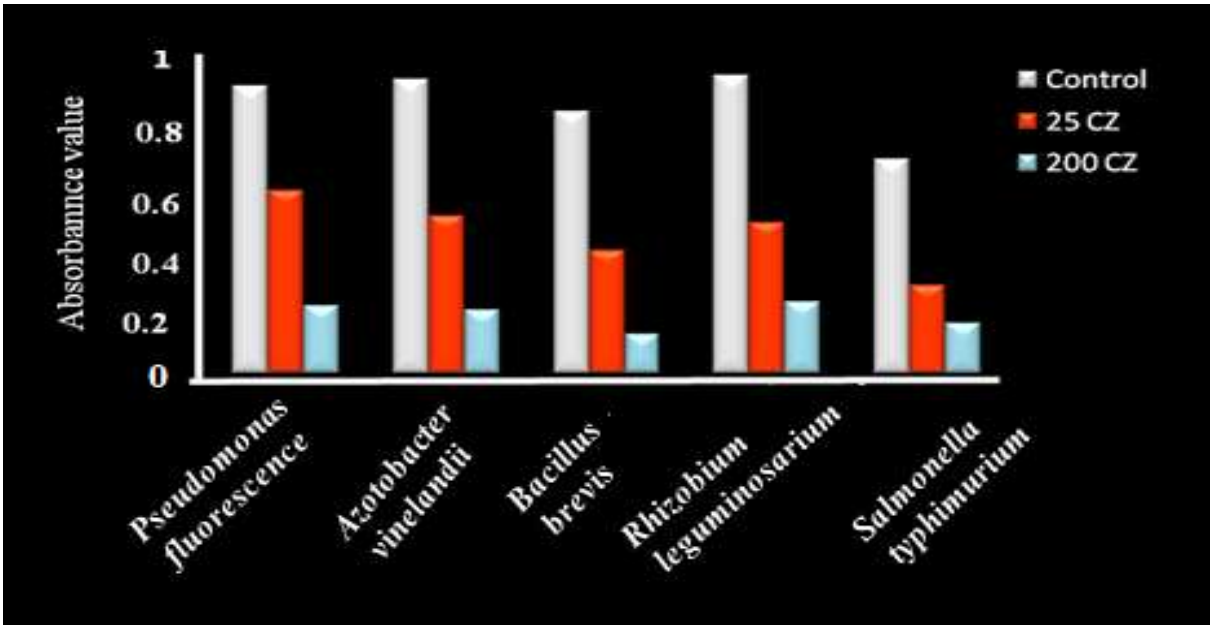


Figure 3.10: Change in the absorbance value of siderophores in presence of CZ carbamate.

The negative influence of the carbendazim on siderophores iron metal binding in percentage is deduced as followed for 25 ppm: 59% (*Salmonella typhimurium*) > 53% (*Bacillius brevis)*> 49% (*Rhizobium leguminosarium*)> 47% (*Azotobacter vinelandii*)> 35% (*Pseudomonas*

fluorescence) with respect to control. At 250 ppm concentration level the deduced harmful effect was as followed: 85% (Bacillius brevis)> 78% (*Azotobacter vinelandii*)> 77% (*Salmonella typhimurium*) ~ 77% (*Pseudomonas fluorescence*) and76% (*Rhizobium leguminosarium*) with respect to control as shown in Figure 3.11.

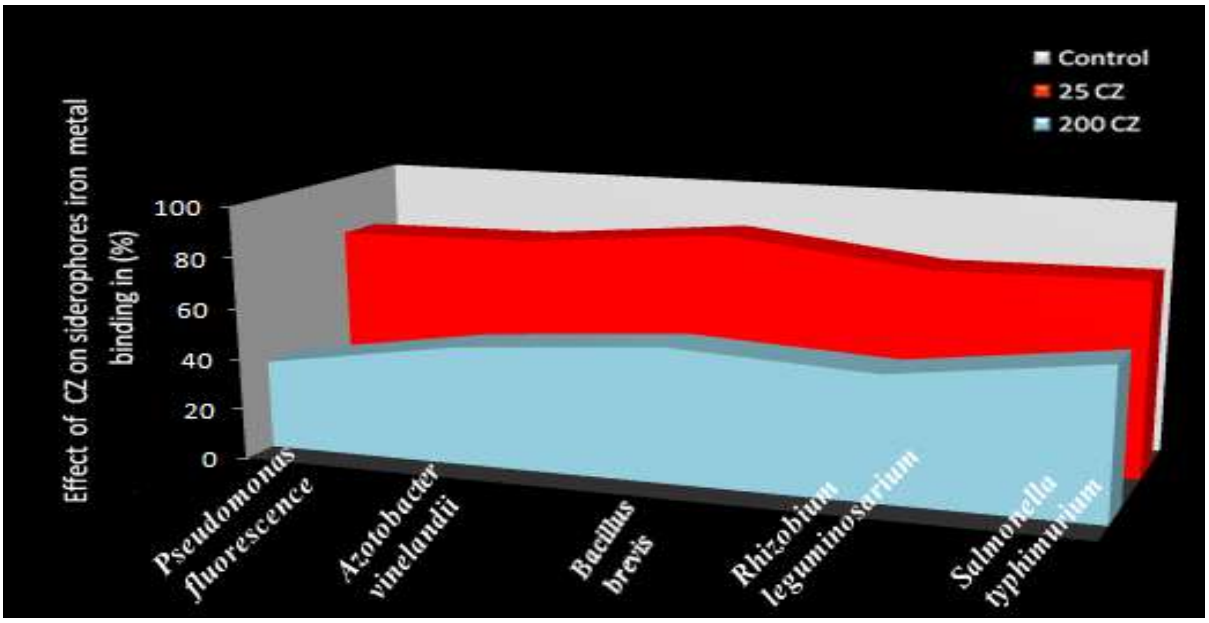


Figure 3.11: Harmful effect of CZ on siderophores iron metal binding in percentage.

3.3.2.2.5. Quantitative analysis of siderophores in presence of Methomyl (M).

Methomyl pesticide was found to be most opposing pesticides for siderophores metal binding capacity. To ascertain the fact, deduced absorbance value in presence of methomyl carbamate with respect to control are represented graphically in Figure 3.12

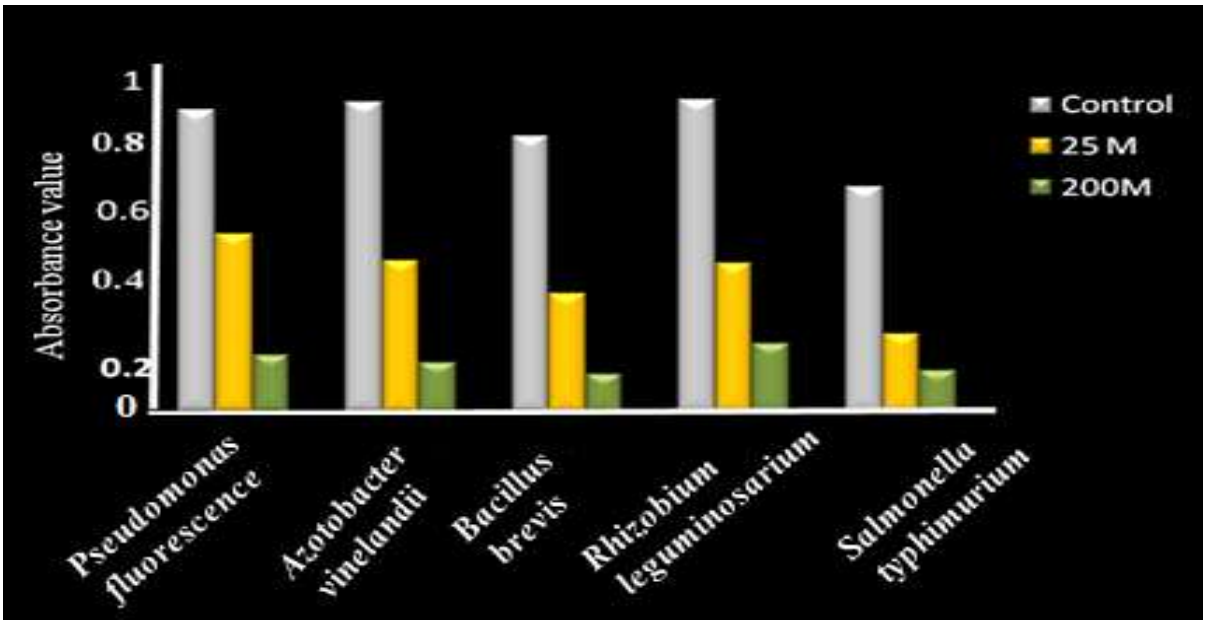


Figure 3.12: Change in the absorbance value of siderophores in presence of M carbamate.

In percentage, the adverse effect of the methomyl is concluded as follows, at 25ppm concentration level: 65% (*Salmonella typhimurium*)> 57% (*Bacillius brevis)*> 52% (*Rhizobium leguminosarium*)> 51% (*Azotobacter vinelandii*)> 42% (*Pseudomonas fluorescens*) with respect to control. At 250 ppm concentration level, the deduced harmful effect was 86% (Bacillius *brevis*)> 83% (*Azotobacter vinelandii*) > 81% (*Salmonella typhimurium*) ~ 81% (*Pseudomonas fluorescens*) and 78% (*Rhizobium leguminosarium*) with respect to control as shown in Figure 3.13.

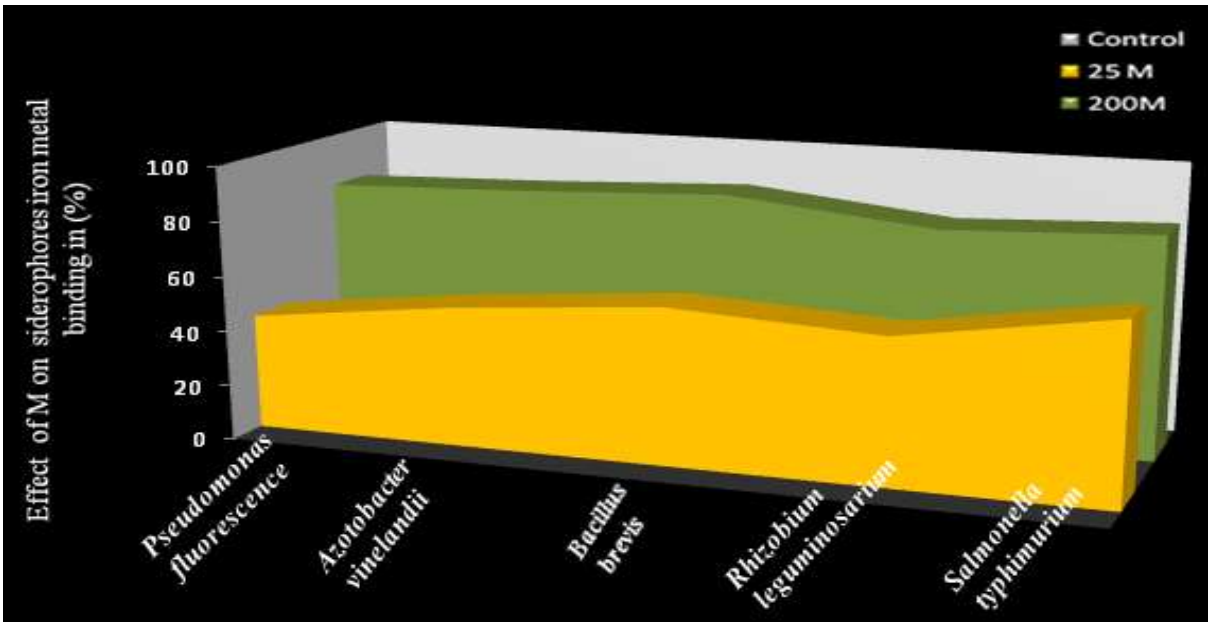


Figure 3.13: Harmful effect of M on siderophores iron metal bimding in percentage.

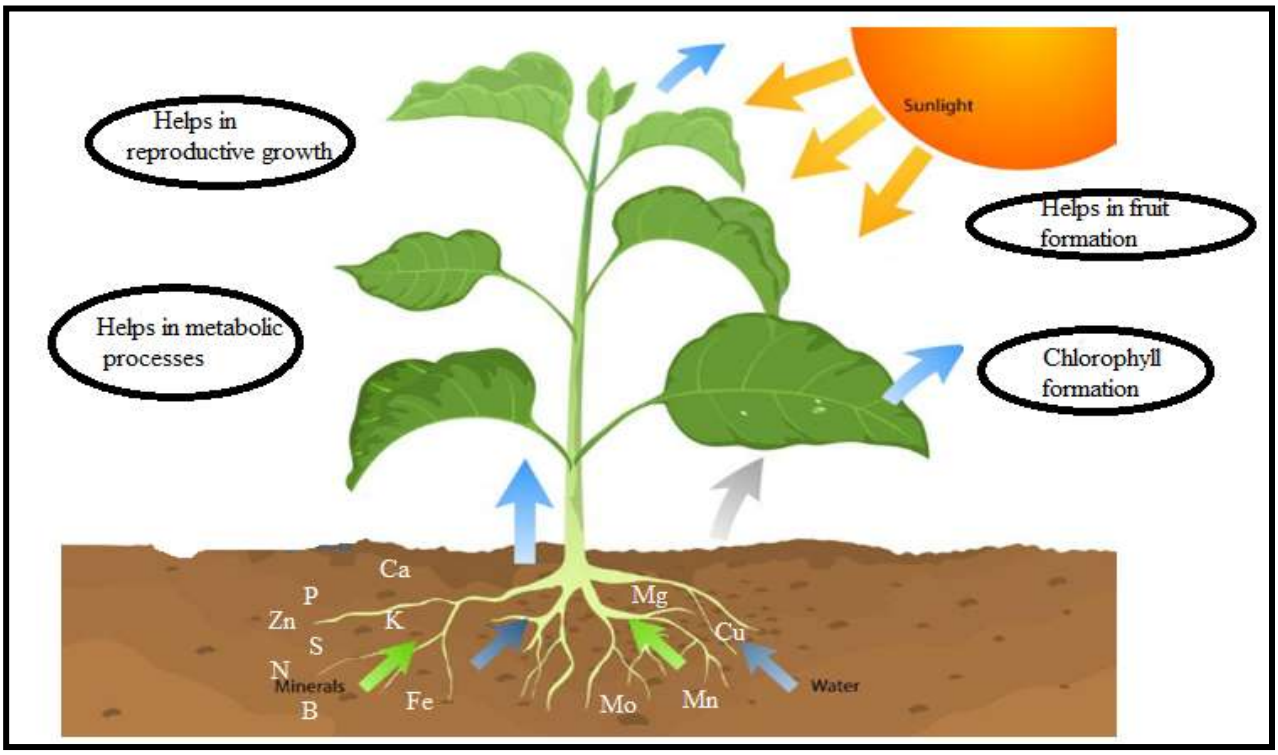
Conclusion

Each of the selected carbamate pesticides, were found hampering the growth of the plant growth promoting bacteria (*Azotobacter vinelandii, Pseudomonas fluorescence, Salmonella typhimurium, Rhizobium leguminosarum* and *Bacillus brevis*). Their adverse effects on the beneficial bacteria were not restricted up to limiting their growth. Above that, found malicious for siderophores production as observed in qualitative analyses. On evaluating their influence on siderophores metal binding ability attained by quantitative analyses, the data revealed that carbamates were found successful in declining it. The negative influence of each carbamates on the siderophores metal binding ability of the plant growth promoting bacterial strains ranging 24-81% for *Pseudomonas fluorescens*, 19-83% for *Azotobacter vinelandii*, 26-86% for Bacillius *brevis*, 15-78% for *Rhizobium leguminosarum* and 21-81% for *Salmonella typhimurium*. The maximum adverse effect was observed in case of the methomyl and the minimum effect was found in the case of carbofuran. One of the recognizable fact deduced

from the experimentation was that, the inhibition abiity of the carbamates are directly related to its complex forming ability with the Fe(II) metal ion.

Chapter-4

Effect of the pesticides on the nutrients of the agricultural crop (Maize plant)



INTRODUCTION

Agriculture has to face the challenging demands of the rising population at the global level and the possibility of the expansion of agriculture land is limited. At the same time, one third of the crop yield is expected to be destroyed by different type of pests.[119]Thus, subjected to limitations, sustainable agriculture with increased productivity and profit level is only possible by the usage of the pesticides. As,they act instrumental in limiting the losses from the weeds, diseases, insects etc. that can markedly reduce the amount of harvestable produce.[120]

Despite the fact crop productivity has increased, the world population is dealing with problem of the micronutrient malnutrition. According to the WHO reports more than 3.7 million people are affected by the deficiencies of the micronutrients. In which, deficiencies from the iron and zinc are among the top. Respective issue has become the major impediment to socioeconomic development.[121] Since, absolute concentration of the nutrition in human comes directly and indirectly from food crops and they are dependent on the soil;therefore, the root cause of scarcity of micronutrients must lies in the soil. What's more, the analysis of soil sample in India after green revolution suggested that 49% of soils have become potentially deficient in Zn, 12% in Mn, 3% in Fe, 5% in Cu, 11%in Mo and 33%in B.[122]

Such shocking revelations need to be addressed, as it is the major constrained in the production of the quality food. Certain parameters like soil type, soil pH and organic matter are mostly consider as the cause for micronutrient deficiency.[79] But, here question arise that if it would be the sole reason than why scarcity of the micronutrient in soil as well as plant would have rose only after the green revolution? To look upon the fact, attention must be shifted to the key contender which made green revolution successful. The one of the component is the introduction of the pesticides. Although, through the efforts of the scientist, pesticides progressed from more toxic to less toxic and more water soluble to less water soluble, but in terms of their effect on the micronutrient of the plant very minimal research is known.

To unearth the effect,whether the presence of the pesticides is affecting the morphological parameter and metal ions content in the plants,investigation is done in presence of carbamate pesticides (Methomyl (M), Thiophanate methyl (TM), Carbofuran (CF), Carbendazim (CZ) and Thiodicarb (TC) carbamates) on the Maize plant (*Zea mays*). The main objective of the study is (i) Evaluation of the morphological changes observed in presence of carbamate

pesticides and(ii) Investigation of effect of metal ions content in seeds of*Zea mays* after application of carbamate(s).

4.2. EXPERIMIENTAL

4.2.1 Materials

98% pure Carbofuran, Carbendazim,Thiodicarb, Thiophanatemethyl andMethomyl was provided by Gautmi Ltd, Hyderabad (India). The other laboratory chemicals of AR grade were purchased from LobaChemie which included NaOH, HCl, DMSO, acetic acid, and metal salts (Iron).

4.2.2 Methodology for Experimentation.

Experiment was conducted using, *Zea mays*(9108) under natural condition from months of May 2014 to August 2014. Entire soil was first dried, filtered and autoclaved. Soil experiments were randomized designs, and each treatment group consisted of four pot replicates with each containing individual plants. During the experiment, each pot was filled with 3.0 Kg of air dried soil. In the soil, no external minerals and nutrients were added. The maize crops plants were grown till maturation and were watered with distilled water throughout the experiment. They were treated with the pesticides after the 15 days of the sowing. And with additional metal ions solution were added after 15 days of adding carbamate(s) pesticides.The experiment segregated into the three groups: first group was left untreated with pesticides and tagged as control. In the second group, individual carbamate (Carbofuran, Carbendazim,Thiodicarb, Thiophanate methyl and Methomyl) were sprayed separately in the pot by 50.0mL with two different concentration level of 25.0mg/L and 50.0mg/L. The third group was treated with 50.0mL solution of 50.0mg/L dose of the individual metal ion (Mn(II), Fe(II) or Zn(II)), along with the carbamate doses at two distinct concentrations of 25.0mg/L and 50.0mg/L.

When the maize plants were fully grown, the shoot length and the head length of the maize were measured and compared with control. Furthermore, kernels of corn per row and shoot weight were measured with respect to the control. The harvested seeds were washed twice using the distilled water and dried at 70°C for 2 days. The dried samples were grounded, digested and analyzed for metal ions concentration. For sample digestion, 0.3g of crushed corn seeds and shoots of each plant were taken into the test tubes. To each tube 5.0 mL concentrated nitric acid and 2.0 mL hydrogen peroxide were added. All the test tubes were

shaken after regular interval of time. After 24hour, the extract was filtered by using Whatmann-1 filter paper. 2.0mL of the extract was then diluted to 10.0 mLby using triple distilled water. The prepared solutions were used for detecting the metal ion concentration using ICP-AES analytical technique.

4.2.3. Statistical analysis

Origin software 6.0 and 8.0 were used for the ANOVA statistical analysis. Use of statistical analysis was done for quantitative analysis of siderophore production and metal ions content variations observed in maize plant growth experiments. The difference among treatment means was compared by high range statistical domain using ANOVA test at ($p \leq 0.05$) level.

4.3. RESULTS AND DISCUSSIONS

4.3.1. Effects of the applied carbamates on the morphological parameters of the *Zea mays* plant.

From the investigation it was found that, each of the carbamates pesticides has adversely affected the maize plant morphology with respect to the control. The negative impact on the growth of the carbamates pesticides doses (25.0 ppm and 50.0 ppm) was experimentally found reducing the plant growth in increasing order as follows: carbofuran>thiophanate methyl>thiodicarb>carbendazim> methomyl. Deduced data also revealed that, the influence of the pesticides not remained limited up to the growth of the maize plant but also the growth of the food crop was retarded by their usage.

From the investigation it is found that, at the dose level of 25.0mg/L of carbofuran pesticide, 30% of seed weight, 21% of shoot weight, 12% stem length and 10% of head length were reduced with respect to the control. In presence of the higher dose of carbofuran (CF) i.e. at 50.0mg/L dose level - 41% of seed weight, 34% of shoot weight, 31% of the stem length and 19% of the head length was decreased, compared to the control. In a similar manner, the result deduced for the thiophanate methyl (TM) as, at the dose level of 25.0mg/L–decrease in 36% of seed weight, 32% of shoot weight, 29% of the stem length and 27% head length was observed. Moreover, at 50.0mg/L-51% of seed weight, 45% of shoot weight, 41% of the stem length and 42% of the head length got reduced. In case of the thiodicarb (TC), the negative influence on the maize plant was found higher with respect to the use of same concentration CF and TM against control as, at the dose of 25.0mg/L; 38% growth in seed weight, 36% in shoot weight, 37% in stem length and 33% in head length was

decreased. At 50.0mg/L - 53% of the seed weight, 47% of the shoot weight, 48% of the stem length and 48% of the head length decreased. In case of carbendazim (CZ),use of 25.0mg/L, resulted decrease in 44% for the seed weight, 42% for the shoot weight, 44% for the stem length and 43% for the head length with respect to control. At dose level of 50.0mg/L- 63%decrease in the seed weight, 52% in shoot weight, 53% in the stem length and 53% for the head length was found with respect to control. The maximum harm on the physical parameter of maize crop growth was found in presence of the methomyl pesticide. On application of 25.0 mg/L, decrease in 55% of the seed weight, 52% of the shoot weight, 51% of the stem length and 56% of the head length with respect to control was observed. At its level of 50.0mg/L - 67% of the seed weight, 62% of the shoot weight, 60% of the stem length and 63% of the head length was decreased with respect to control. The impact of the low dose of carbamate on the Zea*Mays* plant is showcased below in Figure 4.1 with respect to control.

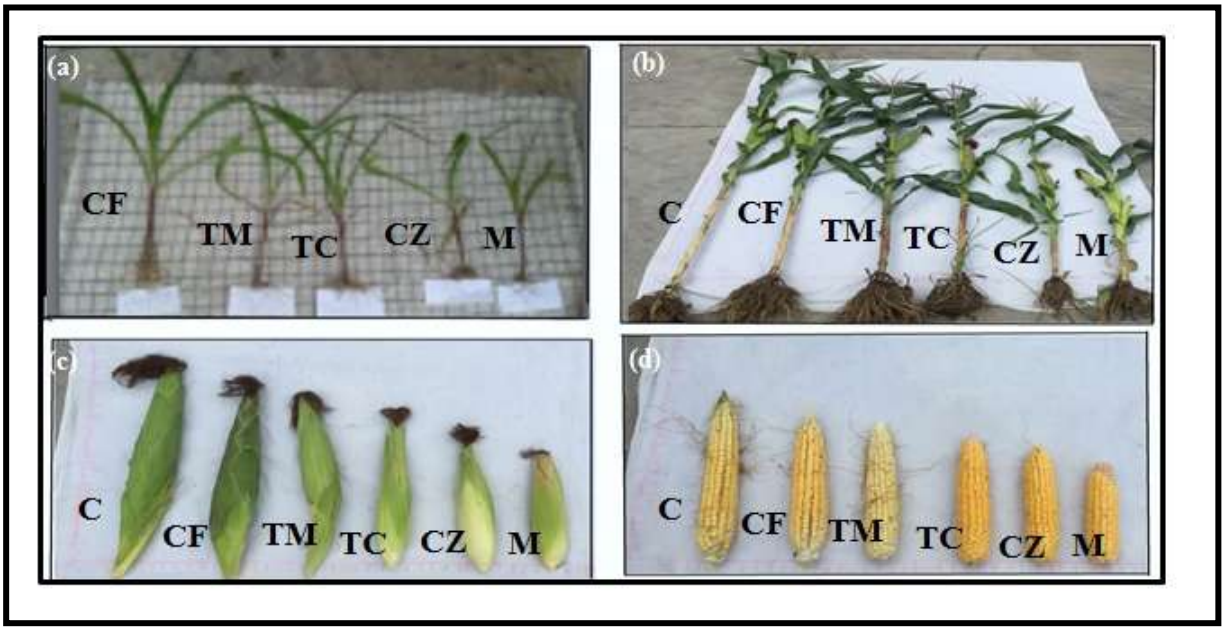


Figure 4.1: The effect of the carbamates pesticides- (a) and (b) on the growth of the maize plant (*ZeaMays*(9108)) and (c) and (d) on the growth of food crops. The effect observed under the carbofuran (CF),thiophanate methyl (TM), thiodicarb (TC), carbendazim (CZ) and methomyl (M) with respect to control (C) at 25.0mg/L.

The combined effect of the low dose (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of individual carbamate on the *Zea mays* on seed weight, shoot weight, head length and stem length with respect to control for all the carbamate pesticides (Carbofuran, Carbendazim, Thiodicarb, Thiophanate methyl and Methomyl) is graphically represented in Figure 4.2, which showcased the impact of the carbamates in decreasing the growth of the *Zea mays* plant

and represented in increasing order as follows: carbofuran>thiophanate methyl>thiodicarb> carbandazim > methomyl with respect to the control.

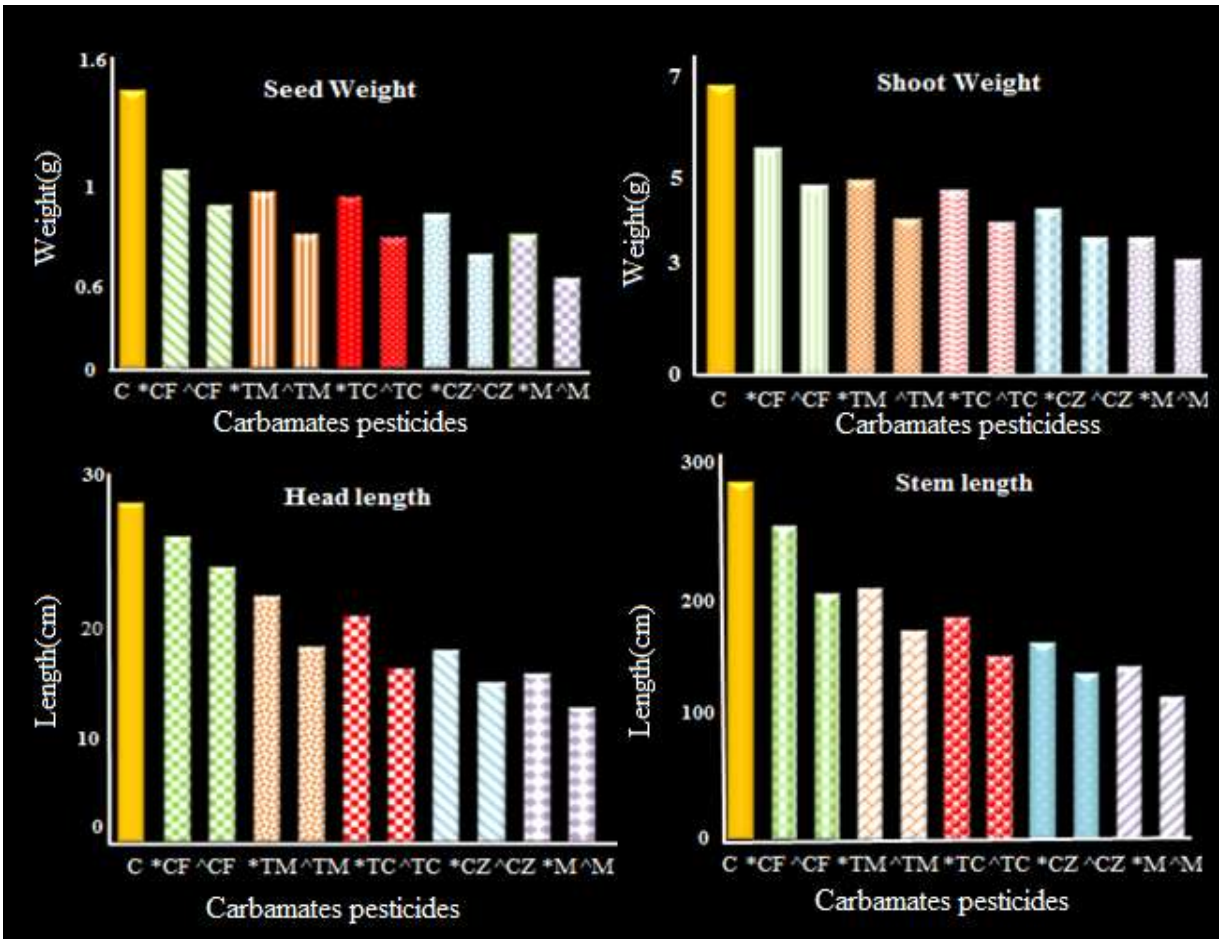


Figure4.2: The mean values± standard deviation values obtained under the influence of (*) low dose (25.0 mg/L) and high dose (^) (50.0mg/L) of the carbamate(Carbofuran (CF), Thiophanate methyl (TM), Thiodicarb (TC), Carbendazim (CZ) , and Methomyl (M)) on the mature maize plant's (*ZeaMays* (9108)) seed weight, shoot weight, head length and stem length with respect to control.

The observed mean value± standard deviation of the maize plant(*Zea mays* (9108)), seed weight, shoot weight, head length and stem length with respect to control for all the carbamates pesticides (Carbofuran, Carbendazim , Thiodicarb , Thiophanate methyl and Methomyl) are compiled in tabular form as Table 4.1 .

Table 4.1: Mean ± standard deviation values of the maize plant (*Zea mays* (9108) seed weight, shoot weight, head length and stem length with respect to control under the influence of the carbamates pesticides (Carbofuran (CF), Thiophanate methyl (TM), Thiodicarb (TC), Carbendazim (CZ) , and Methomyl (M) at low (25.0mg/L) and high (50.0mg/L) doses with respect to control.

Sample	Dose (mg/L)	Seed Weight (g)	Shoot Weight (g)	Shoot Length (cm)	Head Length (cm)
Control		**1.45±0.01**	**`6.48±0.02**	**296.2±0.2**	**28±0.2**
CF	25.0	1.03±0.03	5.08±0.01	260.1±0.3	25.2±0.4
	50.0	0.85±0.03	4.23±0.05	203.7±0.4	22.6±0.5
TM	25.0	0.92±0.02	4.37±0.03	207.3±0.3	20.3±0.4
	50.0	0.70±0.04	3.50±0.01	172.1±0.2	16.1±0.1
TC	25.0	0.89±0.02	4.11±0.04	184.3±0.1	18.6±0.4
	50.0	0.68±0.05	3.42±0.03	151.2±.3	14.3±0.2
CZ	25.0	0.80±0.03	3.72±0.03	163.0±0.4	15.8±0.3
	50.0	0.59±0.02	3.08±0.05	138.0±0.2	13.1±0.6
M	25.0	0.65±0.03	3.06±0.03	143.4±0.3	12.2±0.4
	50.0	0.47±0.06	2.44±0.02	118.2±0.2	10.3±0.2

4.3.2. Effects of the applied carbamates pesticides doses on the morphological parmeters of the *Zea mays* in presence of the metal ions.

On evaluating the morphological parameter of Zea *mays* plant, in presence of carbamate, after the addition of the Mn(II), Fe(II), Cu(II) and Zn(II) metal ions, it was found that, different metal ions act as growth enhancer for the Zea *mays* plant and suppressed the effect of the applied carbamates on the plant growth. The result obtained for each of the pesticides is discussed below:

4.3.2.1 Effect of the of metal ions on the *Zea mays* treated with low and high doseof CF.

In presence of the (50.0mL,50.0mg/L) metal ions dose on the *Zea mays* plant, which is already treated with (50.0mL, 25.0mg/L or 50.0mL, 50.0mg/L) doses of the CF, it was found that the metal ions doses have subdued the effect of the CF. The observed mean value± standard deviation value for the lower and higher doses of carbofuran with respect to control in presence of the different metal ions are compiled in tabular form below i.e. in Table 4.2.

Table 4.2:Mean ± standard deviation value of the seed weight, shoot weight, stem length and head length of the mature *Zea mays* (9108) plant with/without 50.0mL, 50.0mg/L dose of the individual metal ion (one among Mn(II),Fe(II),Cu(II) and Zn(II) at a time) treated with low dose (50.0mL, 25.0 mg/L) and high dose (50.0mL, 50.0mg/L) of the CF with respect to control.

Sample	Dose (mg/L)	Seed Weight (g)	Shoot Weight (g)	Stem Length (cm)	Head Length (cm)
Control		**1.45±0.01**	**`6.48±0.02**	**296.2±0.2**	**28±0.2**
CF	25.0	1.03±0.03	5.08±0.01	260.1±0.3	25.2±0.4
	50.0	0.85±0.03	4.23±0.05	203.7±0.4	22.6±0.5
Mn(II)+ CF	25.0	1.1 ± 0.01	5.61±0.01	271.5±0.5	26.0±0.2
	50.0	0.96±0.01	4.69±0.01	216.6±0.4	24.1±0.1
Fe(II) + CF	25.0	1.20±0.04	5.76±0.04	277.7±0.4	26.2±0.1
	50.0	0.99±0.05	4.74±0.02	224.7±0.5	24.0±0.1
Cu(II) +CF	25.0	1.09±0.05	5.32±0.02	267.6±0.5	25.5±0.2
	50.0	0.89±0.04	4.53±0.05	210.11±0.3	23.0±0.2
Zn (II) +CF	25.0	1.08±0.06	5.44±0.01	264.7±0.3	25.7±0.1
	50.0	0.89±0.04	4.43±0.02	210.2±0.4	23.08±0.2

The compiled effect of the each of the metal ion on the *Zea mays* plant was deduced in percentage. From the experimentation, it was found that when(50.0mL,25.0mg/L) dose of CF was applied to the Zea *mays*, it reduced the seed weight by 29%, but when the same was treated with 50.0mL, 50.0mg/L of metal ions, decrease in seed weight was observed by, 24% for Mn(II), 17% for of Fe(II), 24% for Cu(II) and 25% for Zn(II)with respect to the control, showing slight betterment on use of metal ions with respect to the use of 50.0mL, 25.0mg/L, CF. In a similar manner, on use of 50.0mL, 50.0mg/L of CF, on the Zea *mays* plant, seed weight was reduced by 41%,on which use of 50.0mL of 50.0mg/L metal ions doses, the reduction with respect to control was found 35% for Mn(II), 31% for Fe(II), 38% for Cu(II) and 38% for Zn(II).

The use of 50.0mL, 25.0mg/L CF, decreased shoot weight by 16%with respect to control; whereas on additional dose of 50.0mL, 50.0mg/L of metal ions, decrease in shoot weight was found as:13% for Mn(II),11% for Fe(II), 19% for Cu(II) and 16% for Zn(II) metal

ions with respect to control.At the same time on application of 50.0mL, 50.0mg/L of CF, shoot weight was decreased by 34%; where on 50.0mL,additional application of 50.0mg/L of metal ions,resulted reduction in shoot weight as: 27% for Mn(II),26% for Fe(II), 30% for Cu(II) and 31% for Zn(II) metal ion with respect tothe control.

Application of 50.0mL, 25.0mg/L CF leads to decrease in stem length by 13%; on which by application of 50.0mL, 50.0mg/L metal ions, stem length was observed to be decreased by 7% for Mn(II), 7% for Fe(II), 9% for Cu(II) and 11% for Zn(II) metal ion with respect to control.In parallel, a dose of 50.0mL, 50.0mg/L of CF reduced growth of stem length by 31%; where on additional application of 50.0mL, 50.0mg/L individual metal ion resulted decrease in stem length by 26% for Mn(II), 24% for Fe(II), 29% for Cu(II) and 29% for Zn(II) metal ion with respect to control.

Head length of *Zea mays* was reduced by 10% with respect to control on application of 50.0mL, 25.0mg/L CF. However, when 50.0mL dose of 50.0mg/L metal ionswas applied to the CF treated plant (25.0mg/L), result depicted that addition of metal ions has slightly improved the *Zea mays* plant growth and reduction in, head length was found as: 8% for Mn(II),4% for Fe(II), 9% for Cu(II) and 8% for Zn(II) metal ion with respect to control was observed. While, in case of the,50.0mL,50.0mg/L CF, reduction in head length was 19%; on which 50.0mL additional supply of 50.0mg/L metals ion leads to decrease head length by 14% for Mn(II),14% for Fe(II), 17% for Cu(II) and 17% for Zn(II) metal ion with respect to control.

On analysis of above obtained results, it can be concluded that, growth of *Zea mays* largely depends on amount of CF supplied over the crop. On application of higher dose of CF (50.0mL, 50.0mg/L) more reduction in seed weight, shoot weight, stem length and head length of *Zea mays* were obtained. On additional supply of metal ions, Fe(II) and Mn(II) largely repair the reduction in physical growth of plant, while, additional supply of Cu(II) and Zn(II) hardly showed any change in morphological parameter of CF effected crop. Both Fe(II) and Mn(II) form quick and very stable complex with CF and therefore as expected on application of CF, supply of Fe(II) and Mn(II) should highly hamper, the additional dose of which repairs largely the loss in growth parameters of *Zea mays.* Additional supply of Fe(II) or Mn(II) do not completely repair, effect of CF, may indicate two possibilities, either depletion in metal ion by pesticide is not the sole reason of loss in growth of plant, or loss in growth of plant may be a result of multiple metal ion loss cause by the application of CF and

additional supply of a single metal ion is not enough to repair the entire loss (as shown in Figure 4.3) .

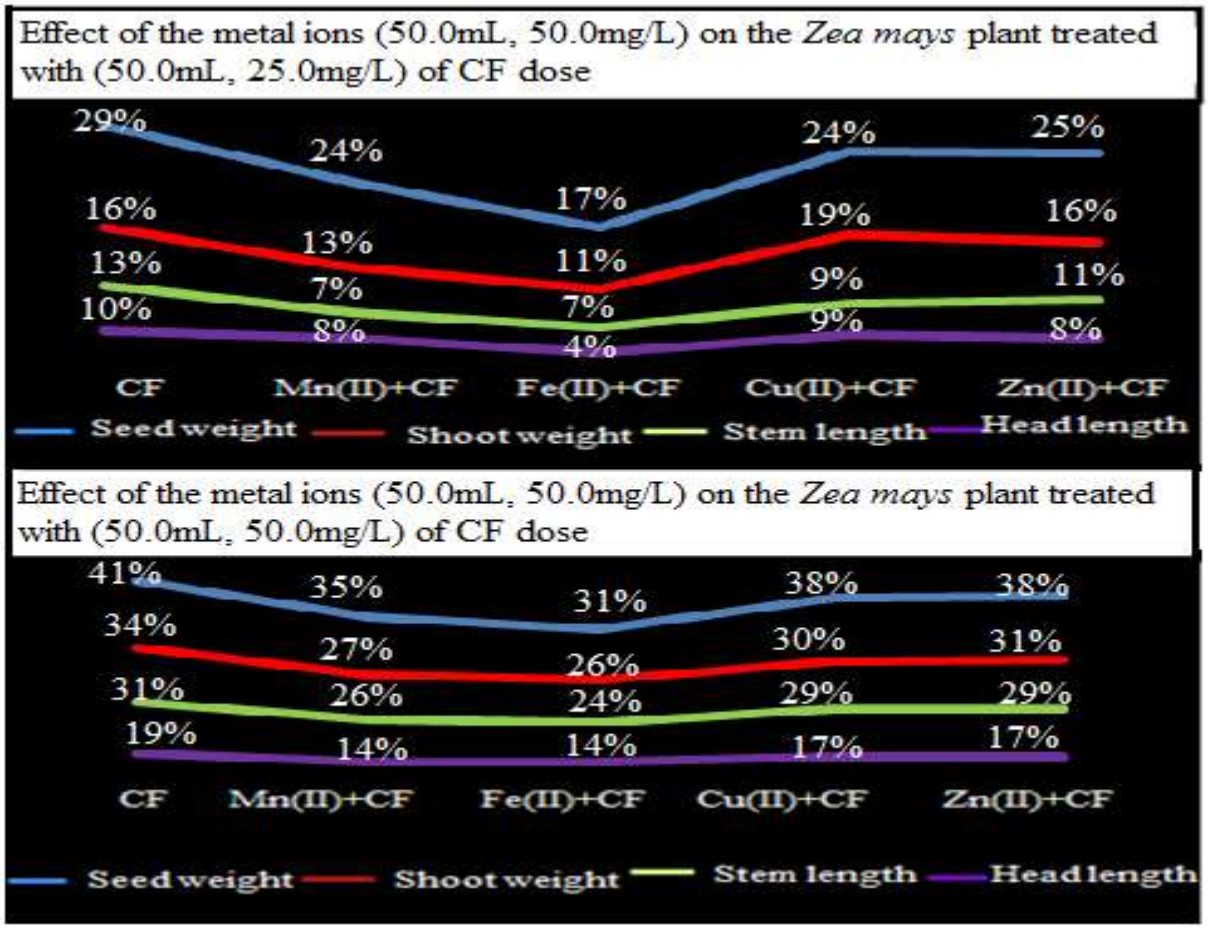


Figure 4.3: Morphological effect of additional supply of metal ions (50.0mL, 50.0mg/L) on *Zea mays* treated with low dose (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of CF with respect to control

4.3.2.2. Effect of the of metal ions on the *Zea mays* plant treated with TM low and high dose.

In a similar manner, when the morphological effect of the supply of 50.0mL,50.0mg/Lmetal ions dose was studied on the Zeamays,that was already treated with the lowdose (50.0mL, 25.0 mg/L) and high dose (50.0mL, 50.0mg/L) of TM; it was found that metal ions found beneficial in suppressing the effect of the TM dose on the *Zea mays* plant.The achieved mean ± standard deviation value for the lowand high doseof TM on the seed weight, shoot weight, stem length and head length of the *Zea mays* plant in presence of the different metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) are compiled in tabular form in Table 4.3.

Table 4.3: Mean ± standard deviation value of the seed weight, shoot weight, stem length and head length of the mature *Zea mays* ((9108) plant with/without the dose of the metal ions (Mn

(II),Fe(II),Cu(II)and Zn(II) treated with low dose (50.0mL, 25.0 mg/L) or high dose (50.0mL,50.0mg/L) of the TM with respect to control.

Sample	Dose[#] (mg/L)	Seed Weight (g)	Shoot Weight (g)	Stem Length (cm)	Head Length (cm)
Control		**1.45±0.01**	**`6.48±0.02**	**296±0.2**	**28±0.2**
TM	25.0	0.92±0.02	4.37±0.03	207.3±0.3	20.3±0.4
	50.0	0.70±0.04	3.50±0.01	172.1±0.2	16.1±0.1
Mn(II)+ TM	25.0	0.98± 0.02	4.70±0.02	218.4±0.1	22.2±0.2
	50.0	0.76±0.01	3.92±0.03	190.0±0.4	18.0±0.7
Fe(II) + TM	25.0	0.97±0.02	4.75±0.02	215.2±0.2	22.5±0.3
	50.0	0.75±0.03	3.98±0.01	188.6±0.1	18.7±0.4
Cu(II) +TM	25.0	1.08±0.02	4.92±0.02	235.3±0.2	23.4±0.2
	50.0	0.86±0.01	4.27±0.04	204.2±0.4	19.0±0.2
Zn (II) +TM	25.0	0.97±0.01	4.73±0.04	216.3±0.2	21.8±0.3
	50.0	0.76±0.01	3.94±0.02	189.2±0.3	23.1±0.4

At the dose of 50.0mL, 25.0mg/L of TM to the Zea *Mays*, seed weight reduced by 36%, but when the same was treated with 50.0mL of 50.0mg/L of metal ions, seed weight was observed decrease by 32% for Mn(II), 33% for of Fe(II), 25% for Cu(II) and 33% for Zn(II) with respect to the control. In a similar manner, on use of 50.0mL, 50.0mg/L of TM, on the Zea *Mays* plant, seed weight was reduced by 51%, which on use of 50.0mL of 50.0mg/Lof metal ions, was found reduced by 47% for Mn(II), 48% for Fe(II), 40 % for Cu(II) and 47% for Zn(II) metal ion with respect to control.

On use of 50.0mL, 25.0mg/L TM, decrease in shoot weight by 32% was observed; whereason applying the additional dose of50.0mL of50.0mg/L metal ions, decreased shoot weight was found limited to 27% for Mn(II),26% for Fe(II), 23% for Cu(II) and 26% for Zn(II) metal ion with respect to control.At the same time on application of 50.0mL, 50.0mg/L of TM,45% decrease in shoot weight was observed;where 50.0mL additional use of 50.0mg/L metal ions, leads to reduction ofshoot weight upto39% for Mn(II),38% for Fe(II), 34% for Cu(II) and 39% for Zn(II) with respect of control.

In case of stem length, on application of 50.0mL, 25.0 mg/L of TM, decreased stem length was found as 29%; while after application of 50.0mL, 50.0mg/L metal ions, decrease in stem length was observed by 26% on use of Mn(II), 27% on use of Fe(II), 20% on use of Cu(II) and 27% on use of Zn(II) with respect to control. In parallel,50.0mg/L TM reduced growth of stem length by 41%; where on additional application of 50.0mL, 50.0mg/L individual metal ion resulted in the lower decrease in stem length by 35% for Mn(II), 36% for Fe(II), 31% for Cu(II) and 36% for Zn(II) metal ion with respect to control.

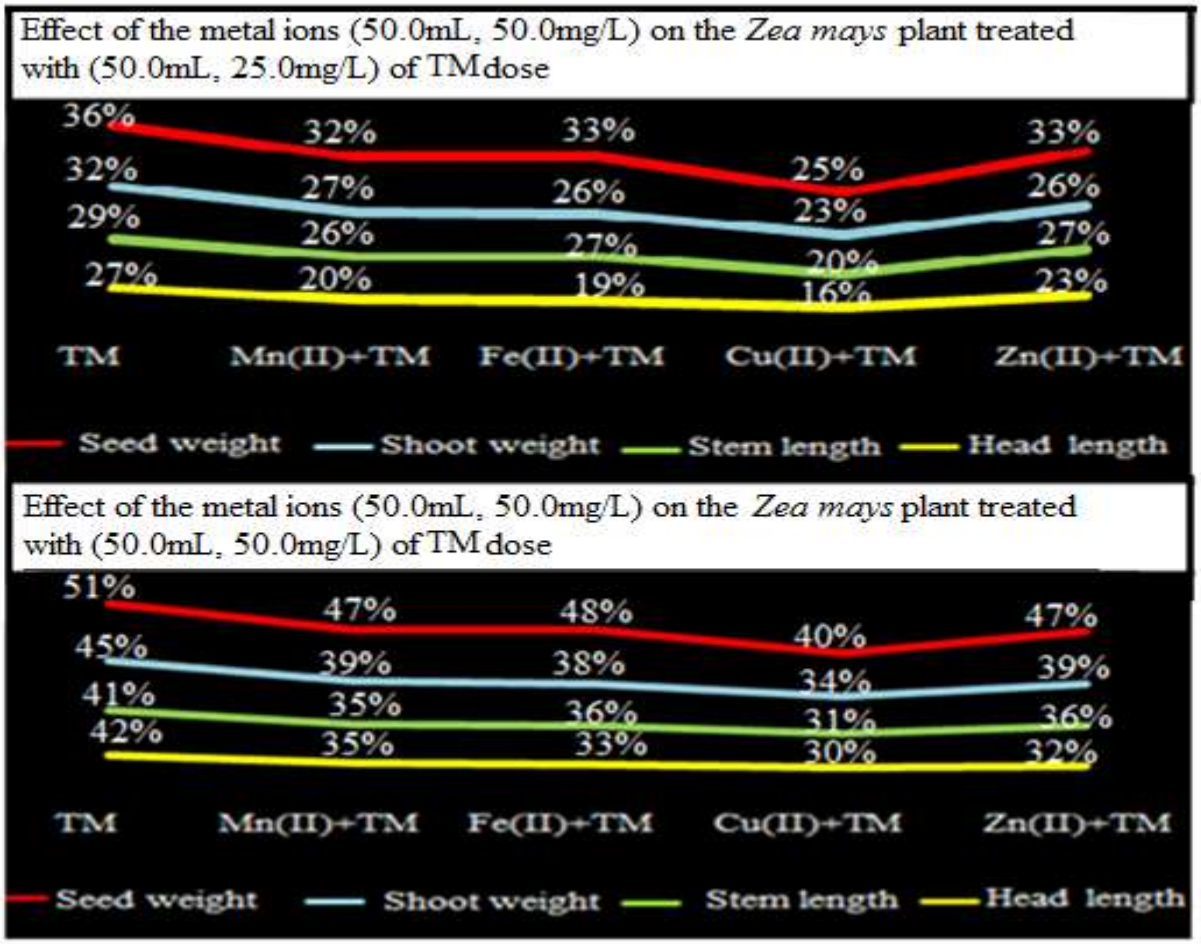


Figure 4.4: Morphological effect of additional supply of metal ions (50.0mL, 50.0mg/L) on*Zea mays* treated with low dose (50.0mL, 25.0 mg/L) and high dose (50.0mL, 50.0mg/L)of TM with respect to control.

Head length of *Zea mays* was reduced by 27% with respect to control on application of 50.0mL, 25.0 mg/Lof TM. However, when the 50.0mL dose of 50.0mg/L metal ion was applied to the TM treated plant (50.0mL, 25.0 mg/L), result depicted that addition of metal ions has slightly improved the *Zea mays* plant growth, and decrease in head length was found as: 20% for Mn(II),19% for Fe(II), 16% for Cu(II) and 23% for Zn(II) metal ion with respect to control. While, in case of the, 50.0mg/L TM, reduction in head length was 42% ; on which

50.0mL additional supply of 50.0mg/L metal ion leads to decrease in head length by 35% for Mn(II),33% for Fe(II), 30% for Cu(II) and 32% for Zn(II) metal ion with respect to control.

On evaluating the data, it was observed that the high dose of (50.0mL, 50.0mg/L) of TM causes more reduction in seed weight, shoot weight, stem length and head length of *Zea mays*plant.On additional supply of metal ions, the rise in the growth parameters was observed. However,additional supply of Cu(II) enhanced the physical parameters, maximum in case of the TM pesticides. As, TM form complex with Cu(II) metal ion on fast rate, the possibility of it to trap the metal ion prevails due to which it is not able to reach the plant.On adding the additional dose of it, helped the plant to consume it for the proper growth (as shown In Figure 4.4).

4.3.2.3. Effect of the of metal ions on the *Zea mays* plant treated with TC low and high dose

Likewise, when the morphological effect of the supply of 50.0mL,50.0mg/Lmetal ions dose was studied on the Zeamays with the lowdose (50.0mL, 25.0 mg/L) and high dose (50.0mL, 50.0mg/L) of TC; it was found that metal ions found beneficial in curbing the effect of the TC dose on the *Zea mays* plant.The achieved mean ± standard deviation value for the lowand high doseof TC on the seed weight, shoot weight, stem length and head length of the *Zea mays* plant in presence of the different metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) are compiled in tabular form in Table 4.4.

Table 4.4: Mean value ± standard deviation of the seed weight, shoot weight, stem length and head length of the mature *Zea mays* ((9108) plant with/without the dose of the metal ions (Mn (II),Fe(II),Cu(II)and Zn(II) treated with low dose (50.0mL, 25.0 mg/L) or high dose (50.0mL,50.0mg/L) of the TC with respect to control.

Sample	Dose (mg/L)	Seed Weight (g)	Shoot Weight (g)	Stem Length (cm)	Head Length (cm)
Control		**1.45±0.01**	**`6.48±0.02**	**296±0.2**	**28±0.2**
TC	25.0	0.89±0.02	4.11±0.04	184.3±0.1	18.6±0.4
	50.0	0.68±0.05	3.42±0.03	151.2±.3	14.0±0.2

Mn(II)+ TC	25.0	0.96±0.04	4.52±0.02	194.2±0.1	19.4±0.2
	50.0	0.77±0.03	3.80±0.05	173.2±0.3	16.1±0.3
Fe(II) + TC	25.0	0.98±0.02	4.45±0.02	194.8±0.2	19.2±0.2
	50.0	0.75±0.03	3.75±0.06	175.8±0.5	15.8±0.1
Cu(II) +TC	25.0	1.01±0.05	4.92±0.03	214.2±0.3	22.1±0.4
	50.0	0.87±0.03	4.15±0.02	185.2±0.3	17.5±0.5
Zn (II) +TC	25.0	1.00±0.02	4.97±0.04	215.4±0.2	21.7±0.3
	50.0	0.86±0.03	4.21±0.02	187.4±0.4	17.0±0.2

At the low dose of the TC, seed weight was reduced by 39% of *Zea mays*. When the same was treated with 50.0mL of 50.0mg/L of metal ions, seed weight was observed limited decrease as, 34% for Mn(II), 34% for Fe(II), 30% for Cu(II) and 31% for Zn(II)supply with respect to the control. In a similar manner, on use of high dose of TC, over *Zea mays*, seed weight was reduced by 53%, which on use of 50.0mL of 50.0mg/Lof metal ions,the decrease was found as 46% for Mn(II), 48% for Fe(II), 40% for Cu(II) and 40% for Zn(II) supply with respect to control.

In case of the shoot weight, at 50.0mL, 25.0 mg/L of TC, 37% reduction was observed with respect to the control; whereas on applying the additional dose of 50.0mL of 50.0mg/L metal ions, shoot weight decrease was found as 30% for Mn(II),31% for Fe(II), 24% for Cu(II) and 23% for Zn(II) metal ion with respect to control. At the same time on application of 50.0mL, 50.0mg/L of TC, 47% decrease in shoot weight was observed; where on 50.0mL additional use of 50.0mg/L metal ions, leads to decrease in shoot weight by 41% for Mn(II),42% for Fe(II), 36% for Cu(II) and 35% for Zn(II) metal ion with respect to the control.

On application of 50.0mL, 25.0 mg/L of TC, decrease in stem length was found as 38%; while after application of 50.0mL, 50.0mg/L metal ions, stem length was observed decrease by 34% for Mn(II), 34% for Fe(II), 28% for Cu(II) and 27% for Zn(II) metal ion with respect to the control. In parallel, application of high dose of TC reduced growth of stem length by 43%; where on additional application of 50.0mL, 50.0mg/L individual metal ion resulted decrease in stem length by 41% for Mn(II), 40% for Fe(II), 37% for Cu(II) and 36% for Zn(II) metal ionsupply with respect to the control.

Head length of *Zea mays* was reduced by 33% with respect to control on application of low dose of TC. However, when the 50.0mL dose of 50.0mg/L metal ion was applied to

the TC treated plant (50.0mL, 25.0 mg/L), result depicted that addition of metal ions has slightly improved the *Zea mays* plant growth, and head length was found a decrease by: 30% for Mn(II),31% for Fe(II), 21% for Cu(II) and 22% for Zn(II) metal ionsupply with respect to the control. While, in case of the, application of high dose of TC, reduction in head length was 50%; on which 50.0mL additional supply of 50.0mg/L metal ion leads to decrease in head length as: 42% for Mn(II),43% for Fe(II), 37% for Cu(II) and 39% for Zn(II) metal ion with respect to the control.

The data suggested that, the high dose of (50.0mL, 50.0mg/L) of TC causes more reduction in seed weight, shoot weight, stem length and head length of *Zea mays* plant.On additional supply of metal ions, it was found the maximum suppress of the harmful effect of TC dose on the *Zea mays* plant was done by Cu(II) and Zn(II) metal ion.As, TC chelate with both the metal ions easily, their additional dose helped plant to enhance the growth (as shown in Figure 4.5).

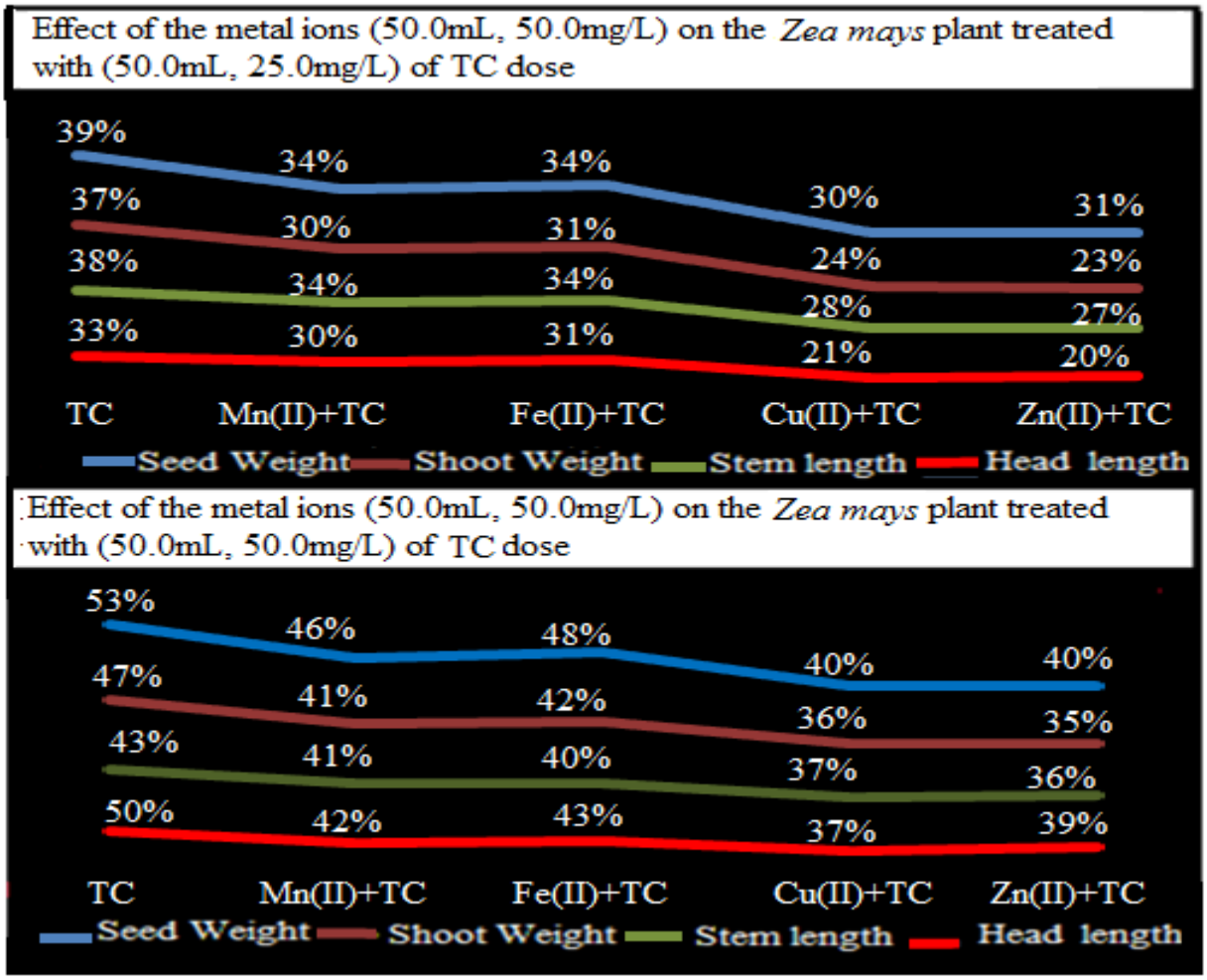


Figure 4.5: Morphological effect of additional supply of metal ions (50.0mL, 50.0mg/L) on *Zea mays* treated with low dose (50.0mL, 25.0 mg/L) and high dose (50.0mL, 50.0mg/L) of TC with respect to the control.

4.3.2.4. Effect of the metal ions on *Zea mays* plant treated withlow and high dose of CZ.

The addition of the metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) to the *Zeamays* plant containinglow dose (50.0mL, 25.0 mg/L) and high dose (50.0mL, 50.0mg/L) of CZ was found beneficial. As, it helped to enhance the growth of the seed weight, shoot weight, stem length and head length of the *Zea mays* plant reduced due to usage of CZ.The observed mean value± standard deviation for the low and high doses of CZ in presence of the different metal ions on the Zeamays are compiled in Table 4.5.

Table 4.5: Mean value ± standard deviation of the seed weight, shoot weight, stem length and head length of the mature *Zea mays*(9108) plant, with/without the dose of the metal ions (Mn (II),Fe(II),Cu(II) and Zn(II) effected by low dose (50.0mL, 25.0 mg/L) and high dose (50.0mL,50.0mg/L) of the CZ with respect to the control.

Sample	Dose (mg/ L)	Seed Weight (g)	Shoot Weight (g)	Stem Length (cm)	Head Length (cm)
Control		**1.45±0.01**	**`6.48±0.02**	**296±0.2**	**28±0.2**
CZ	25.0	0.80±0.03	3.72±0.03	163.0±0.4	15.8±0.3
	50.0	0.59±0.02	3.08±0.05	138.0±0.2	13.1±0.6
Mn(II)+ CZ	25.0	0.88±0.04	3.98±0.03	175.8±0.3	17.4±0.3
	50.0	0.64±0.03	3.40±0.03	149.5±0.2	14.2±0.4
Fe(II) + CZ	25.0	0.98±0.02	4.28±0.02	182.4±0.4	19.2±0.2
	50.0	0.72±0.04	3.90±0.02	169.2±0.3	16.4±0.5
Cu(II) +CZ	25.0	1.00±0.02	4.38±0.04	185.3±0.2	18.9±0.3
	50.0	0.74±0.02	3.85±0.03	172.3±0.2	16.5±0.2
Zn (II) +CZ	25.0	0.92±0.03	4.12±0.02	178.2±0.4	18.0±0.2
	50.0	0.68±0.01	3.76±0.01	163.1±0.3	15.1±0.4

The application of 50.0mL, 25.0 mg/L of CZ, has reduced seed weight by 44% of *Zea mays* with respect to the control. On application of the 50.0mL of 50.0mg/L metal ions, seed weight was observed less decrease i.e. by, 39% for Mn(II), 32% for of Fe(II), 31% for Cu(II) and 36% for Zn(II) with respect to the control. In a similar manner, on use of 50.0mL, 50.0mg/L of CZ, on the *Zea mays* plant, seed weight was reduced by 59%, which on use of 50.0mL of

50.0mg/L of metal ions, the reduction was found by 55% for Mn(II), 50% for Fe(II), 48% for Cu(II) and 53% for Zn(II) metal ion with respect to control.

In case of the shoot weight, at low dose (50.0mL, 25.0mg/L) of CZ, 42% reduction was observed with respect to control; whereas on applying the additional dose of 50.0mL of 50.0mg/L metal ions, shoot weight was reduced by 38% for Mn(II),33% for Fe(II), 32% for Cu(II) and 36% for Zn(II) metal ion with respect to control. At the same time on application of50.0mL, 50.0mg/L of CZ, 52% decrease in shoot weight was observed; where on 50.0mL additional use of 50.0mg/L metal ions, leads to reducethe shoot weight by47% for Mn(II),39% for Fe(II), 40% for Cu(II) and 44% for Zn(II) with respect of control.

At application of 50.0mL, 25.0mg/L of CZ stem length decreased by 44% w.r.t control; where onafter application of 50.0mL, 50.0mg/L metal ions, reduction in stem length was observed by 40% for Mn(II), 38% for Fe(II), 37% for Cu(II) and 39% for Zn(II) metal ion with respect to control.On application of high dose of CZ reduction in growth of stem length was found as 53%; where on additional application of 50.0mL, 50.0mg/L individual metal ion, stem length was found to decrease by 49% for Mn(II), 42% for Fe(II), 41% for Cu(II) and 45% for Zn(II) metal ion with respect to control.

In case of head length of *Zea mays*, 43%reduction with respect to control on application of 50.0mL, 25.0mg/LCZ was observed. However, when the 50.0mL dose of 50.0mg/L metal ion was applied to the low dose CZtreated plant, decrease in head length was found limited by 37% for Mn(II), 31% for Fe(II), 32% for Cu(II) and 35% for Zn(II) metal ion with respect to the control. While, in case of the, high dose application of CZ, reduction in head length was 53% w.r.t. control; on which 50.0mL additional supply of 50.0mg/L metal ion decreased in head length by 49% for Mn(II),41% for Fe(II), 41% for Cu(II) and 46% for Zn(II) metal ion with respect to the control.

At the (50.0mL, 50.0mg/L) dose of CZ, the reduction in seed weight, shoot weight, stem length and head length of *Zea mays* plant was found maximum. Its effect is minimized after the addition of the metal ions. The additional supply ofFe(II) and Cu(II) ions are found most beneficial for increasing the growth of *Zea mays* effected by CZ. These metal ions can easily interact with CZ carbamate due to which their possibility to trap the metal ions exist. When the additional dose of metal ion is supplied, the metal ions become available for plant and helped to enhance the growth (as shown In Figure 4.6).

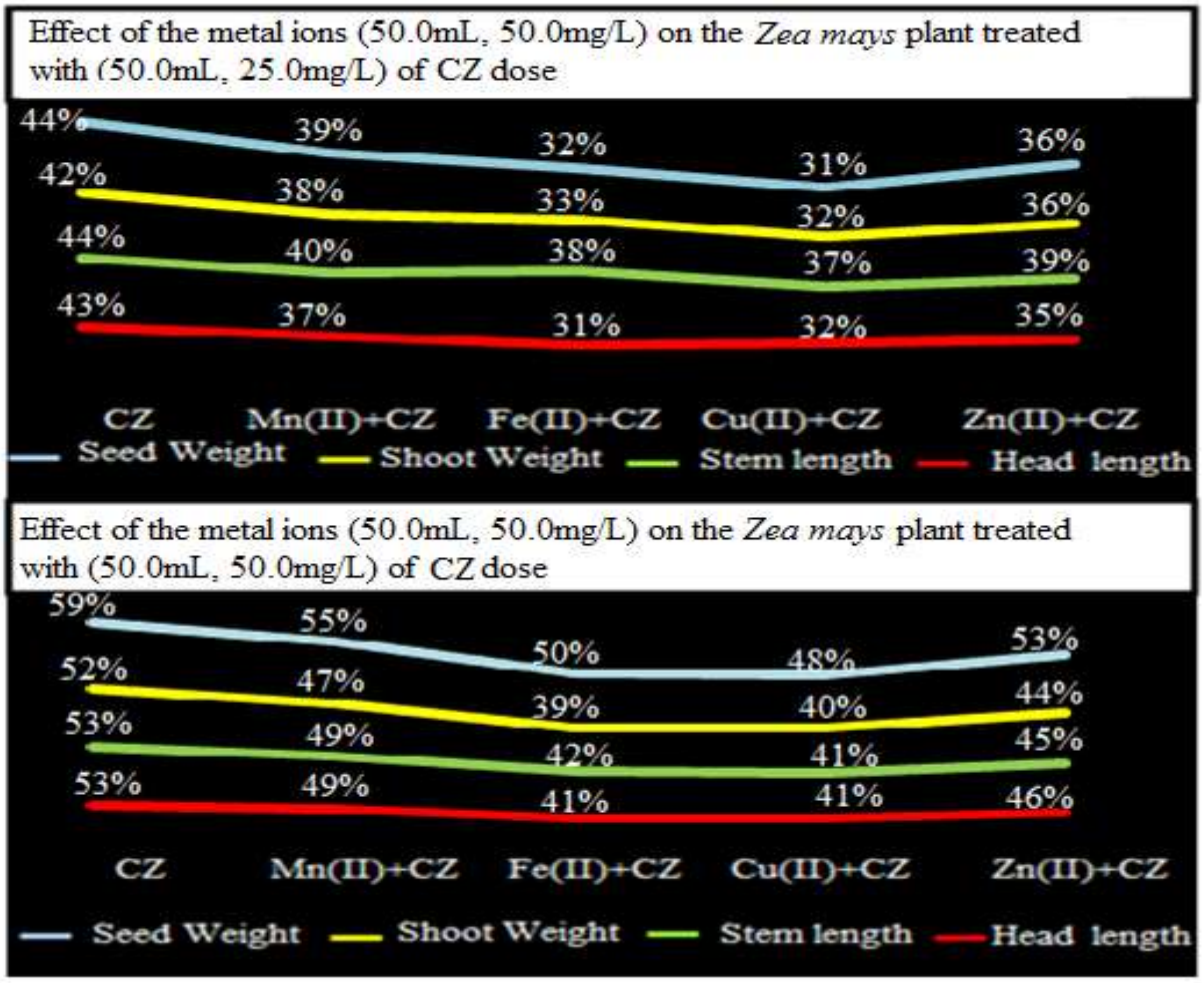


Figure 4.6: Morphological effect of additional supply of metal ions (50.0mL, 50.0mg/L) on *Zea mays* treated with low dose (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of CZ with respect to control.

4.3.2.5. Effect of the metal ions on the *Zea mays* plant treated with M low and high dose.

Although, methomyl (M) has highly retarded the Zea mays plant growth. But, the additional usage of metal ions is found useful for the plant. As, in presence of the additional metal ions, the increase in the growth of seed weight, shoot weight, stem length and head length of the *Zea mays* plant was observed with respect to pesticide effected plant.The observed mean value± standard deviation for the lower and higher doses of M in presence of the different metal ions on the *Zea mays* are compiled in Table 4.6.

Table 4.6: Mean value ± standard deviation of the seed weight, shoot weight, stem length and head length of the mature *Zea mays*(9108) plant with/without the dose of Mn(II),Fe(II),Cu(II) and Zn(II) metal ions, already treated withlow dose (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of the M with respect to the control

Sample	Dose (mg/ L)	Seed Weight (g)	Shoot Weight (g)	Stem Length (cm)	Head Length (cm)
Control		**1.45±0.01**	**`6.48±0.02**	**296±0.2**	**28±0.2**
M	25.0	0.65±0.03	3.08±0.03	143.4±0.3	12.2±0.4
	50.0	0.47±0.06	2.44±0.02	118.2±0.2	10.3±0.2
Mn(II)+ M	25.0	0.82±0.02	3.85±0.01	173.2±0.4	13.1±0.4
	50.0	0.61±0.03	3.27±0.03	146.7±0.4	14.0±0.3
Fe(II) + M	25.0	0.89±0.04	3.91±0.04	175.4±0.3	16.9±0.2
	50.0	0.66±0.03	3.48±0.02	148.2±0.1	14.4±0.3
Cu(II) +M	25.0	0.79±0.04	3.39±0.04	158.2±0.6	14.5±0.4
	50.0	0.57±0.02	2.94±0.02	130.5±0.3	11.6±0.1
Zn (II) +M	25.0	0.73±0.04	3.32±0.05	162.3±.7	14.9±0.3
	50.0	0.55±0.03	2.98±0.04	135.2±0.1	11.5±0.4

At 50.0mL, 25.0mg/L(low dose) of M, seed weight of the *Zea mays* plant was reduced by 55% with respect to control. On application of the 50.0mL of 50.0mg/L of metal ions, seed weight was observed decrease by, 43% for Mn(II), 38% for of Fe(II), 45% for Cu(II) and 49% for Zn(II) with respect to the control. In a similar manner, on use of50.0mL,50.0mg/L(high dose) of M, on the *Zeamays* plant, seed weight was reduced by 67%, where on useof 50.0mL of 50.0mg/L of metal ions, decrease in seed weight was found as 57% for Mn(II), 54% for Fe(II), 60% for Cu(II) and 62% for Zn(II) metal ion with respect to control.

Application of low dose of M reduced shoot weight by 52% with respect to control; whereas on applying the additional dose of 50.0mL of 50.0mg/L metal ions along with M, shoot weight was found decrease by 40% for Mn(II),40% for Fe(II), 47% for Cu(II) and 48% for Zn(II) was observed w.r.t control. At the same time, on application of high dose of M, 62% decrease in shoot weight was observed; where on 50.0mL additional use of 50.0mg/L metal ions, leads to decrease in shoot weight by 49% for Mn(II),46% for Fe(II), 55% for Cu(II) and 50% for Zn(II) with respect to the control.

Stem length was decreased by 51% on applying low dose of M; while with the additional application of 50.0mL, 50.0mg/L metal ions, decrease in stem length was observed

by 41% for Mn(II), 40% for Fe(II), 46% for Cu(II) and 45% for Zn(II) metal ion with respect to control. For, 50.0mL, 50.0mg/L dose of M, reduction in growth of stem length was found as 60%; where on additional application of 50.0mL, 50.0mg/L individual metal ion stem length was found decreased by 50% for Mn(II), 50% for Fe(II), 56% for Cu(II) and 54% for Zn(II) metal ion with respect to the control.

Head length of *Zea mays* was reduced by 56% with respect to control on application of low dose of M. However, when 50.0mL dose of 50.0mg/L metal ion was applied to the low dose M treated plant, head length was found decrease by 53% for Mn(II),39% for Fe(II), 48% for Cu(II) and 46% for Zn(II) metal ion with respect to the control. In case of high dose treated M, reduction in head length of *Zea mays* was found 63%; on which 50.0mL additional supply of 50.0mg/L metal ion, decreased head length by 50% for Mn(II),48% for Fe(II), 58% for Cu(II) and 58% for Zn(II) metal ion with respect to the control.

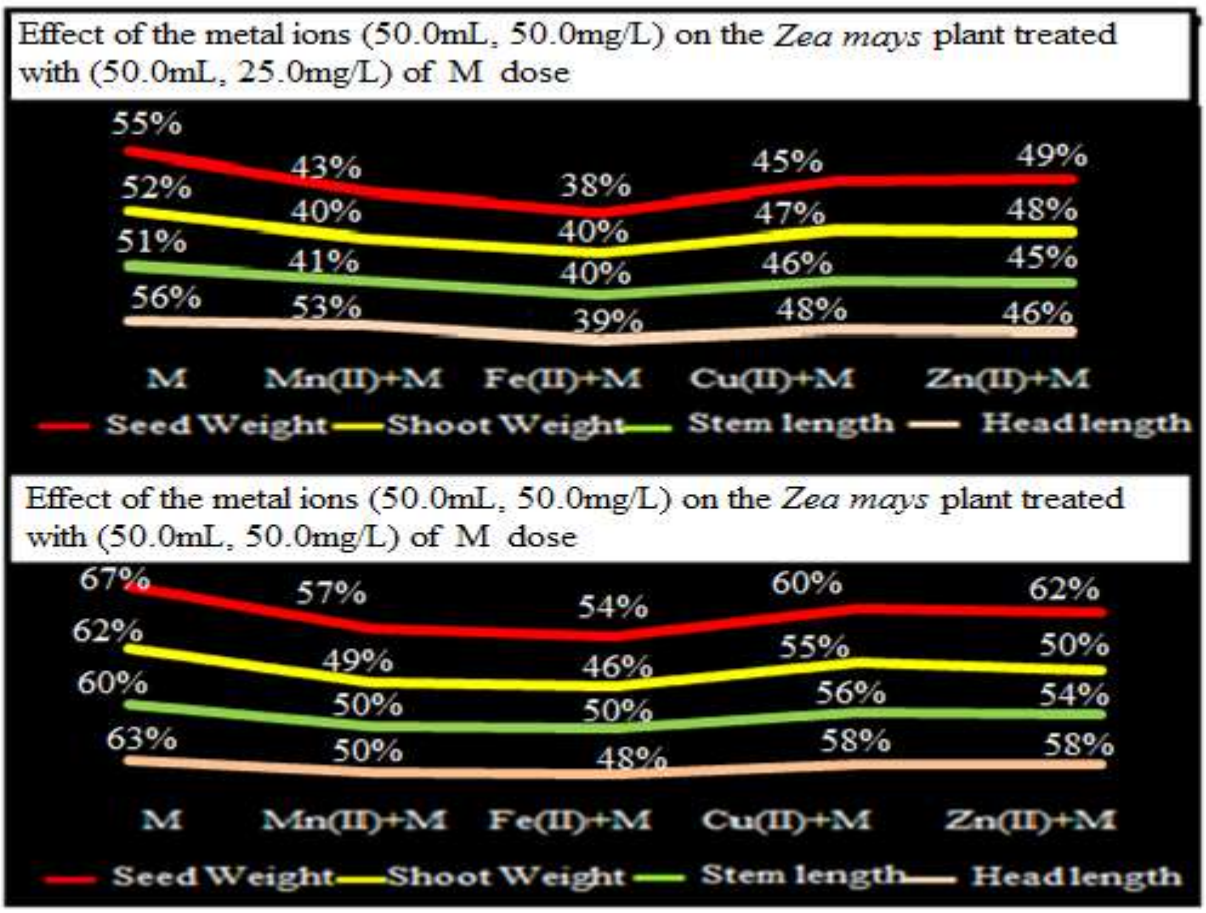


Figure 4.7: Morphological effect of additional supply of metal ions (50.0mL, 50.0mg/L) on *Zea mays* treated with low dose (50.0mL,25.0mg/L) and high dose (50.0mL, 50.0mg/L) of M with respect to the control.

The reduction in seed weight, shoot weight, stem length and head length of *Zea mays* plant was found maximum at high dose (50.0mL of 50.0mg/L) of M. Its effect is minimized after

the additional application of the metal ions. The additional supply of Mn(II) and Fe(II) are found most beneficial in increasing the growth. As, M form chelate quickly with both these metal ions, its tendency to trap them and make it unavailable to the plant is more, on additional supply of Mn(II)/ Fe(II), plants got these nutrients that were used for controlling the retardation of growth of *Zea mays* (as shown in Figure 4.7).

4.3.3. Effects of the applied carbamate pesticide in presence and absence of the additional metal ions on the content of the metal ions in the seeds of *Zea mays* plant.

For evaluating the essential metal ions content of the matured and dry seeds of the *Zea mays* plant, ICP-AES analysis was performed. The inferred data revealed that each of the selected carbamate was found hampering the essential metal ions content in the seeds of the *Zeamays* plant. When the selective carabamte was treated with the dose (50.0mL, 50.0mg/L)of the metal ions, increased amount of the essential metal ion content in the seeds of the *Zeamays* was observed. The obtained data was deduced in percentage separately for each of the selected carbamate pesticide. The results also depicted that each of the metal ions doses were found beneficial for the carbamate effected *Zea mays* plant. The impact of the each metal ions was different in presence of the each of the selected carbamates pesticide.

4.3.3.1 Effect of additional metal ions on the content of the metal ions on the seeds of CF effected *Zea mays* plant.

The investigation of the effect of the carbofuran pesticide on the metal ions content of the seeds was done. From the study, it was revealed that, low dose (50.0mL, 25.0mg/L) of the CF has reduced the metal ions content by 13% for Mn(II), 15% for Fe(II), 12% for Cu(II) and 11% for Zn(II) metal ions. On this low dose CF effected *Zea mays*, additional application of Mn(II) (50.0mL, 50.0mg/L) decreased the percentage of metal ion cotent by 7% for Mn(II), 4% for Fe(II), 2% for Cu(II) and 6% for Zn(II) with respect to the control. Similar amount of addition of Fe(II), resulted drop in metal ion content by 4% for Mn(II), 7% for Fe(II), 3% for Cu(II) and 8% for Zn(II) w.r.t. the control. Similarly, additional application of Cu(II) (50.0mL, 50.0mg/L) leads to decrease in percentage of metal ion by 2% for Mn(II), 2% for Fe(II), 2% for Cu(II) and 2% for Zn(II). While, additional application of Zn(II) on low dose CF effected *Zea mays* plant, and resulted decrease inmetal ion content by 2% for Mn(II), 1% for Fe(II), 1% for Cu(II) and 8% for Zn(II) with respect to the control.

At high dose (50.0mL, 50.0mg/L) of CF, decrease in content of metal ions was observed by 27% for Mn(II), 30% for Fe(II), 18% for Cu(II) and 19% for Zn(II) w.r.t the control. On additional supply of (50.0mL, 50.0mg/L) of Mn(II), decrease of metal ion content get limited by 9% for Mn(II), 3% for Fe(II), 2% for Cu(II) and 4% for Zn(II) w.r.t control. Addition of similar amount of Fe(II) limited the amount of decrease in metal ion content by 7% for Mn(II), 6% for Fe(II), 5% for Cu(II) and 5% for Zn(II) w.r.t. control

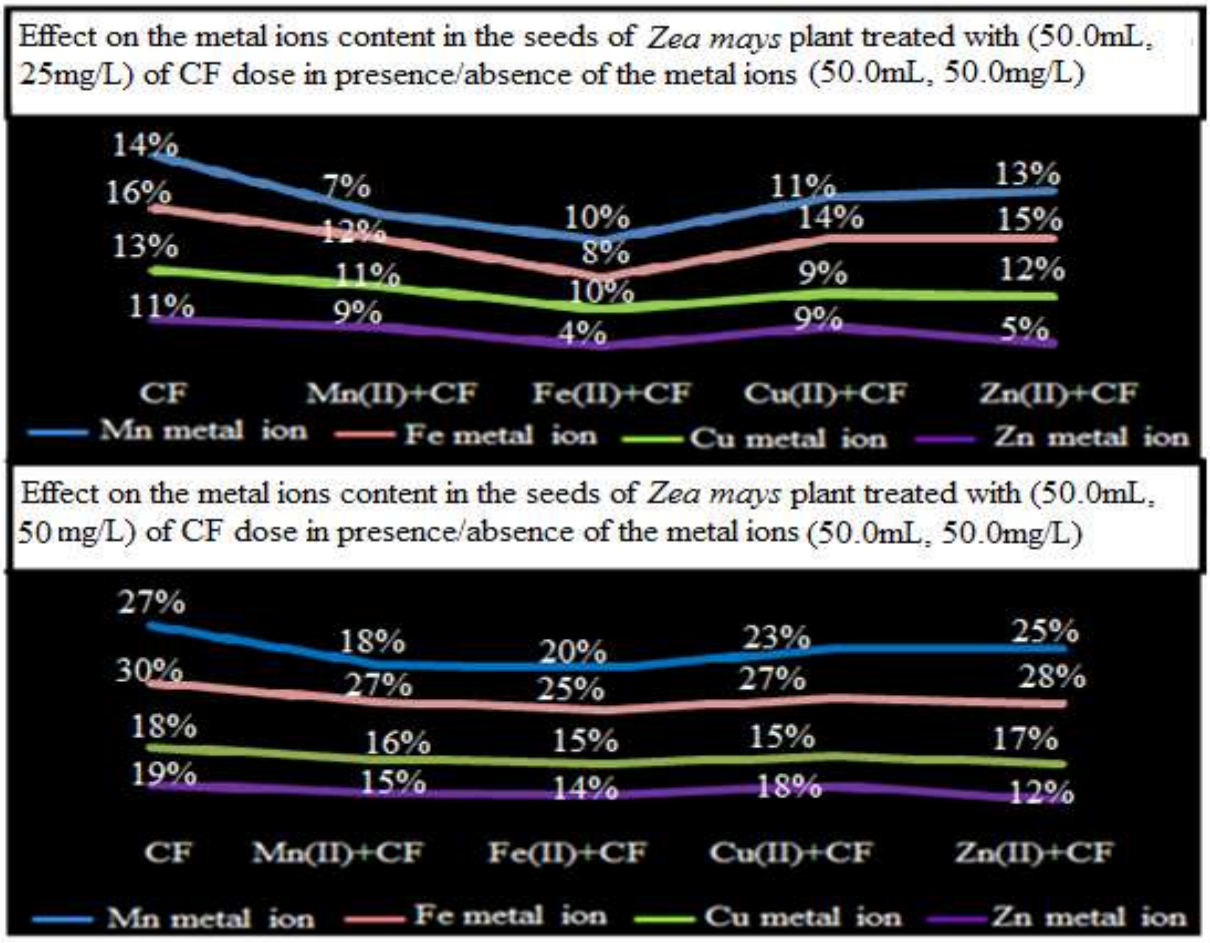


Figure 4.8.: Effect of the low(50.0mL, 25.0mg/L) and high(50.0mL, 50.0mg/L) CF on the metal ions content of the seeds of the *Zea mays*, in presence/ absence of the (50.0mL, 50.0mg/L) of the metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)).

Addition of (50.0mL, 50.0mg/L) Cu(II) on the high dose applied CF, limit the decrease in metal ion content by 5% for Mn(II), 4% for Fe(II), 3% for Cu(II) and 2% for Zn(II) w.r.t. the control. In a similar way additional application of 50.0mL, 50.0mg/L Zn(II) to the high dose CF applied *Zea mays* limit the decrease of metal ion content by2% for Mn(II), 2% for Fe(II), 1% for Cu(II) and 6% for Zn(II) w.r.t. the control.

The compiled effect of lower (50.0mL, 25.0mg/L) and higher (50.0mL, 50.0mg/L) dose of CF in absence/presence of the additional application of 50.0mg/L metal ion (Mn(II), Fe(II), Cu

(II) or Zn(II)) on the content of the *Zea mays* seed metal ions is represented in Figure 4.8 and complete data in tabular form is represented in Table 4.7.

Table 4.7: Mean±standard deviation value of the metal ion content with/ without additional supply of (50.0mL, 50.0mg/L) metal ion (Mn(II),Fe(II),Cu(II) and Zn(II))on low (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of CF effected mature seed of the *Zea mays* (9108).

Sample	Dose of CF (mg/L)	Mn	Fe	Cu	Zn
Control		**0.44±0.03**	**4.34±0.2**	**1.10±0.7**	**0.52±0.02**
CF	25.0	0.38±0.04	3.65±0.4	0.96±0.3	0.46±0.4
	50.0	0.32±0.02	3.02±0.2	0.90±0.3	0.42±0.3
Mn(II)+ CF	25.0	0.41±0.03	3.83±0.4	0.98±0.2	0.48±0.3
	50.0	0.36±0.02	3.16±0.2	0.92±0.5	0.44±0.2
Fe(II) + CF	25.0	0.40±0.01	3.99±0.4	1.0±0.3	0.50±0.3
	50.0	0.35±0.03	3.26±0.3	0.94±0.3	0.45±0.2
Cu(II) +CF	25.0	0.39±0.04	3.74±0.3	0.99±0.2	0.47±0.3
	50.0	0.34±0.06	3.18±0.2	0.93±0.3	0.43±0.4
Zn(II) +CF	25.0	0.39±0.03	3.70±0.4	0.97±0.4	0.50±0.3
	50.0	0.33±0.05	3.12±0.4	0.91±0.3	0.46±0.4

4.3.3.2 Effect of additional metal ions on the content of the metal ions on the seeds of TM effected *Zea mays* plant

When the effect of the thiophanate methyl on the metal ions content of the seeds were investigated, it revealed thatat lower dose (50.0mL, 25.0mg/L) of the TM has decreased the metal ions content by 20% for Mn(II), 17% for Fe(II), 31% for Cu(II) and 15% for Zn(II) metal ions. On additional supply (50.0mL, 50.0mg/L) of Mn(II), percentage of metal ions was decreasedby 4% for Mn(II), 12% for Fe(II), 27% for Cu(II) and 9% for Zn(II) w.r.t the control. In a similar manner, addition of (50.0mL, 50.0mg/L) Fe(II), reduced metal ion content by 6% for Mn(II), 11% for Fe(II), 25% for Cu(II) and 5% for Zn(II) w.r.t. control. Cu(II) (50.0mL, 25.0mg/L) decreased percentage of metal ion by 11% for Mn(II), 14% for Fe(II), 18% for Cu(II) and 11% for Zn(II) compared to the control. While, additional application of (50.0mL, 50.0mg/L) Zn(II) on low dose TM effected*Zea mays* plant, resulted

decreased metal ion content by 13% for Mn(II), 15% for Fe(II), 29% for Cu(II) and 5% for Zn(II) with respect to the control.

At the high dose of TM, reduced amount of metal ions was observed: 31% for Mn(II), 31% for Fe(II), 47% for Cu(II) and 26% for Zn(II) with respect to the control. On additional supply of (50.0mL, 50.0mg/L) of Mn(II), decreased amount of metal ion content come to 9% for Mn(II), 26% for Fe(II), 42% for Cu(II) and 21% for Zn(II) w.r.t control. On applying similar amount of Fe(II), metal ion content reduced by 11% for Mn(II), 25% for Fe(II), 40% for Cu(II) and 9% for Zn(II) w.r.t. control. Addition of (50.0mL, 50.0mg/L) of Cu(II) on the high dose (50.0mL, 50.0mg/L) of TM, limited the decrease metal ion content by 18% for Mn(II), 28% for Fe(II), 35% for Cu(II) and 23% for Zn(II) w.r.t. the control. In a similar way additional application of 50.0mL, 50.0mg/L Zn(II) to the high dose TM applied *Zea mays* reduced metal ions content by 20% for Mn(II), 29% for Fe(II), 44% for Cu(II) and 17% for Zn(II) w.r.t. the control.

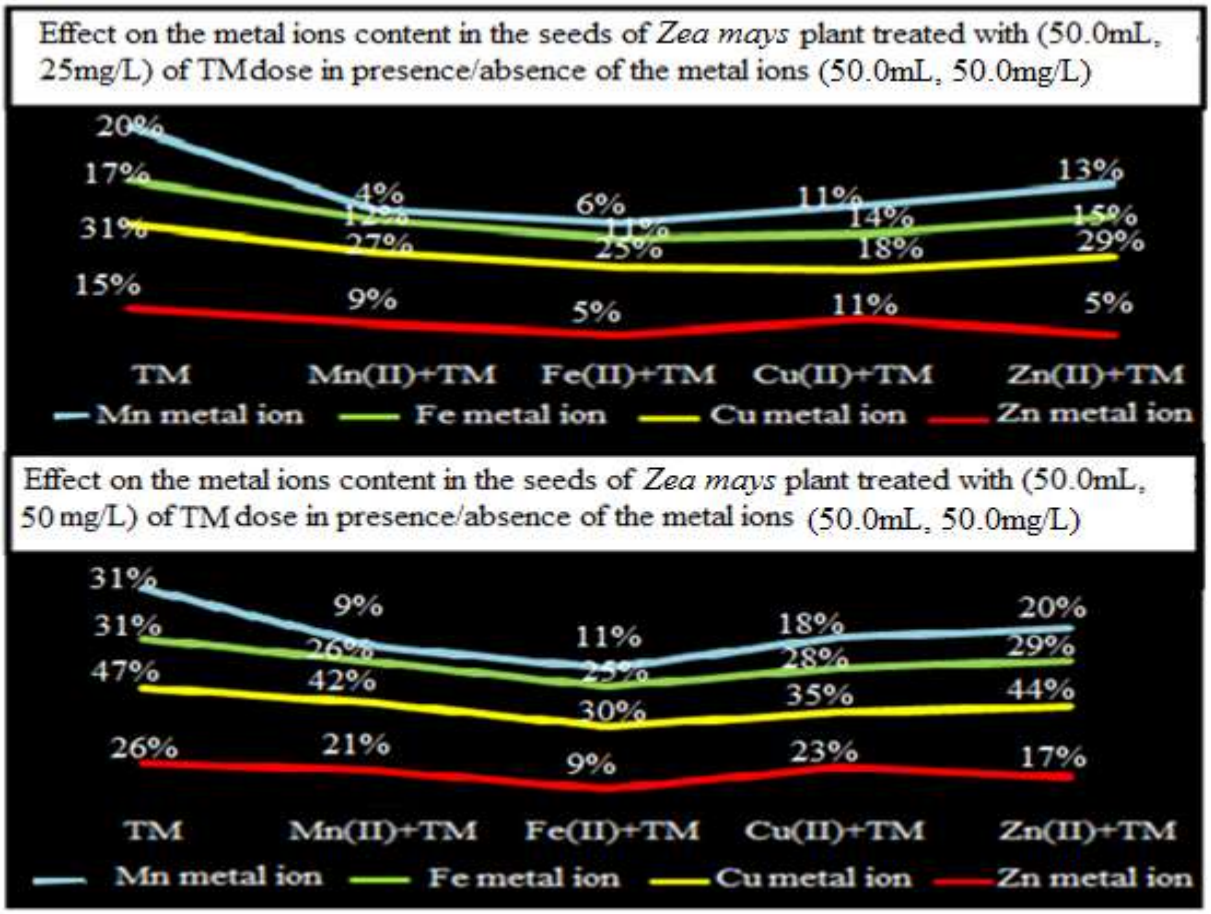


Figure 4.9: Effect of the low (50.0mL, 25.0mg/L) and high(50.0mL, 50.0mg/L) TM doses in presence/ absence of the (50.0mL, 50.0mg/L) metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) on the metal ions content of the seeds of the *Zea mays* plant.

The summed up effect of lower (50.0mL, 25.0mg/L) and higher (50.0mL, 50mg/L) dose of TM in absence/presence of the additional application of 50.0mg/L metal ion (Mn(II), Fe(II), Cu (II) or Zn(II)) on the content of the *Zea mays* seed metal ions is represented in Figure 4.9 and complete data in tabular form is represented in Table 4.8.

Table 4.8: Mean ± standard deviation value of the metal ion content with/ without additional supply of (50.0mL, 50.0mg/L) metal ion (Mn (II), Fe(II), Cu(II) and Zn(II)) on low (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of TM effected mature seed of the *Zea mays* (9108).

Sample	Dose of TM (mg/L)	Mn(II)	Fe(II)	Cu(II)	Zn(II)
Control		**0.44±0.03**	**4.34±0.2**	**1.10±0.7**	**0.52±0.02**
TM	25.0	0.35±0.06	3.60±0.5	0.76±0.4	0.40±0.04
	50.0	0.29±0.02	3.06±0.5	0.55±0.2	0.28±0.06
Mn(II)+ TM	25.0	0.38±0.05	3.62±0.3	0.80±0.3	0.47±0.03
	50.0	0.33±0.03	3.11±0.2	0.59±0.6	0.41±0.05
Fe(II) + TM	25.0	0.37±0.04	3.83±0.3	0.82±0.5	0.49±0.02
	50.0	0.31±0.06	3.45±0.4	0.63±0.8	0.42±0.04
Cu(II) +TM	25.0	0.36±0.04	3.74±0.2	0.85±0.3	0.46±0.03
	50.0	0.30±0.03	3.28±0.5	0.66±0.5	0.40±0.04
Zn (II) +TM	25.0	0.38±0.05	3.66±0.3	0.78±0.2	0.49±0.03
	50.0	0.35±0.04	3.09±0.3	0.63±0.3	0.43±0.04

4.3.3.3. Effect of additional metal ions on the content of the metal ions in the seeds of TC effected *Zea mays* plant.

The effect of the doses of thiodicarb on the metal ions content of the seeds were investigated.It revealed thatat low dose (50.0mL, 25.0mg/L) of the TC, reduction in metal ions content was found as: 18% for Mn(II), 16% for Fe(II), 20% for Cu(II) and 12% for Zn(II) metal ions w.r.t the control. On the additional supply of 50.0mL, 50.0mg/LMn(II) metal ion, drop in the percentage of metal ions was found as: 14% for Mn(II), 13% for Fe(II), 14% for Cu(II) and 5% for Zn(II) with respect to the control. In a similar manner, after application ofFe(II) dose (50.0mL, 50.0mg/L), decrease in metal ion content was found as: 10% for Mn(II), 10% for Fe(II), 9% for Cu(II) and 7% for Zn(II). In a similar manner, Cu(II)

(50.0mL, 50.0mg/L), decreased percentage of metal ion was found as: 12% for Mn(II), 10% for Fe(II), 12% for Cu(II) and 10% for Zn(II) w.r.t the control. The additional application 50.0mL, 50.0mg/L of Zn(II) on low dose TC, decreased metal ion content by 8% for Mn(II), 14% for Fe(II), 13% for Cu(II) and 10% for Zn(II) w.r.t. the control.

At the high dose (50.0mL, 50.0mg/L) of TC, decreased amount of metal ions was observed as: 41% for Mn(II), 35% for Fe(II) , 27% for Cu(II) and 19% for Zn(II) with respect to the control. On additional supply of 50.0mL, 50.0mg/L Mn(II), reduction in metal ion content was found as: 37% for Mn(II), 22% for Fe(II), 26% for Cu(II) and 10% for Zn(II) w.r.t control. On applying similar amount of Fe(II), metal ion content dropped by 35% for Mn(II), 31% for Fe(II), 18% for Cu(II) and 14% for Zn(II) w.r.t. control. At50.0mL, 50.0mg/Lapplication of Cu(II),reduction in metal ion content was found as:35% for Mn(II), 29% for Fe(II), 22% for Cu(II) and 14% for Zn(II) w.r.t. the control. On application of50.0mL,50.0mg/LZn(II), drop in metal ion content was found as 31% for Mn(II), 33% for Fe(II), 22% for Cu(II) and 16% for Zn(II) w.r.t. the control.

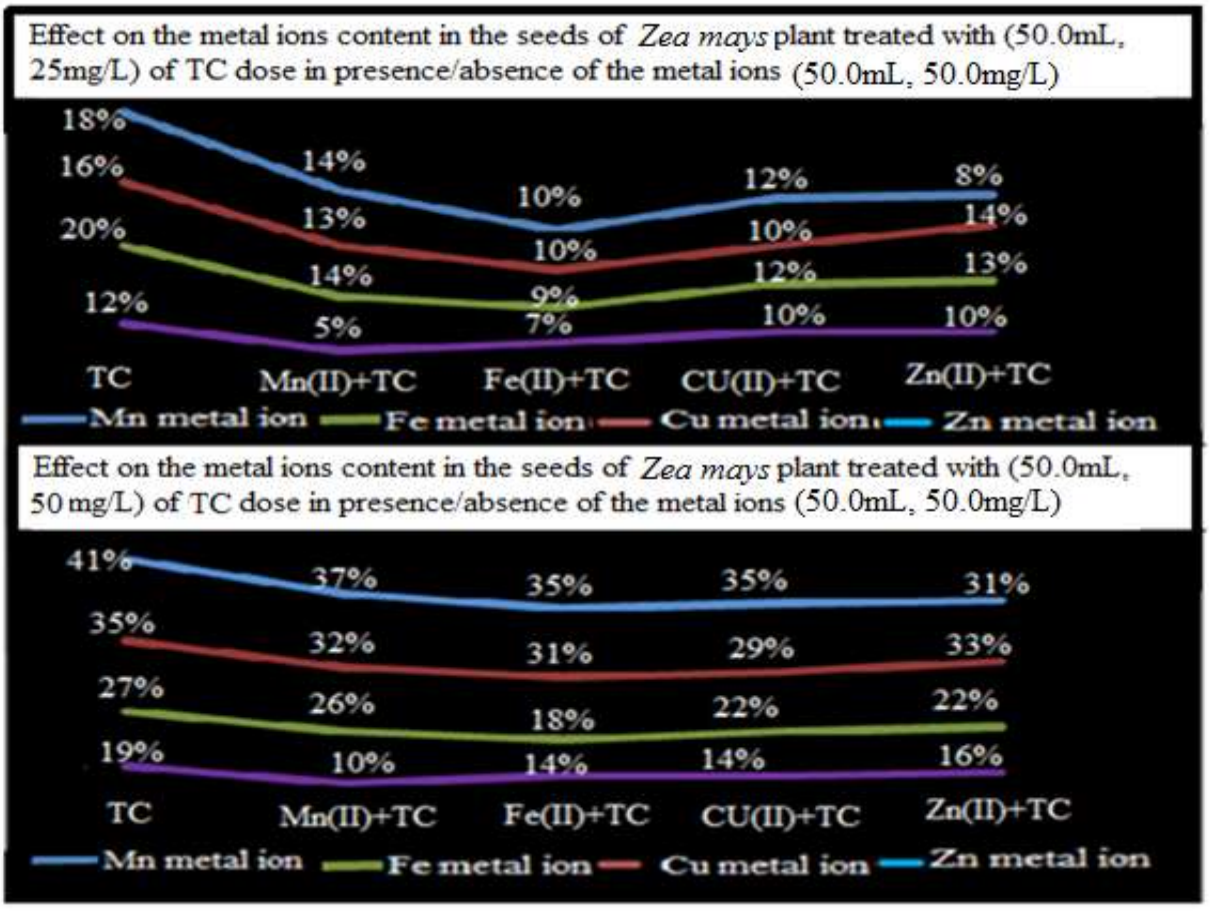


Figure 4.10: Effect of the low (50.0mL, 25.0mg/L) and high(50.0mL, 50.0mg/L) TC doses in presence/ absence of the (50.0mL, 50.0mg/L) of the metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) on the metal ions content of the seeds of the Zea mays plant.

The summed up effect of lower (50.0mL, 25.0mg/L) and higher (50.0mL, 50.0mg/L) dose of TC in absence/presence of the additional application of 50.0mL, 50.0mg/L metal ion (Mn(II), Fe(II), Cu(II) or Zn(II)) on the content of the *Zea mays* seed metal ions is represented in Figure 4.10 and complete data in tabular form is represented in Table 4.9.

Table 4.9:Mean value± standard deviation of the metal ion content with/ without additional supply of (50.0mL, 50.0mg/L) metal ion (Mn (II), Fe(II), Cu(II) and Zn(II)) on low (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of TC effected mature seed of the *Zea mays* (9108).

Sample	Dose (mg/L)	Mn	Fe	Cu	Zn
Control		**0.44±0.03**	**4.34±0.2**	**1.10±0.7**	**0.52±0.02**
TC	25.0	0.39±0.04	3.48±0.4	0.93±0.5	0.43±0.04
	50.0	0.36±0.03	3.20±0.3	0.72±0.3	0.31±0.02
Mn(II)+ TC	25.0	0.42±0.02	3.76±0.2	0.96±0.1	0.45±0.06
	50.0	0.40±0.04	3.22±0.3	0.75±0.6	0.33±0.04
Fe(II) + TC	25.0	0.41±0.05	3.96±0.2	0.99±0.4	0.47±0.04
	50.0	0.38±0.03	3.56±0.5	0.76±0.3	0.35±0.02
Cu(II) +TC	25.0	0.40±0.05	3.85±0.4	1.00±0.2	0.46±0.03
	50.0	0.38±0.04	3.41±0.4	0.79±0.3	0.34±0.03
Zn (II) +TC	25.0	0.40±0.03	3.80±0.2	0.95±0.3	0.48±0.03
	50.0	0.37±0.04	3.39±0.5	0.74±0.5	0.36±0.03

4.3.3.4. Effect of additional supply of metal ions on the metal ion content of the CZ effected seeds of *Zea mays* plant.

The CZ carbamate found to curb the metal ion content in the seeds, while on investigation it was found that, at low dose (50.0mL, 25.0mg/L) of the CZ decreased metal ions content as: 36% for Mn(II), 34% for Fe(II), 36% for Cu(II) and 28% for Zn(II) metal ions with respect to the control. On supplying the additional dose (50.0mL, 50.0mg/L) of Mn(II) metal ion, reduction in the percentage of metal ions was found limited as: 30% for Mn(II), 30% for Fe(II), 32% for Cu(II) and 25% for Zn(II) with respect to the control. When similar dose of

Fe(II) was supplied, drop in metal ion content was found as: 27% for Mn(II), 28% for Fe(II), 29% for Cu(II) and 21% for Zn(II) w.r.t. control. In a similar manner, on Cu(II) dose (50.0mL, 25.0mg/L), reduced percentage of metal ion content in seed was found as: 31% for Mn(II), 31% for Fe(II), 25% for Cu(II) and 25% for Zn(II) w.r.t. control. With the additional application (50.0mL, 50.0mg/L) of Zn(II), decreasein metal ion content was found as: 34% for Mn(II), 32% for Fe(II), 34% for Cu(II) and 17% for Zn(II) with respect to the control.

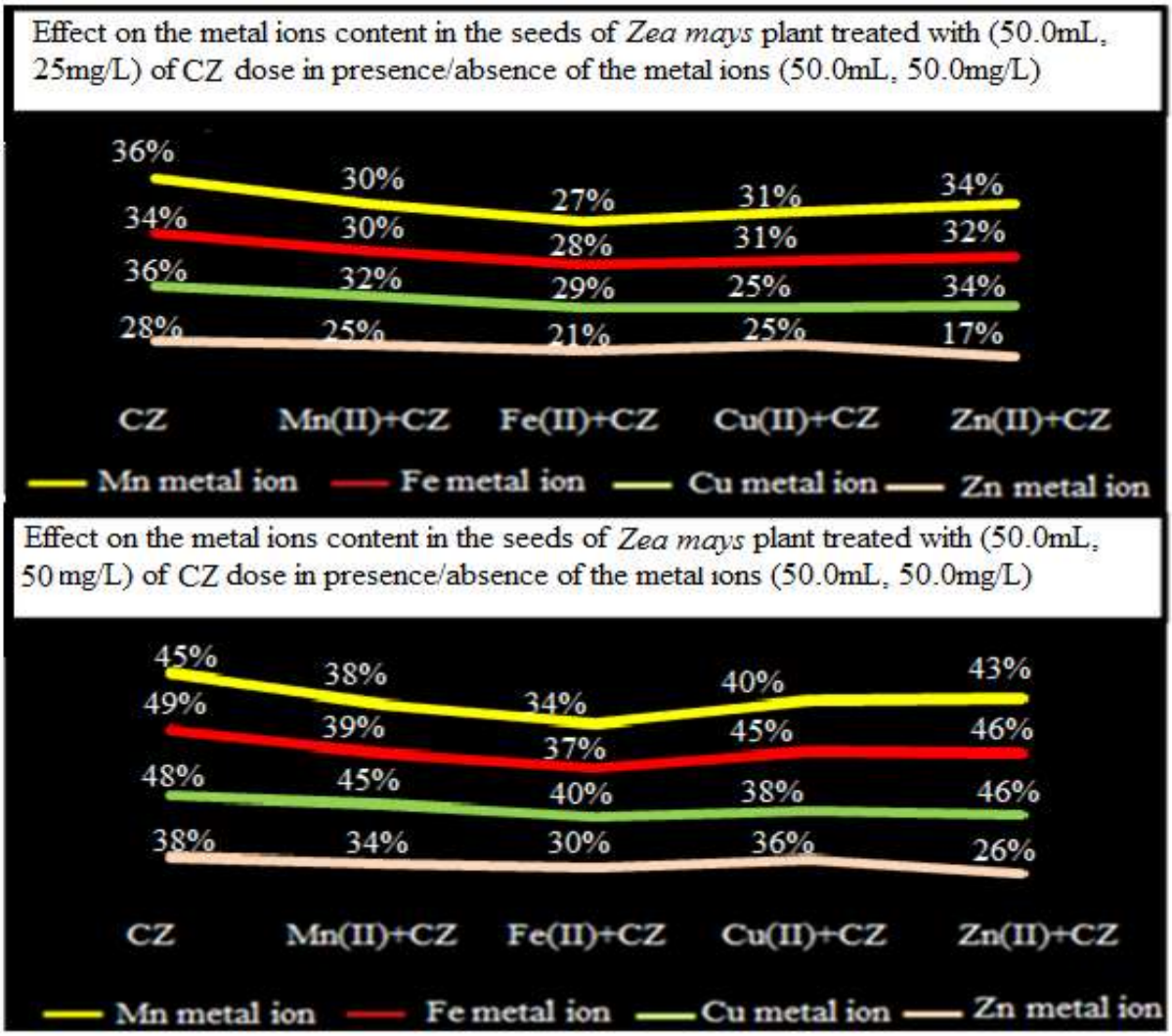


Figure 4.11: Effect of the low (50.0mL, 25.0mg/L) and high(50.0mL, 50.0mg/L) CZ doses in presence/ absence of the (50.0mL, 50.0mg/L) of the metal ions (Mn(II), Fe(II), Cu(II) and Zn(II)) on the metal ions content of the seeds of the *Zea mays* plant.

For the high dose (50.0mL, 50.0mg/L) of CZ, reduction in the metal ions content was observed as: 45% for Mn(II), 49% for Fe(II), 48% for Cu(II) and 38% for Zn(II) with respect to the control. On additional supply of 50.0mL, 50.0mg/L Mn(II), drop in metal ion content was found as: 38% for Mn(II), 39% for Fe(II), 45% for Cu(II) and 34% for Zn(II) w.r.t control. In a similar manner on additional application of Fe(II), reduction in metal ion content

was observed as: 34% for Mn(II), 37% for Fe(II), 30% for Cu(II) and 30% for Zn(II) w.r.t. control. When similar dose of Cu(II) was applied, drop in metal ion content was found as: 40% for Mn(II), 45% for Fe(II), 38% for Cu(II) and 36% for Zn(II) w.r.t. the control. At the dose of (50.0mL, 50.0mg/L) of Zn(II), decreased metal ion content was found as 43% for Mn(II), 46% for Fe(II), 46% for Cu(II) and 26% for Zn(II) w.r.t. the control.

Table 4.10:Mean ± standard deviation value of the metal ion content with/without additional supply of (50.0mL, 50.0mg/L) metal ion (Mn(II), Fe(II), Cu(II) and Zn(II)) on low (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of CZ effected mature seed of the *Zea mays* (9108).

Sample	Dose[#] (mg/L)	Mn	Fe	Cu	Zn
Control		**0.44±0.03**	**4.34±0.2**	**1.10±0.7**	**0.52±0.02**
CZ	25.0	0.28±0.06	2.85±0.3	0.70±0.4	0.37±0.05
	50.0	0.24±0.02	2.20±0.4	0.57±0.2	0.32±0.02
Mn(II)+ CZ	25.0	0.31±0.04	3.01±.0.3	0.74±0.4	0.39±0.03
	50.0	0.27±0.03	2.64±0.4	0.60±0.3	0.34±0.04
Fe(II) + CZ	25.0	0.32±0.03	3.10 ± 0.5	0.78±0.3	0.41±.0.02
	50.0	0.29±0.02	2.73±0.3	0.65±0.2	0.36±0.01
Cu(II) +CZ	25.0	0.30±0.04	2.98±0.3	0.82±0.4	0.39±0.03
	50.0	0.26±0.02	2.40±0.4	0.68±0.3	0.33±0.04
Zn (II) +CZ	25.0	0.29±0.02	2.93±0.3	0.72±0.6	0.43±0.02
	50.0	0.25±0.04	2.34±0.2	0.59±0.4	0.38±0.03

The collaborative effect of lower (50.0mL, 25.0mg/L) and higher (50.0mL, 50.0mg/L) dose of CZ in absence/presence of the additional application of 50.0mL, 50.0mg/L metal ion (Mn(II), Fe(II), Cu(II) or Zn(II)) on the content of the *Zea mays* seeds metal ions is represented in Figure 4.11 and complete data in tabular form is represented in Table 4.10.

4.3.3.5. Effect of additional metal ions on the metal ion content of the seeds of M affected *Zea mays* plant.

In case of methomyl, when the low dose of (50.0mL, 25.0mg/L) of the M was supplied, decreased metal ions content was found as: 45% for Mn(II), 49% for Fe(II), 43% for Cu(II) and 30% for Zn(II) metal ions. When the additional supply of (50.0mL, 50.0mg/L) of Mn(II) metal ion was provided, drop in the percentage of metal ions was found as: 36% for Mn(II), 43% for Fe(II), 33% for Cu(II) and 27% for Zn(II) with respect to the control. For the similar dose of Fe(II) metal ion, reduction in metal ion content was found as: 38% for Mn(II), 39% for Fe(II), 36% for Cu(II) and 25% for Zn(II). Similarly on additional application of Cu(II), decreased percentage of metal ion content in seed was found as: 40% for Mn(II), 40% for Fe(II), 31% for Cu(II) and 28% for Zn(II). In a similar manner for (50.0mL, 50.0mg/L) of Zn(II), decreased metal ion content by 43% for Mn(II), 46% for Fe(II), 39% for Cu(II) and 23% for Zn(II) with respect to the control.

At the high dose of (50.0mL, 50.0mg/L) of M, decreased metal ions content was observed as: 59% for Mn(II), 60% for Fe(II), 54% for Cu(II) and 48% for Zn(II) with respect to the control. On additional supply (50.0mL, 50.0mg/L) of Mn(II), drop in metal ion content was found as: 47% for Mn(II), 54% for Fe(II), 48% for Cu(II) and 44% for Zn(II) w.r.t control.

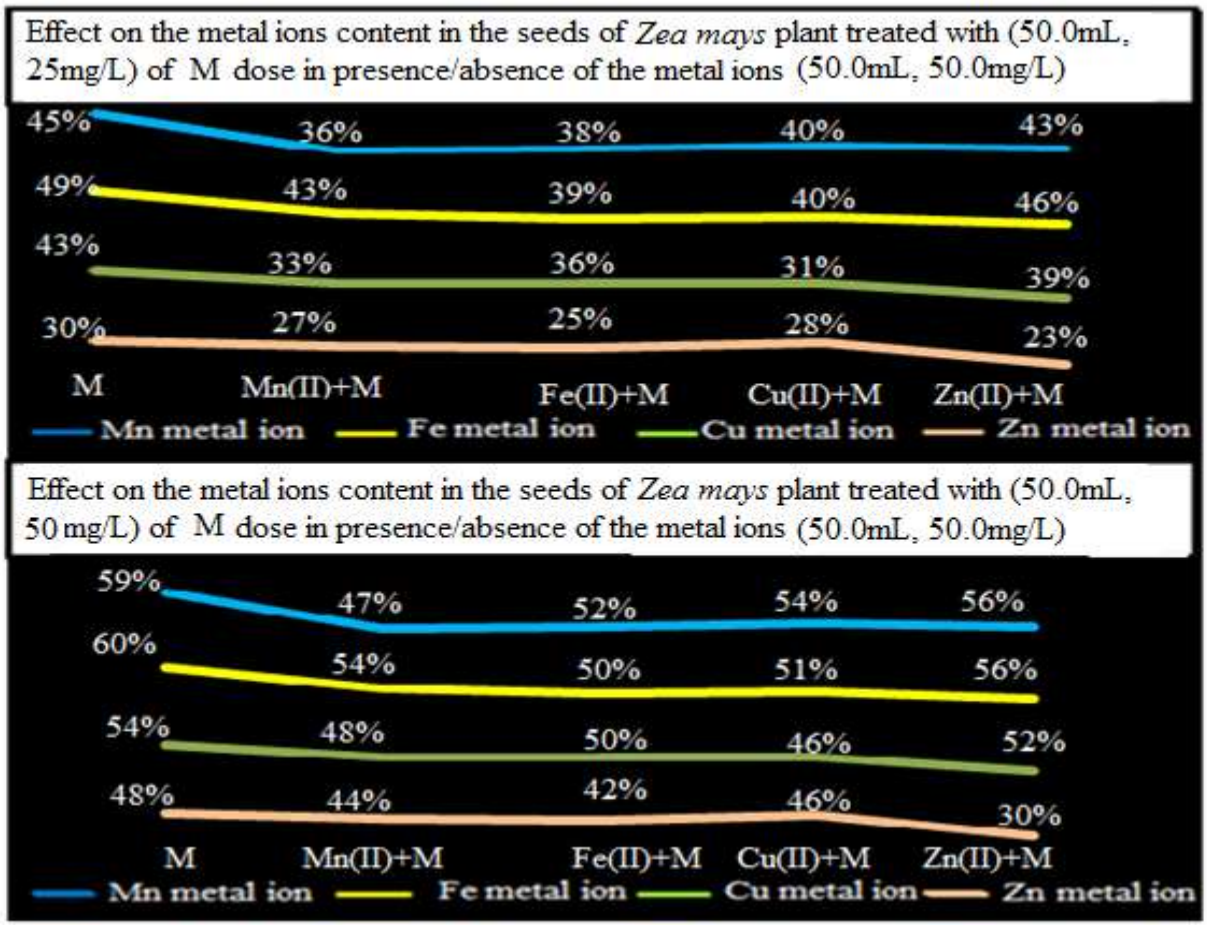


Figure 4.12: Effect of the low (50.0mL, 25.0mg/L) and high(50.0mL, 50.0mg/L) M doses in

presence/ absence of the (50.0mL, 50.0mg/L) of the metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) on the metal ions content of the seeds of the *Zea mays* plant.

On application of Fe(II), reduction in metal ion content was observed as: 52% for Mn(II), 50% for Fe(II), 50% for Cu(II) and 42% for Zn(II) w.r.t. control. In a similar manner, application of same amount ofCu(II), decreased metal ion content as: 54% for Mn(II), 51% for Fe(II), 46% for Cu(II) and 46% for Zn(II) w.r.t. the control. On additional application of Zn(II), reduction in metal ion content was found limited by 56% for Mn(II), 56% for Fe(II), 52% for Cu(II) and 30% for Zn(II) w.r.t. the control.

The combined effect of lower (50.0mL, 25.0mg/L) and higher (50.0mL, 50.0mg/L) dose of M in absence/presence of the additional application of (50.0mL, 50.0mg/L) metal ion (Mn(II), Fe(II), Cu (II) or Zn(II)) on the content of the *Zea mays* seed metal ions is represented in Figure 4.12 and complete data in tabular form is represented in Table 4.11.

Table 4.11: Mean ± standard deviation value of the metal ion content with/ without additional supply of (50.0mL, 50.0mg/L) metal ion (Mn (II), Fe(II), Cu(II) and Zn(II)) on low (50.0mL, 25.0mg/L) and high dose (50.0mL, 50.0mg/L) of M effected mature seed of the *Zea mays* (9108).

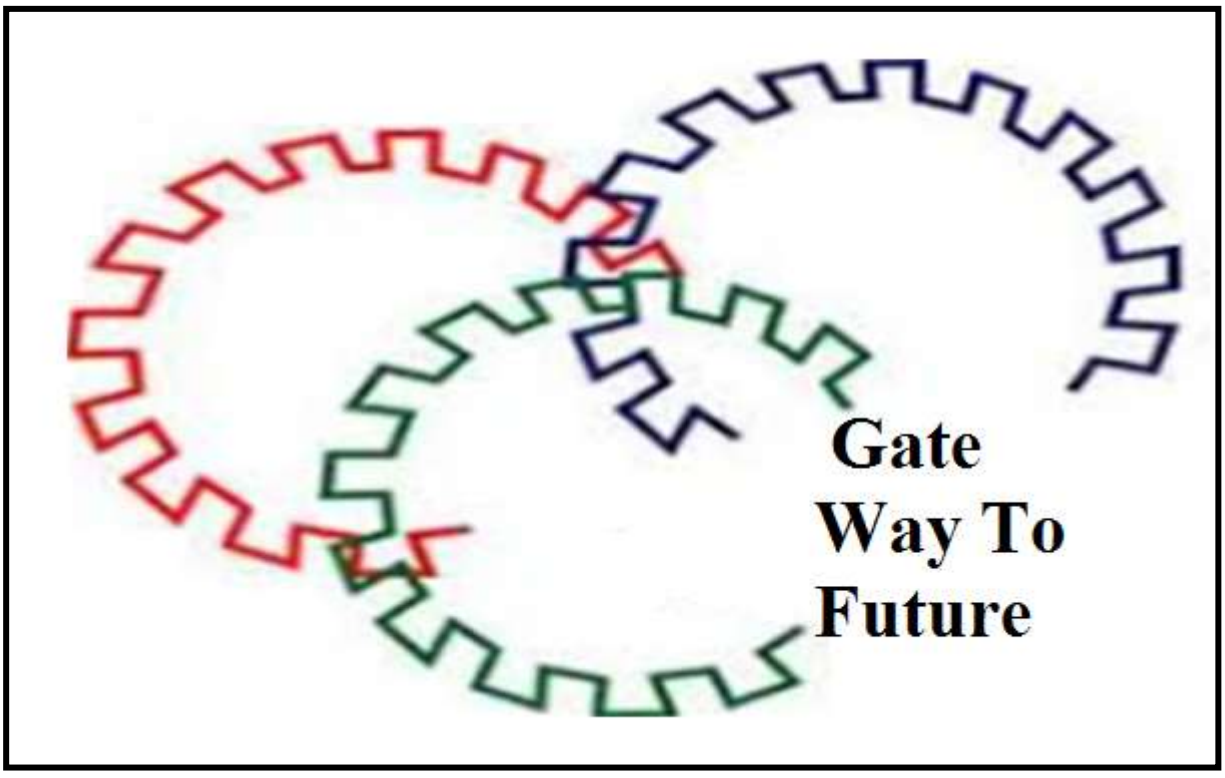
Sample	Dose (mg /L)	Mn	Fe	Cu	Zn
Control		**0.44±0.03**	**4.34±0.2**	**1.10±0.7**	**0.52±0.02**
M	25.0	0.24±0.04	2.21±0.3	0.63±0.4	0.36±0.03
	50.0	0.18±0.03	1.73±0.4	0.50±0.2	0.27±0.07
Mn(II)+ M	25.0	0.28±0.04	2.45±0.6	0.73±0.4	0.38±0.03
	50.0	0.23±0.02	1.96±0.3	0.57±0.6	0.29±0.02
Fe(II) + M	25.0	0.27±0.05	2.65±0.3	0.70±0.4	0.39±0.04
	50.0	0.21±0.04	2.17±0.4	0.55±0.3	0.30±0.02
Cu(II) +M	25.0	0.26±0.03	2.60±0.4	0.75±0.5	0.37±0.05
	50.0	0.20±0.06	2.09±0.2	0.59±0.4	0.28±0.03
Zn (II) +M	25.0	0.25±0.03	2.32±0.4	0.67±0.2	0.40±0.04
	50.0	0.19±0.05	1.89±0.3	0.52±0.6	0.36±0.03

Conclusion

From the experimentation, it was accomplished that the usage of the selective carbamate pesticide ((Carbofuran (CF), Thiophanate methyl (TM), Thiodicarb (TC), Carbendazim (CZ) , and Methomyl (M)) low (50.0mL, 25.0mg/L) and high (50.0mL, 50.0mg/L) doses has decreased the growth of the *Zea mays* plant by virtue of its seed weight, shoot weight, stem length and head length. After the addition of individual metal ion (Mn(II), Fe(II), CuII) and Zn(II)), increase in growth of these parameters of *Zeamays* plant were observed. Similar affect was observed when the metal ion content of the *Zea mays* plant was investigated. The rise in the growth and the metal ions content in the seeds of the Zea *mays* plant in presence of the doses of the metal ions (Mn(II), Fe(II), Cu (II) and Zn(II)) is directly related to the chelating ability of the carbamate pesticide with the metal ions. Therefore, unveiling the fact that interaction of the carbamate pesticides with the metal ions act as obstruction for the metal ions availability to the *Zea mays* plant.

Chapter-5

Conclusion & Significance



Carbamates contain carbonyl and amine group as active cite for interaction with acetylcholinesterase enzyme of the pest/ animal body. At the same time, oxygen and nitrogen act as a good ligating cite and may undergo complexation with metal ions. Bioavailability of essential metal ions are extremely important and mostly transported directly or indirectly to the food chain of animals by virtue of uptake by the plant from soil. In addition, for the survival of plant itself, essential trace metal ions are extremely important as are used from respiration to excretion of toxic elements from plant body. Keeping possibility of metal complexation by carbamate in mind, interaction of carbamates specifically CF/M/TC/TM/CZ with essential trace metal ions were checked. On essential trace metal ion adsorbed on silica surface, when carbamates on investigation was passed, found to form quick complexes (observed by color change), which leaches out on additional supply of solvent on it, because of probable formation of induced dipole- dipole interaction (between carbamate complex and silica) from ion-dipole interaction (between metal ion-silica). In a similar manner rate of interaction between carbamate and essential metal ion was also investigated in a liquid media (mostly methanol), where rate of interaction were found comparatively slower than on metal impregnated silica surface. The investigation of rate of interaction (between carbamate and metal ion), in liquid media has shown that the rate of interaction depends on hard-soft acid-base interaction. Also, it was observed that the rate of interaction increases with increase in pH and temperature that indicate metal-carbamate complex will quickly form in basic soil and in hot environment. Also, the formed complexes are insoluble in almost all known laboratory solvents. Most of the reported methods of recovery and residue analyses of pesticide depends on extraction of pesticides in the solvents where they are soluble in. So, after the report of metal- carbamate complexation, all the methods of recovery and residue analysis of pesticide from soil is in a big doubt.

As far as stability is concerned, the formed complexes are very- very stable and mostly do not decompose below 850°C. Although, stability of such complexes was found entirely dependent on interaction of hard and soft acid base. For example, thiophanate methyl form very stable complex with Cu(II) ion that do not completely decompose below 900°C, but form unstable complex with Fe(II). The complex formed after precipitation were found insoluble in almost all known laboratory solvents, unaffected by the pH of the medium and observed to be in nano particle size. Therefore the stable metal- carbamate complexes are expected to remain in the soil for the long duration in the form of nano- particles.

Plant growth promoting bacteria plays the role of the transporter of the metal ions, by secreting the siderophores, which bind with the metal ions of the soil and reaches plant. The highlighted fact is that, it is the same soil on which the pesticides are applied and exhibited the tendency to adversely influence growth of the plant growth promoting bacteria. Moreover, our observation clearly suggests that carbamates form quick and stable complex with the trace essential metal ions; thus, exhibited the possibility to compete with the siderophoresfor the metal ions. On our investigation of effect of selected carbamates(CF/M/TC/TM/CZ)on plant growth promoting bacteria (*Salmonella typhimurium, Azotobacter vinelandii*, *Pseudomonas fluorescence*, *Rhizobium leguminosarum* and *Bacillus brevis*), it was observed that the growth of PGPR and siderophore production were largely effected by use of carbamate(s).The harmful effect of investigated carbamate on the siderophores binding ability is found directly linked with their ability to form the quick and stable complex with the Fe(II) metal ion.

As, CF/M/TC/TM/CZ carbamates were found pernicious for both the metal ions and the PGPR, their effect on the *Zea mays* plantwas investigated. Astonishingly, it depicted that usage of the carbamates has adversely affected the different growth parameters of plant (seed weight, shoot weight, head length and stem length). Moreover, it presence hampered the metal ion content in seeds. The fact was authentified, by parallelly investigating the effect of the added metal ions on the *Zea mays* plants and the metal ions content of the seeds treated with the selected carbamates. Their results suggested that, metals doses were found successful in suppressing the effect of the carbamtes to the some extent and growth as well as metal ion content increased on adding the metal ions. Current study has proved that CF/M/TC/TM /CZ carbamates could strongly interact with the metal ions. In the real world, the outcome of such interaction is visible on the plant growth. As, they inhibits the plant growth by curbing essential metal ions uptake in four ways: (1) through direct interactions with metal ions, (2) through interactions with bounded metal ions of siderophores, (3) through the direct inhibition of PGPR strains, and (4) through direct/indirect inhibition of the plant growth.

Our research has input the maiden efforts, to aware the society regarding the effects of the pesticides on the metal ion which could make the soil less fertile. Moreover, it opens the new area of research in which, one of the parameter to evaluate the pesticides should be, to check their interaction with the metal ions. As interactions have been checked on the soil level the possibility of such metal-pesticides complex inside the plant and its consequences on the plant health is still unknown. In a similar manner, its effects on the soil micro flora and physical as well as chemical properties of the soil are needed to be addressed. One of the

underlined point, extracted out from the research is that for sustainable agriculture, more emphasis should be put on delivering the quality of food rather than of the quantity. As, it is well quoted by eminent personality that, “Our lives are well connected. How healthy the plants and animals are today determines how healthy we will be tomorrow”.

Chapter-6

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1. Sukhmanpreet Kaur , Vijay Kumar , Mohit Chawla, Luigi Cavallo, Albert Poater and Niraj Upadhyay, "Pesticides Curbing Soil Fertility :Effect of complexation of Free Metal Ions".*Front.Chem*, **2017**, 5:43, 1-10.

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TITLE OF PAPER UNDER REVIEW

1." Simultaneous determination of carbamate pesticides residue in food grain using U.V spectrophotometer." is under process.

2."Chemical and biological interaction of catecholate sidrophores with carbamate and organophospahte pesticides." under process.