

**Efficacy of Transition Metal Complexes of Some  
Polydentate Ligand Systems As Potential Plant  
Nutrient Supplements: A Morphological And  
Biochemical Study**

*A Thesis submitted to the*  
**UNIVERSITY OF NORTH BENGAL**

*For the Award of*  
**DOCTOR OF PHILOSOPHY**  
in  
**CHEMISTRY**

By  
**KAUSHIK ACHARJEE**

*(M.Sc. in Chemistry)*

Supervised by

**Prof. BISWAJIT SINHA**

**DEPARTMENT OF CHEMISTRY**

**UNIVERSITY OF NORTH BENGAL**

Co- Supervised by

**Dr. PRAHLAD DEB**

**DIVISION OF HORTICULTURE**

**VISVA BHARATI UNIVERSITY**

July, 2021

*Dedicated*  
*to*  
*My Beloved Parents*

## **DECLARATION**

I declare that the thesis entitled **“Efficacy of Transition Metal Complexes of Some Polydentate Ligand Systems As Potential Plant Nutrient Supplements: A Morphological And Biochemical Study”** has been prepared by me under the supervision of Prof. Biswajit Sinha, Professor, Department of Chemistry, University of North Bengal and Dr. Prahlad Deb, Assistant Professor, Department of Horticulture, Visva Bharati University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

*Kaushik Acharjee*

**KAUSHIK ACHARJEE**

Department of Chemistry  
University of North Bengal  
Darjeeling-734013  
West Bengal, India

DATE: *07/07/2021*

# UNIVERSITY OF NORTH BENGAL

Prof. Biswajit Sinha, Ph. D  
Professor  
Department of Chemistry  
E-mail: biswachem@nbu.ac.in  
biswachem@gmail.com  
M: 9932738973, 9641967211



‘समानो मन्त्र समिति समानी’  
Accredited by NAAC with Grade A

Ph: +91-353-2776381  
University of North Bengal  
Darjeeling 734 013  
INDIA  
July 7, 2021

## CERTIFICATE

I declare that **Mr. KAUSHIK ACHARJEE** has prepared the thesis entitled “**Efficacy of Transition Metal Complexes of Some Polydentate Ligand Systems As Potential Plant Nutrient Supplements: A Morphological And Biochemical Study**” for the award of Ph. D degree on University of North Bengal under joint supervision of me and Dr. Prahlad Deb of Department of Horticulture, Visva Bharati University. He has carried out most of the works at the Department of Chemistry, University of North Bengal.

*B. Sinha*  
07-07-2021

*Prof. Biswajit Sinha*  
Prof. Biswajit Sinha  
Department of Chemistry  
University of North Bengal  
Department of Chemistry  
University of North Bengal  
Darjeeling-734013  
West Bengal, India

**DEPARTMENT OF HORTICULTURE &  
POSTHARVEST TECHNOLOGY**

**PALLI SIKSHA BHAVANA**  
(INSTITUTE OF AGRICULTURE)

**FOUNDED BY:**  
RABINDRANATH TAGORE

**ACHARYA:**  
SRI NARENDRA MODI

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POST: SRINIKETAN  
DIST: BIRBHUM, WB  
PIN: 731 236  
STD: 03463  
☎: 264248/787  
FAX : 91(03463)  
PSB 264118/264771

From:

**Dr. Prahlad Deb**

Assistant Professor

**CERTIFICATE**

I declare that **Mr. KAUSHIK ACHARJEE** has prepared the thesis entitled “**Efficacy of Transition Metal Complexes of Some Polydentate Ligand Systems as Potential Plant Nutrient Supplements: A Morphological and Biochemical Study**” for the award of Ph. D degree on University of North Bengal under joint supervision of me and Prof. Biswajit Sinha of Department of Chemistry, University of North Bengal. He has carried out some of the works at Department of Horticulture & Postharvest Technology, Institute of Agriculture, Visva Bharati.

*Prahlad Deb*

(Prahlad Deb)

**Dr. Prahlad Deb**  
Assistant Professor  
Dept. of Horticulture & Post-Harvest Technology  
Palli-Siksha Bhavana(Institute of Agriculture)  
Visva-Bharati, Sriniketan-731236  
West Bengal, India

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(Signature of the candidate)

A handwritten signature in blue ink, appearing to read "Biswajit Sinha".

*Prof. Biswajit Sinha  
Department of Chemistry  
University of North Bengal*

(Signature of the Supervisor)

## **ABSTRACT**

The study the efficacy of polydentate transition metal complexes of essential trace elements over conventional micronutrient supplements is an emergent field of research for solving micronutrient deficiency in the plants. The vast interest in this field has stemmed from the fascinating features (like less toxicity, less reactivity and environment friendly nature, *etc.*) of these polydentate transition metal complexes. In the present dissertation three first row transition elements, *viz.*, Zn, Cu and V (which are micronutrients for plants) have been chosen for complexation with some polydentate ligands and their effects on various morphological and biochemical parameters are thoroughly studied with four plant subjects, *viz.*, papaya, chickpea, rice and mung bean.

Chapter I is an introductory one that describes micronutrients, their role in plants body, reasons of micronutrient deficiencies, problems of conventional micronutrient supplements and the methods to solve the micronutrient deficiencies in an alternative environment friendly pathway using transition metal complexes of some polydentate Schiff base ligands as potent micronutrient supplements.

Chapter II details the chemicals and materials used in this research work. A brief description of various physico-chemical and spectroscopic techniques, *viz.*, elemental analysis, FTIR spectroscopy, UV-Visible spectroscopy, AAS, specific conductance *etc.*, used for the physico-chemical characterization of the synthesized complexes and various scientific protocols for different biochemical analyses have also been given.

In chapter III, morphological and biochemical effects of four N<sub>2</sub>O<sub>2</sub> donor Schiff bases and their Zn<sup>2+</sup> complexes on *Carica papaya* L. and *Cicer arietinum* L. are presented. Four different Schiff base ligands and their Zn<sup>2+</sup> complexes are first synthesized and then characterized by different analytical and spectroscopic techniques. To investigate their effects papaya and local chickpea seeds are treated with each ligands and their Zn<sup>2+</sup> complexes and different morphological and biochemical parameters are monitored. Among all the ligands (L1-L4) and their complexes (C1-C4) it is found that complex (C4) shows the maximum efficacy when treated as a micronutrient supplement for *Carica papaya* L. and *Cicer arietinum* L.

In Chapter IV, morphological and biochemical effects of two azo functionalized N<sub>2</sub>O<sub>2</sub> donor Schiff bases and their Cu<sup>2+</sup> complexes on rice plants

(*Oryza sativa* L.) are presented. Two different ligands and their copper complexes were synthesized and then characterized by both spectroscopic and elemental analyses. Investigations were done by taking rice seeds as plant material. Various growth and biochemical parameters were monitored by taking different concentrations of  $\text{CuSO}_4$ , the prepared ligands (L5 and L6) and their Cu(II) complexes (C5 and C6). Analysis of various biochemical results revealed that the Schiff base Cu(II) complexes (C5 and C6) have less toxic effects than copper sulfate on rice seedlings and thus facilitates better tolerance to copper toxicity than copper sulfate.

In Chapter V, efficacy of a  $\text{N}_2\text{O}_2$  donor Schiff base (L7) and its vanadyl complex (C7) on various morphological and biochemical parameters of mung bean (*Vigna radiata* L.) are presented. The Schiff base polydentate ligand is synthesized by the condensation of benzidine and benzil in ethanolic medium. The formed ligand was condensed further with  $\text{VO}_2\text{SO}_4 \cdot x\text{H}_2\text{O}$  to get the corresponding vanadyl complex. Both the ligand and the complex were characterized by spectroscopic and elemental analyses. Mung bean was selected as a plant material. Various morphological and biochemical parameters, *e.g.*, leaf senescence assay, chlorophyll content, different reactive oxygen species (ROS) were estimated and were compared to those with ammonium vanadate. Outcomes of the experiments revealed that the Schiff base complex has less toxic effects than ammonium vanadate on mung bean seedlings and provide better tolerance to vanadium toxicity. Though different stress marker and reactive oxygen species accumulation were less and minimum pigment damage was noticed in the Schiff base complex (C7) treated seedlings but the optimum positive impact largely depends on the dose. Beyond certain concentration the complex may show inhibitory effects on the plants. Therefore the present study revealed that heavy metal Schiff base complexes can be used as potential supplement to meet up micronutrient deficiency.

Finally chapter VII contains the concluding remarks of the research works embodied in this thesis.

## **PREFACE**

I started the research work presented in this thesis entitled **“Efficacy of Transition Metal Complexes of Some Polydentate Ligand Systems As Potential Plant Nutrient Supplements: A Morphological And Biochemical Study”** in the year 2014 under the supervision of Prof. Biswajit Sinha and the co-supervision of Dr. Prahlad Deb in the Department of Chemistry, University of North Bengal, India.

Deficiency of micronutrients in soil is a global problem. It is always challenging to solve the micronutrient deficiency by adding supplements without hampering the soil nature and the environment. Conventional micronutrient supplements, being mostly ionic in form, in most of the cases are responsible for the alteration of the pH of the medium. Such challenges always bring the opportunities to develop unique alternative methods having synergistic behavior. The synergistic behavior leads to improve performance of materials and substantially make the environment sustainable.

Due to the versatility in the donor sites, Schiff bases can form complexes with almost all the transition metal ions. Although they were very well known but their applications in various fields of chemistry make them interesting till today. Complexes of transition metal ions with Schiff bases provide a large and growing class of compounds with both stereochemical and magnetochemical interests. They can act as synthetic models of metal containing sites for various metalloproteins and metalloenzymes. Unsymmetrical Schiff base ligands have many advantages over their symmetrical counterparts in the elucidation of the composition and the geometry of the metal binding sites in the metalloprotein and the metalloenzymes and the selectivity of the natural systems with synthetic materials. Schiff base transition metal complexes are often superior to conventional micronutrient supplements in various concentration ranges. The procedures of preparations of these compounds are very simple and thus can be synthesized in large scale. Therefore heavy metal Schiff base complexes can be used as supplements to meet up micronutrient deficiencies and at the same time they can minimize the toxicity generated by application of different heavy metals. However these aspects must be further enquired at multi-locations and varied environmental conditions to draw a direct conclusion about the superiority.

The present dissertation is focused on the synthesis and physico-chemical characterization of some polydentate Schiff base metal complexes of Zn, Cu and V and their application as potent micronutrient supplier in some plants.

## Acknowledgement

I take this opportunity to articulate my heartfelt gratitude and deepest sense of indebtedness to my supervisors, **Prof. Biswajit Sinha**, Professor, Department of Chemistry, University of North Bengal and **Dr. Prahlad Deb**, Assistant Professor, Horticulture and Post Harvest Technology, Visva-Bharati University for their valuable advice, constant encouragement and insightful guidance through this path of my research with great endurance. I convey my sincere gratitude to **Prof. Usha Chackraborty**, Professor (Retired), Department Of Botany, University of North Bengal for her timely help and advice regarding the various biochemical studies.

I am highly obliged to **Prof. A. Misra**, HEAD, Department of Chemistry, N.B.U, **Prof. P. Ghosh**, Professor, Department of Chemistry, N.B.U, **Prof. M. N. Roy**, former Coordinator, SAP-DRS-III, N.B.U. and vice chancellor, Alipurduar University for their kind support and valuable suggestions. I am also thankful to the other faculty members and the non-teaching staffs of the Department of Chemistry, N.B.U and Horticulture and Post Harvest Technology, Institute of Agriculture, Visva-Bharati University for their cordial support during my research period. I am highly obliged to the Departmental Special Assistance Scheme under the University Grants Commission, New Delhi, India (SAP-DRS-III, NO.540/12/DRS/2013) and UGC-BSR scheme for providing instrumental and financial assistance. I must convey my special thanks to **Miss. Jayanwita Sarkar**, SRF, Department of Botany, for her continuous support in accomplishing various biological analyses. I am also thankful to my senior and junior lab mates for their valuable suggestions and cooperation.

I also express my heartfelt gratitude to my parents (**Mr. Khagesh Acharjee and Lt. Jaya Acharjee**), my wife (**Satarupa De**) and my 'Dada' (**Surajit Konar**) for their love, affection and continuous support during this period. Above all I pay my heartfelt gratitude to Almighty God who guided and provided me the strength, patience and required courage to complete this work after all the challenges and difficulties. Without his blessings, all my endeavours would not turn into achievements.

**Mr. Kaushik Acharjee**

**Research Scholar**

**Department of Chemistry**

**University of North Bengal**

**Darjeeling -734013, INDIA**

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# **CHAPTER I**

## **1. Introduction**

Of the ninety two naturally occurring elements nature has preferred to engage only twenty seven elements in order to sustain life. These chosen elements are important for development of body and hence are termed as essential elements. Of the 27 essential elements 15 are metals. Among these metals the transition metals like Cu, Zn, Fe, Ni, Mn, Mo, V, Cr are very important for plants. Since they are required in very trace amount, they are called micronutrients. The micronutrient deficiency and micronutrient toxicity in plants are of major concern for both the chemists and biologists. Micronutrients play pivotal role in cellular metabolism.<sup>1-3</sup> Some biological processes such as protein and carbohydrate metabolism, anti-oxidative defense and various enzyme activities are largely dependent upon micronutrients.<sup>4</sup> They serve as structural and catalytic components of several proteins and enzymes which are required for growth and development. In 1972, Emanuel Epstein defined micronutrients as:<sup>5</sup>

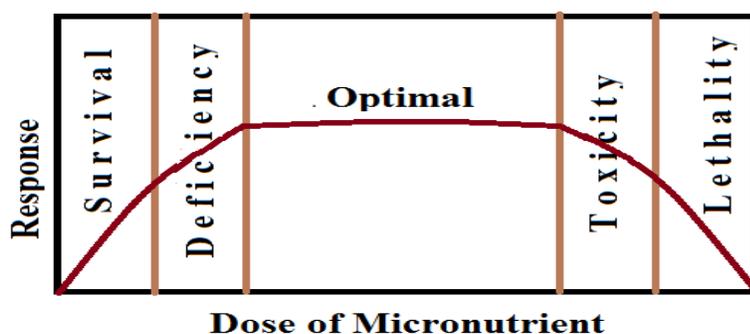
“It is an element, absolutely required for the maintenance of life, its absence results several malfunction of body and ultimately resulting death.”

Several additional criteria regarding micronutrients are as follows:

- a) A physiological deficiency must appear when the element is removed from the diet.
- b) The deficiency can be relieved by the addition of specific element.
- c) A specific biochemical function is associated with a particular element.
- d) The effect of essential element cannot be fully replaced by any other element.
- e) The element should have direct influence on the organism and be involved in the metabolism.

## **2. The Dose-Response Curve of essential micronutrients:**

The ‘Dose-Response’ curve shows a correlation between the ‘Dose’, *i.e.*, the concentration of essential element and its effects on living system termed as the ‘Response’ of that particular living system. Every micronutrient has an optimal concentration for a particular living system. Above that optimal concentration the system will become toxic even death can happen and below that optimal range there will be some deficiency symptoms. For example, Fluorine is an essential element and its daily intake of 0.5 mg will enable human body to survive normally. But an intake



**Fig 1.1.** Dose-Response curve for a particular micronutrient.

of 2 mg/day of Fluorine will show some deficiency symptoms. However, if the intake is raised up to 10 mg/day, then it will be optimum for performing normal biochemical functions. Further increase of its concentration will make the body toxic and if the dose is raised to 100 mg/day death may occur.<sup>6</sup>

### **2.1. Role of Selected Micronutrients**

In this dissertation three essential transition metals, *viz.*, Zn, Cu and V are selected for the study. These three metals are chosen due to their wide and diverse role in plant's body. All the three metals serve as structural and catalytic component of several proteins and enzymes required for the growth and development.

#### **2.1.1. Role of Zinc**

Zinc being one of the most important micronutrient in plants, acts as cofactors of enzymes like dehydrogenases, anhydrases, peroxidases and oxidases.<sup>7-9</sup> Several biochemical pathways such as anti-oxidative defense and various enzyme activities are largely dependent upon Zn. Zinc has a major role to play in carbohydrate and protein metabolism process.<sup>10,11</sup> It is found that zinc can increase the biosynthesis of chlorophyll and carotenoid-these two are associated in photosynthetic pathways.<sup>12</sup> But in reality it is found that 51% of world's soil is deficient of Zinc.<sup>13</sup> So Zn deficiency is rather a global threat in agricultural field, since this deficiency can reduce the crop yields and crop quality. Some of the symptoms of zinc deficiency in plants are listed below:

- a) Stunting and reduced height of plants
- b) Intervenal chlorosis
- c) Brown spot on upper leaves
- d) Distorted leaves.



**Fig 1.2.** Zinc deficiency in maize plants (foreground) with healthier maize plants with no zinc deficiency (background). This figure has been adapted from Ref. No 14.

### **2.1.2. Role of Copper**

Copper is one of the important redox active transition metal for plants. It mainly acts as a structural component of electron transfer proteins and enzymes like plastocyanin, cytochrome oxidase, *etc.*<sup>15</sup> It plays important role in photosynthetic pathways, respiration and protein metabolism.<sup>16</sup> In plants, copper is necessary for performing the normal role of cytosol, endoplasmic reticulum, chloroplast stroma, thylakoid lumen, *etc.* Although it is required in very trace amount, its deficiency can reduce the nitrogen fixation, thereby lowering the protein levels. Some of the symptoms of copper deficiency in plants are as follows:

- a) Dieback of stems and twigs
- b) Yellowing of leaves
- c) Stunted growth of plants
- d) Pale green leaves that can be wither easily.

### **2.1.3. Role of Vanadium**

Vanadium is one of the important trace elements for plants. Although it is toxic to terrestrial plants but it is very influential for plant growth and development if applied in trace level.<sup>18</sup> Vanadium can initiate the rate of photosynthesis. It has large effect on the synthesis of chlorophyll, carotene and it participates in nitrogen metabolism.<sup>19</sup> It also triggers the germination stages.



**Fig 1.3.** Copper deficient rose leaves in right (smaller size, chlorosis) compared to normal leaves. This figure has been adapted from Ref. No 17.

Some of the symptoms of vanadium deficiency in plants are as follows:

- a) Growth reduction in plants.
- b) Changed color of the leaves, particularly from the leaf margin towards the midrib.
- c) Intervential chlorosis.



**Fig1.4.** Intervential chlorosis in tomato leaf due to vanadium and magnesium deficiency. This figure has been adapted from Ref. No 20.

### **2.2. Micronutrient deficiency: a global problem**

Deficiency of micronutrients in soil is a global problem. A study in India, with 14683 soil samples collected from all over the country, reveals 49% of the soil samples are deficient of Zn, 12% in Fe and 3% in Cu.<sup>21</sup> No doubt that the green revolution helped the production of the crops in last few decades but this also depletes the micronutrients pool in drastic level. Farmers have a tendency to apply micronutrients only when the deficiency symptoms are observed but not preparing the soils first. Moreover some common farming practice is also responsible for the degradation of micronutrient pool. To solve the micronutrient deficiency the most conventional and straight forward way is to apply metal salts to the soil. Most of the metal contained fertilizers are inorganic in origin. Although these metal contained fertilizers work well to solve the problem but they lead to certain changes in soil that ultimately make the soil infertile after few decades.<sup>22</sup> The repeated use of inorganic fertilizers may lead to following problems:

#### **2.2.1. Increase of salt concentration in soil**

Excessive use of metal salts of inorganic origin may increase the salt concentration in soil and such a situation is very harmful for the crops. This compels plants to spend more energy to drag water from soil and as a result their growth becomes ceased and their morphology gets changed. This effect is significant for plants which are sensitive in high salinity soils.

#### **2.2.2. Chance of contamination**

Inorganic salts being ionic in nature are generally water soluble. So after rainfall the salts can be washed away from soils and can be mixed with the rivers and ponds, *etc.*, and thus the situation will make the water toxic and can be harmful for aquatic systems.

#### **2.2.3. Toxicity for plants**

Micronutrients are required by plants in very trace level. Since inorganic fertilizers are ionic they are highly reactive. So if they are slightly overdosed then plants will undergo stress and the body will become very toxic. This happens frequently for the inorganic supplements and thus some common symptoms like defoliation, wilting or decreased growth are observed in plants.

#### **2.2.4. Leaching**

Leaching is observed for the inorganic fertilizers when used in sandy soils. The inorganic metal salts can be leached away from the plant's root. If this happens then the applied salt will no longer be available for plants.

#### **2.2.5. Change of soil pH and free electron activity**

Most of the metal fertilizers contain oxide or sulphate or nitrate as counter anions. For these types of fertilizers the metal ion is incorporated in plants body but the counter anions remain in soil and ultimately converts to corresponding acid, which will alter the pH of the medium. Moreover they can hamper the free electron activity of the soil.<sup>23</sup>

### **2.3. Solving micronutrient deficiency: A modern approach**

To solve the micronutrient deficiency an alternative is the use of metal chelates instead of inorganic fertilizers. Metal chelates have the benefit that unlike the inorganic fertilizers they are less reactive and thereby less toxic.<sup>24</sup> At the early stages the most common chelators that were used extensively were ethylenediamine tetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA).<sup>25</sup> EDTA type of chelates due to their multiple binding sites (Hexadentate) can bind with almost all the transition metals and their metal complexes are very stable. That's why EDTA is used in the complexometric titrations in analytical chemistry. It is found that most of the EDTA containing metal chelates are soluble in water. These water soluble metal chelates can be absorbed in root pours and can be transmitted to cells.<sup>26</sup> They have the advantage that they are not responsible for drastic pH change of the medium. But recent study reveals that although they are useful to minimize the micronutrient deficiency but chelators like EDTA, DTPA have certain disadvantages which cannot be overlooked. The disadvantages are as follows:

- a) Since EDTA type of ligands can bind with almost all the transition metals, so after the incorporation of the desired metal to plants body, the free ligand can bind with the other metals present in system. This will lower the availability of the other metals in plants.<sup>27</sup>
- b) Another disadvantage of these metal chelates is that they undergo photo degradation in the medium in presence of UV radiation. The photo degradation leads to some undesirable products like glyoxylic acid, formaldehyde, *etc.*, that are harmful for plant growth and development.<sup>28</sup>

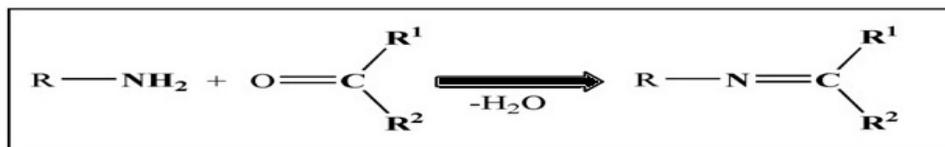
Besides the EDTA type of ligands literature study reveals the use of amino acids as a ligand. Ghasemi *et al.*<sup>29</sup> in their work made Fe- amino acid complexes and found their positive effect over Fe-EDTA when applied to the tomato seedlings. Same types of observations were found for Zn-amino acid complexes when they were treated in lettuce plant. But the problem of using amino acid type of ligands is that since they are water soluble they can be leached from the soil if remain unused and can contaminate other systems.

### 2.4. Tusk specific polydentate ligands: an alternative

From the above discussions it is clear that in order to act as a potential plant micronutrient supplier, the chelates must be selective as well specific. Otherwise they will solve one specific micronutrient deficiency but can lead deficiencies for other micronutrients. Another important factor is the solubility of the metal chelates. Solubility will be such that the leaching should be minimal. In view of these special type of tailor made polydentate ligands like Schiff bases have drawn the attentions of researchers recently. Schiff base is the condensation product of an amine and aldehyde. It acts as an important class of ligands since it can bind the metal ion through the azomethine (-C=N) group.<sup>30,31</sup> Due to easy preparative way and vast applications in different fields they are often be called as 'privileged ligands'. Schiff base metal complexes show catalytic properties in different homogeneous and heterogeneous reactions.<sup>32</sup> Just altering the functional groups, Schiff base and their derivatives may become at the same time 'specific and selective' to perform number of task specific applications.

#### 2.4.1. Schiff base chemistry

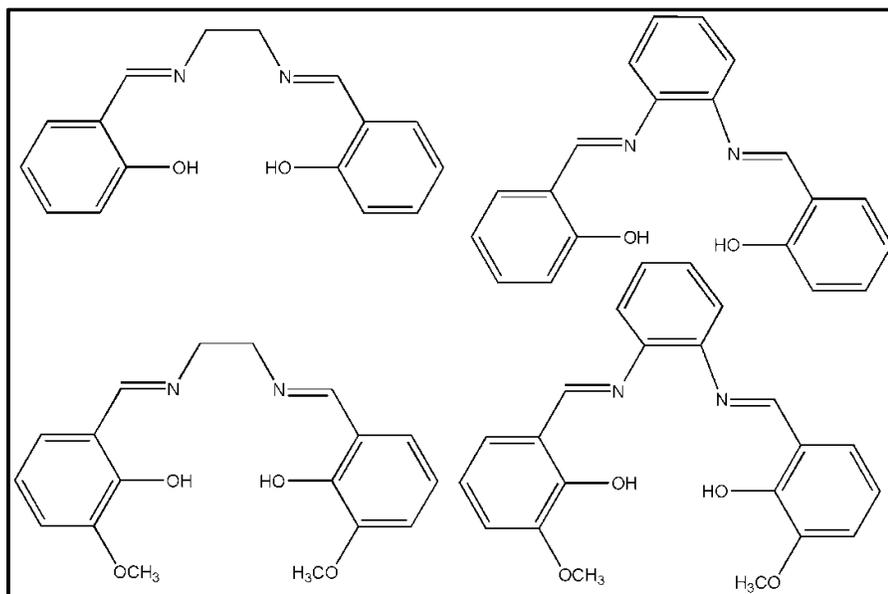
Schiff bases containing azomethine group (-HC=N-) have now been proved to be privileged compounds and such compounds were first prepared by Hugo Schiff in 1864. The general synthetic route of formation of Schiff base is as follows:



**Fig 1.5.** Condensation of amine and aldehyde to form Schiff base. Here R<sup>1</sup> and R<sup>2</sup> are different substituents like alkyl, aryl, cycloalkyl and heterocyclic groups, *etc.*

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Investigation suggests that not only aldehydes but also ketones can form Schiff bases, although the rate of formation is somewhat lower than those with the aldehydes. Literature suggests that Schiff base derived from aromatic aldehydes are supposed to be more stable than aliphatic aldehydes.<sup>33</sup> If one or more donor atom is present near to the azomethine group the chelating power of such ligands increases very much. Depending on the donor sites Schiff bases may act as bi-, tri-, tetra- or polydentate ligands. Schiff base derived from salicylaldehyde and 1, 2-diamines such as ethylene diamine, phenylene diamine results tetradentate ligand system, commonly known as “Salen” or “Salophen” are highly potential ligand systems in coordination chemistry.

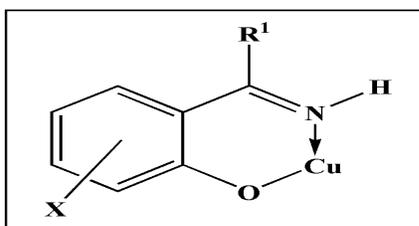


**Fig 1.6.** Examples of some Schiff bases derived from the condensation of different aromatic aldehydes and diamines.

These types of  $N_2O_2$  ligands (Fig 1.6) show excellent chelating effects. This is due to the fact that the phenolate oxygen is a hard donor which can stabilize the higher oxidation state of the metal, whereas nitrogen being the borderline donor can stabilize the lower oxidation state of the metal.<sup>34</sup> Another key factor of these types of complexes is that they can undergo keto-enol tautomerism. Not only that by taking suitable formyl- or keto- and primary amines a large number of Schiff base macrocycles can be synthesized. These macrocycles have vast applications in supramolecular chemistry.<sup>35,36</sup>

**2.4.2. Schiff base transition metal complexes**

Due to the versatility in the donor sites Schiff bases can form complexes with almost all the transition metal ions. Although they were very well known but their application in various fields of chemistry made them interesting till now.<sup>37,38</sup> Organometallic compounds of Schiff bases have played a vital role in chemistry, mainly due to their ease of preparation, structural variability and widespread applications such as catalyst, functional materials and antibacterial reagents, *etc.*<sup>39-41</sup> The first synthesized Schiff base complex was dark green bis(salicyldiamino)Cu(II). This was prepared by the condensation of cupric acetate, salicylaldehyde and aqueous ammonia.<sup>42</sup>



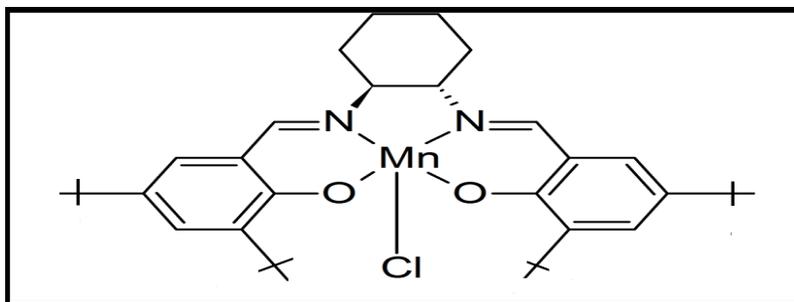
**Fig 1.7.** Structure of bis(salicyldiamino)Cu(II)

Although synthesized very long ago, the Schiff base metal complexes have not lost their significance because of the following factors:

- a) They can stabilize various oxidation states of metal ions by altering the hardness and softness of the binding sites.
- b) They can enhance the stability and solubility of the homogeneous and heterogeneous catalysts by altering the substituents.<sup>43</sup>
- c) They can act as synthetic models of metal containing sites for various metalloproteins and metalloenzymes.<sup>44-45</sup>
- d) Complexes of transition metal ions with Schiff bases provide a large and growing class of compounds with both stereochemical and magnetochemical interest.
- e) Unsymmetrical Schiff base ligands have exhibited many advantages over their symmetrical counterparts in the elucidation of the composition and the geometry of the metal binding sites in the metalloprotein and the metalloenzymes and the selectivity of the natural systems with synthetic materials.<sup>46</sup>

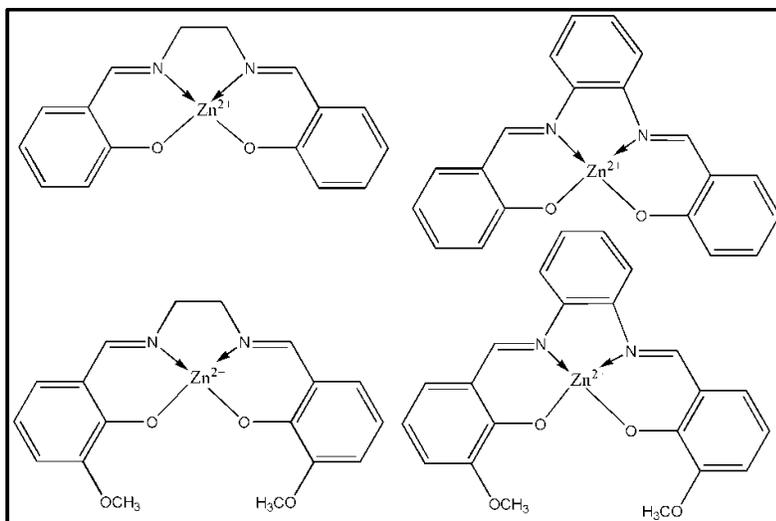
## Chapter I

Although Schiff base metal complexes were discovered since the mid nineteenth century, after the work of Jorgensen and Werner these types of complexes have gained interest of many chemists in the field of coordination chemistry.<sup>47</sup> One of the crucial discovery of Schiff base complex is the formation of “Jacobsens’s catalyst” where the Schiff base was used obtained by the condensation of trans-1,2-diaminocyclohexane and 3,5-di-tert-butyl-2-hydroxybenzaldehyde followed by the treatment of ligand with Mn(III) acetate in open air.



**Fig 1.8.** Jacobsen's catalyst

Not only with Mn(III), different Schiff complexes of transition metals like Co(II), Ni(II), Fe(III), Cr(III), Zn(II) were prepared with the aid of different techniques.<sup>48</sup>



**Fig.1.9.** Various Schiff base-Zn<sup>2+</sup> complexes.

Actually Schiff base metal complexes can be prepared without any difficulty and they offer diverse applications and most importantly they act as artificial models of various biologically active compounds.

**2.4.3. Applications of Schiff base transition metal complexes**

Schiff bases and their metal complexes not only have played an important role in the development of modern coordination chemistry, but they can also be found at key points in the development of inorganic biochemistry, catalysis, optical materials, *etc.*<sup>49,50</sup> Some important applications of Schiff base metal complexes are as follows:

**2.4.3.1. As Catalysts**

A large number of homogenous and heterogeneous reactions are affected by Schiff base metal complexes. Such complexes play major roles and their effects can be varied by altering metal ions, coordination sites and the ligand types. A literature survey revealed that chiral Schiff base complexes are more selective in various reactions like hydroxylation, oxidation, epoxidation and aldol condensation, *etc.*<sup>51,52</sup> Schiff base complexes of Cr(III), Mn(III), Fe(II), Co(II), Ni(II) and Zn(II) are used for the oxidation of cyclohexane.<sup>53</sup> Several substituted salen type Mn(III) can be encapsulated in Zeolites-Y for aerobic oxidation of styrene to benzaldehyde, styrene oxide and phenyl acetaldehyde.<sup>54</sup> Silyl ketone undergoes asymmetric oxidation with high enantioselectivity in presence of Mn(II) salen complexes. Literature reveals that dinuclear Schiff base complexes of transition metal ions are more capable to act as a catalyst than the mononuclear complexes due to the synergistic effect of two metal ions.<sup>55</sup> This observation is justified for dinuclear complexes of Cu(II) that can act as effective catalyst in oxygen activation.<sup>56</sup> Literature is full of examples in which complexes of Schiff base ligands were employed as catalyst in polymerization of ethylene.<sup>57</sup> The ring opening polymerization of cycloalkenes is now-a-days efficiently catalyzed by Schiff base metal complexes at low temperature.<sup>58</sup> The ring opening of large cycloalkanes is usually difficult process but Schiff base complexes of Co(II) and Cr(III) were effective in these reactions with significant enantioselectivity.<sup>59</sup> In Michael addition reaction Schiff base complexes of salen type is used as an efficient catalyst. The epoxidation of non-functionalized trans-olefins is catalyzed by Cr(III) complexes.<sup>60</sup> The asymmetric nucleophilic ring opening reaction of meso-epoxides in the presence of benzoic acid is catalyzed by Co(II) salen complexes.<sup>61</sup> Costa *et. al.* observed the catalytic activity of Pd(II) complexes of Schiff bases possessing nitrogen donors during hydrogenation of alkenes and alkynes under mild conditions.<sup>62</sup> A very efficient catalytic activity is exhibited by mono and dinuclear Pd(II) complexes of Schiff bases possessing tert-butyl groups on the salicyl

ring with steric constraints towards reduction of cyclohexene and nitrobenzene. Thus the transition metals in combination with different Schiff base ligands showed high catalytic activities in reactions of industrial importance and academic interests.

### **2.4.3.2. As Electroluminescent Materials**

Of late, organic electroluminescence (EL) devices have attracted large interest in recent years due to flat-panel display applications. Organic dyes, chelate metal complexes and polymers are three major categories of materials used in the fabrication of organic electroluminescent (EL) devices. Literature reveals that many salicylaldehyde-derived Schiff base metal complexes with the formation of inner complex have been investigated as EL materials. Schiff base Zn(II) complexes can act as electroluminescent materials.<sup>63</sup> Blue luminescent Schiff base Zn(II) and Be(II) complexes were prepared from calixarene by Wei *et al.*<sup>64</sup> Zn complex like bis[salicylidene-(4-dimethylamino)aniline]Zn(II) shows charge transporting performance in organic light emitting diodes (OLEDs).

### **2.4.3.3. Biological Activities**

Literature shows that in many enzymatic reactions for which interaction of an enzyme with an amine or carbonyl group of the substrate is involved, Schiff base intermediates are formed.<sup>65</sup> Over the years, a number of transition metal complexes have been exploited for the design of new drugs due to their diverse biological activities. Schiff base ligands with various coordination modes can bind with different metals and can form many nature identical homo and heterometallic complexes with varied stereochemistry. This unique feature is used for modeling active sites in biological systems.<sup>66</sup> Many redox enzymes like catalase and peroxidase which are responsible of the dismutation of H<sub>2</sub>O<sub>2</sub> and the reduction of alkyl hydroperoxide, respectively are made up by the combination of porphyrin group and Fe(II) or Fe(III). Wang *et al.*<sup>67</sup> reported that Fe(salen) can effectively catalyze the reduction of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>. Literature survey reveals that salen type metal complexes can induce specific damages in DNA or RNA. In the middle of 1980s there was an important discovery that Schiff base transition metal complexes can show chemical nuclease activity and thereby they are vastly used in bio-engineering. Literature also reveals that Schiff base metal complexes are excellent DNA cleavers.<sup>68</sup> The potent biological activity can be related to the presence of nitrogen atom with a lone pair of electron in it and

that the nitrogen can participate in hydrogen bonding with –NH or –OH groups present in biomolecules.

#### **2.4.3.4. Antimicrobial Activities**

Schiff bases and their metal complexes have drawn the attention of chemists for their potential antimicrobial activities. Furyl glyoxal and *p*-toluidene made Schiff base shows antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris*.<sup>69</sup> Thallium (I) complexes of benzothiazolines shows significant antibacterial activity against the pathogenic bacteria.<sup>70</sup> Complexes of Mo (IV) and Mn(II) of ligands hydrazine carbonyl amide and hydrazine carbonyl thioamide exhibit significant antibacterial activity against *Xanthomonas compestris*.<sup>71</sup> Literature reveals that tridentate Schiff base metal complexes show extensive antimicrobial activities against *E. coli*, *S. aureus*, *B. subtilis*.<sup>72-74</sup> Schiff bases derived from Isatin shows excellent anti-HIV activities.<sup>75</sup> Various Schiff bases derived from various sources (benzimidazole, quin-azolinones, pyrazolone, thio-semi-carbazone, etc.) show good antibacterial activities.<sup>76-79</sup> Cyclo-butane and thiazole ring contained Schiff bases exhibit various biological activities. Cu and Ni complexes of chloro-salicylidene and taurine obtained Schiff bases possess significant activities to *Colibacillus* and *Pseudomonas aeruginosa*.<sup>80</sup> Fe and Co containing Schiff base complexes derived from Lysine exhibit antibacterial activities towards *B. Subtilis*, *S. Aureus* and *E. coli*.<sup>81</sup> Schiff bases obtained by the condensation of semicarbazide and furfurylidene diamine and their Zn(II), Cu(II), Cd(II) and Ni(II) complexes exhibit good antimicrobial activities. Bidentate Schiff bases containing Si(IV) having at least one nitrogen donor sites exhibit antibacterial activities.<sup>82</sup>

#### **2.4.3.5. Antifungal Activities**

Literature is full of examples where Schiff bases and their metal complexes act as antifungal agents. Schiff bases obtained from thiazole and benzothiazole exhibit antifungal activities.<sup>83</sup> It is found that if any methoxy or naphthyl or halogen group is attached with the Schiff base the fungicidal activities increase.<sup>84</sup> Schiff bases obtained from pyrandione and quinazolinones show physiological activities against *A. niger* and *candida albican*, respectively.<sup>85</sup> Literature reveals that *Helminthosporium gramineum* which is responsible for strip disease in barely and *Syncephalostrum racemosus* which causes fruit rot in tomato are affected significantly by furan and various amines made Schiff bases.<sup>86</sup> Carbothioamide made Schiff bases and their

metal complexes exhibit fungicidal activities against *H. graminicium*.<sup>87</sup> *A. alternata* which is responsible for disease in brinjal can be controlled by Molybdenum and manganese Schiff base complexes.

### **2.4.3.6. Antiviral Activities and Synergistic Action**

Schiff base metal complexes containing Ag(I) exhibit inhibition against Cucumber mosaic virus.<sup>88</sup> Literature reveals that glycine, salicylaldehyde made Schiff base metal complex of Ag(I) gives positive outcomes up to 74.7% towards Cucumber mosaic virus. Not only that sulfane thiadizole and salicylaldehyde made Schiff bases are quite capable of exhibiting toxicity towards insects. Experiment proves that fluorination on aldehyde part of Schiff base enhances the insecticidal activity.<sup>89</sup> Condensation products of thiadiazole derivatives and o-vanillin show good insecticidal activities against bollworm and enhance the cell survival rate of mung bean sprouts.<sup>90</sup>

### **2.4.3.7. Other Therapeutic Activities**

Literature is full of examples where Schiff bases and their metal complexes show anti-inflammatory, analgesic and anti-oxidative actions. Analgesic and anti-inflammatory activities are shown by thiazole derived Schiff bases.<sup>91</sup> Superoxide and hydroxyl scavenging may be done by taking carboxy methylchitosan containing Schiff bases.<sup>92</sup> Anthelmintic and analgesic activity is shown by furan semicarbazone made Schiff base metal complexes.<sup>93</sup> Salicylidine and anthranilic acid made Schiff bases possess antiulcer activities and if complexation is done with Cu(II) then antiulcer activity further increases.<sup>94</sup> Cu, Ni, Zn, Co containing Schiff bases made from salicylaldehyde and glycine exhibit antitumor activities.<sup>95</sup> Literature depicts that antitumor activity of metal complexes follow the order Ni > Cu > Zn > Co. Human tumor cells are also affected by Schiff bases derived from aromatic and heterocyclic amines.<sup>96</sup>

## **2.5. Objective of the research work and Method of Investigations**

Inspired by the varied use of Schiff bases and their metal complexes, present dissertation is mainly focused on the use of some transition metal complexes as potential micronutrient supplement for selected plants. Although only few examples of this type of works were found in the literature, some notable works have drawn our attention, *e.g.*, N-acetylated Schiff bases were reported to exhibit growth inhibitory activity with seedling of wheat, rye and barley.<sup>97</sup> Schiff bases were reported to initiate

the plant hormone auxin required for the root growth.<sup>98</sup> Therefore the main objectives of this research work are as follows:

- To synthesize polydentate Schiff base-transition metal complexes using normal conventional protocols with slight alterations as required.
- To characterize the synthesized polydentate Schiff base metal complexes with different physicochemical and spectroscopic techniques.
- To study the effect of these polydentate metal complexes on various morphological and biochemical parameters of some selected plants.
- To study whether the synthesized polydentate metal complexes can solve micronutrient deficiency in plants without hampering the environment.

The present dissertation includes a total of six chapters including this introductory one. Chapter II contains details of chemicals and the various physico-chemical techniques used. The applications of the synthesized complexes as potential micronutrient supplier on selected plants are discussed in Chapters III-V, followed by concluding remarks in Chapter VI.

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## CHAPTER II

### 2. Experimental Section

#### 2.1. Source and purification of the chemicals used

All the chemicals used in various works embodied in this dissertation were of analytical reagent grade (A. R) and were used without further purification as procured from various commercial sources. The chemicals used are listed in the Table 2.1.

**Table 2.1.** Purity and source of the chemicals used.

Chemical compound	Mol. Formula	Purity	Source	CAS NO
Salicylaldehyde	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	99%	S. D. Fine Chemicals, India	90-02-08
<i>o</i> -vanillin	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	99%	Sigma-Aldrich, Germany	148-53-8
1,2-ethylenediamine	C <sub>2</sub> H <sub>8</sub> N <sub>2</sub>	99%	S. D. Fine Chemicals, India	107-15-3
1,2-phenylenediamine	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>	99%	S. D. Fine Chemicals, India	95-54-5
Aniline	C <sub>6</sub> H <sub>7</sub> N	99%	S. D. Fine Chemicals, India	62-53-3
Cupric acetate monohydrate	C <sub>4</sub> H <sub>8</sub> CuO <sub>5</sub>	99%	S. D. Fine Chemicals, India	6046-93-1
Zinc acetate dihydrate	C <sub>4</sub> H <sub>10</sub> O <sub>6</sub> Zn	99%	S. D. Fine Chemicals, India	5970-45-6
Methanol	CH <sub>3</sub> OH	99.8%	Sigma-Aldrich, Germany	67-56-1
Ethanol	C <sub>2</sub> H <sub>5</sub> OH	99%	Sigma-Aldrich, Germany	64-17-5
Vanadyl sulfate hydrate	VO <sub>2</sub> SO <sub>4</sub> ·xH <sub>2</sub> O	99%	Sigma-Aldrich, Germany	123334- 20-3
Benzil	C <sub>14</sub> H <sub>10</sub> O <sub>2</sub>	98%	Sigma-Aldrich, Germany	134-81-6
Benzidine	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub>	99%	Sigma-Aldrich, Germany	92-87-5

<b>Dimethyl sulfoxide</b>	C <sub>2</sub> H <sub>6</sub> OS	98%	S. D. Fine Chemicals, India	67-68-5
<b>Acetonitrile</b>	C <sub>2</sub> H <sub>3</sub> N	98%	S. D. Fine Chemicals, India	75-05-08

## 2.2. Experimental Methods

### 2.2.1. Different physico-chemical methods used

Different physico-chemical methods have been used to characterize the structure of Schiff base ligands and their transition metal complexes. A brief account of these methods is given below:

**a) Elemental analysis:** Elemental micro-analyses (C, H and N) of all the synthesized compounds were performed by using Perkin–Elmer (Model 240C) analyzer. The metal contents in the transition metal complexes were determined with the help of Atomic Absorption Spectrophotometer (Varian SpectrAA 50B as shown below in Fig 2.1) by using standard metal solutions (1000 ppm) procured from Sigma-Aldrich, Germany.



**Fig 2.1.** Atomic Absorption Spectrophotometer (Varian SpectrAA 50B).

**b) Melting point:** The melting point of the ligand and complexes were determined by open capillary method with the aid of a melting point apparatus as shown below in Fig 2.2.



**Fig 2.2.** Melting point apparatus.

**c) Infrared spectra:** Infrared spectra of the dried samples (as KBr pellets) were recorded on a Perkin-Elmer Spectrum FT-IR spectrometer (RX-1) operating in the region  $4000$  to  $400\text{ cm}^{-1}$ . IR spectroscopic grade KBr from Sigma-Aldrich, Germany was used for preparing the pellets after drying the salt (KBr) in a drying pistol over anhydrous  $\text{CaCl}_2$  for 24 hours and then kept in a vacuum desiccator over anhydrous  $\text{CaCl}_2$  for using it during recording the IR spectra of the samples.



**Fig 2.3.** Perkin-Elmer Spectrum FT-IR spectrometer (RX-1).

**d) Electronic spectra:** Electronic spectra of the ligands and their transition metal complexes dissolved in a suitable solvent methanol were recorded on a Jasco V-530 double beam UV-VIS spectrophotometer (as shown in Fig 2.4) at ambient



**Fig 2.4.** Jasco V-530 double beam UV-VIS spectrophotometer.

temperature. The instrument was coupled with a thermostatic arrangement to maintain the temperature at 298.15 K. Quartz cells of 1 cm path length were used for holding the sample and the reference solvent during the spectral measurements.

**e) Conductivity:** Specific conductances were measured at  $(298.15 \pm 0.01)$  K with a Systronic conductivity TDS-308 meter (shown in Fig 2.5). The conductance measurements were carried out by using a dip-type immersion conductivity cell (CD-10) with a cell constant of  $1.0 \pm 10\%$   $\text{cm}^{-1}$ . The instrument was standardized by using 0.1 (M) KCl aqueous solution. Measurements were made in a thermostatic water bath maintained at the experimental temperature with an accuracy of  $\pm 0.01$  K.



**Fig 2.5.** Systronic conductivity meter (TDS-308).

**f) Mass measurements**

Mass measurements were carried out on digital electronic analytical balance (Mettler Toledo, AG 285, Switzerland) as shown in Fig 2.6. This Digital balance can measure mass to a very high precision and accuracy. The mass measurements were accurate to  $\pm 0.01$  mg.



**Fig 2.6.** Digital electronic analytical balance (Mettler Toledo, AG 285).

**2.2.2. Synthesis of the ligands and their transition metal complexes****a) Synthesis of  $N_2O_2$  donor ligands and their Zn(II) complexes**

Two different aldehydes, *viz.*, salicylaldehyde and *o*-vanillin, each of purity level 99% were used without any further purification.  $Zn(AcO)_2 \cdot 2H_2O$ , 1,2-ethylenediamine, 1,2-phenylenediamine and spectroscopic grade methanol were used for the synthesis of the ligands and their transition complexes. Slight alterations from standard or literature procedures were adapted during the preparation of both the ligands and their Zn(II) complexes.<sup>1-3</sup> Two aldehydes (Salicylaldehyde/*o*-vanillin) were alternatively treated in 2:1 molar ratio in MeOH with two corresponding amines (1,2-ethylenediamine/1,2-phenylenediamine) to prepare four ligands [**L1-L4**] with yields approximately 90-95%. The four prepared ligands were individually treated with  $Zn(AcO)_2 \cdot 2H_2O$  in MeOH in a molar ratio of 1:1 molar ratio and the respective mixtures were refluxed at temperatures 40-60 °C to obtain four corresponding Zn(II) complexes [**C1-C4**]. Characterization of these ligands and their Zn(II) complexes were done by elemental microanalyses and IR spectroscopic measurements. The

results were found to be more or less similar to those already reported in the literature.<sup>3</sup>

### **b) Synthesis of azo-functionalized Schiff base ligands and their Cu(II) complexes**

0.1 mol of aniline was dissolved first in concentrated HCl. Next 8 g of analytical grade NaNO<sub>2</sub> (dissolved in bi-distilled water) was then added to the aniline solution drop wise with constant stirring for about 1 hour at 0 °C. A solution of 0.1 mol salicylaldehyde, sodium carbonate (36 g) and water was added drop wise in the mixture with constant stirring. The reagents were allowed to react for ~5 hours at 0 °C. After the completion of the reaction light red colored precipitate of 4-(Benzeneazo)salicylaldehyde was filtered off and recrystallized from ethanol. The yield was approximately 80% and the melting point was found to be 118 °C. Treatment of two different amines (1,2-ethylenediamine and 1,2-phenylenediamine) with the azo part in 1:2 ratio in ethanol for ~3 hours at 80 °C to obtain two ligands [L5 and L6]. These ligands were washed and recrystallized from ethanol and the yield was approximately 70%. The two prepared ligands were alternatively refluxed with Cu(AcO)<sub>2</sub>.H<sub>2</sub>O in ethanol in 1:1 molar ratio for ~2 hours at 80 °C yielding two Cu complexes [C5 and C6]. They were filtered, washed and then recrystallized with the yields ~70%. The two ligands L5, *i.e.*, {[N, N'-bis[4-(benzeneazo)salicylaldehyde]ethylenediamine and L6, *i.e.*, [N, N'-bis[4-(benzeneazo)salicylaldehyde]-*o*-phenylenediamine} and two complex C5, *i.e.*, {[N, N'-bis[4-(benzeneazo)salicylaldehyde]ethylenediamine Copper (II) and C6, *i.e.*, [N, N'-bis[4-(benzeneazo)salicylaldehyde]-*o*-phenylenediamine Copper(II)} were characterized by elemental microanalyses, IR spectroscopic measurements, conductometric measurements and electronic spectra. All results were more or less similar to those reported in the literature.<sup>4</sup>

### **c) Synthesis of tetradentate N<sub>2</sub>O<sub>2</sub> donor Schiff base ligand and its vanadium(IV) complex**

Ethanol solution of benzidine (purity > 99%) was refluxed with benzil (purity > 98%) in a round bottom flask in 1:2 molar ratio for ~4 hours at a temperature of 40-60 °C with constant stirring to get the olive green colored ligand L7 [C<sub>40</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>. 2H<sub>2</sub>O]. The ligand was filtered off, washed and recrystallized from ethanol. Then it was dried in a vacuum desiccator. To synthesize the vanadium(IV)

complex the ligand was further refluxed with an ethanolic solution of  $\text{VOSO}_4 \cdot x\text{H}_2\text{O}$  for ~8 hours at 40-60 °C in a molar ratio of 1:1 with constant stirring. The complex **C7** obtained with a molecular formula of  $\text{C}_{80}\text{H}_{56}\text{N}_4\text{O}_6\text{V}_2 \cdot 5\text{H}_2\text{O}$  was washed with ethanol and dried over anhydrous  $\text{CaCl}_2$ . Both the synthesized ligand and the complex were characterized by elemental microanalysis, infrared and electronic spectroscopic spectra. These results were similar to those reported earlier in the literature.<sup>5</sup>

### 2.2.3. Various morphological and biochemical parameters used

All the synthesized ligands and their transition metal complexes were applied to different plant materials. The various morphological and biochemical parameters monitored for the selected plants in different chapters are as follows:

#### a) Germination Index (GI)

Germination Index (GI) was calculated using following formula given by AOSA:<sup>6</sup>

$$\text{GI} = \left[ \frac{a_1}{t_1} \right] + \dots + \left[ \frac{a_n}{t_n} \right]$$

where,  $a_1$ ,  $a_n$ ,  $t_1$  and  $t_n$  stand for the number of seeds germinated in first count, the number of seeds germinated in final ( $n^{\text{th}}$ ) count, the days of first count and the days of final ( $n^{\text{th}}$ ) count, respectively.

#### b) Germination percentage (GP)

Germination percentage was calculated 10 days after the germination by dividing the number of germinated seeds by total number of seeds in each pot, multiplied by hundred.<sup>7</sup>

$$\text{GP} = \frac{x}{y} \times 100$$

where,  $x$  and  $y$  stand for the total number of germinated seeds in each pot and the total number of seeds in each pot, respectively.

#### c) Seedling vigour index (SVI)

Seedling vigour index (SVI) was calculated according to Baki *et al.* as follows:<sup>8</sup>

$$\text{SVI} = L \times P$$

where,  $L$  is seedling length in cm and  $P$  is the germination percentage (GP).

#### d) Coefficient of velocity of germination (CVG)

Formula described by Maguire<sup>9</sup> was followed to calculate coefficient of velocity of germination. The formula is given below:

$$\text{CVG} = \frac{(G_1 + G_2 + \dots + G_n)}{(1 \times G_1 + 2 \times G_2 + \dots + n \times G_n)}$$

where,  $G_n$  is the number of germinated seeds after  $n^{\text{th}}$  day of germination.

**e) Extraction and estimation of total soluble proteins**

Ice cold 0.05 M sodium phosphate buffer solution (pH 7.2) was used for the extraction of total soluble protein from the seed, leaf and root tissues as per the method of Chakraborty *et al.*<sup>10</sup> Total soluble protein was estimated following by the method of Lowry *et al.*<sup>11</sup>

**f) Extraction and estimation of chlorophyll**

Chlorophyll from the leaves was extracted in 80% acetone in dark by following the method of Harborne by measuring the absorbance of the filtrate at 663 nm and 645 nm, respectively in a UV-VIS spectrophotometer.<sup>12</sup>

**g) Extraction and estimation of carotenoids**

Carotenoids from the leaves were extracted in 100% methanol following the method of Lichtenthaler<sup>13</sup> and the absorbance of the filtrate was immediately recorded at 663, 645 and 480 nm in a UV-Visible spectrophotometer.

**h) Extraction and estimation of antioxidative enzymes**

For the extraction of antioxidative enzymes [peroxidase (POX, EC 1.11.1.7), ascorbate peroxidase (APOX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6)], leaf tissues were homogenized in ice cold 50 mM sodium phosphate buffer solution (pH 7.2) with 1% (w/v) poly vinyl pyrrolidone and homogenate was centrifuged at 10000 rpm for ~20 minutes at -4 °C. The supernatant layer was used as crude enzyme extract. POX activity was assayed spectro-photometrically where the oxidation of *o*-dianisidine was observed following the method described by Chakraborty *et al.* with some modifications using UV-VIS spectrophotometer (Cole Parmer, USA). Method described by Asada and Takahashi<sup>14</sup> was followed for the assay of APOX activity, where quantity of ascorbate oxidized was measured spectrophotometrically. Catalase activity was measured by quantifying H<sub>2</sub>O<sub>2</sub> broken down at 240 nm as described by Beers and Sizer.<sup>15</sup> Here 1 enzyme unit was interpreted as 1Δ absorbance per minute.

**i) Extraction and estimation of total sugar**

Total sugar from the leaf was extracted in 95% ethanol using the method of Harborne with minor modifications and Anthrone's method given by Plummer (1978) was followed for the estimation.<sup>16</sup>

**j) Extraction and estimation of Leaf Zinc content**

The Chickpea leaves were plucked off after 30 days from germination and were dried in a forced air oven at 70 °C to a constant weight and grounded to a finely divided powder. The formed dry samples (1 g) were combusted in a muffle furnace at 550 °C for ~8 hour. The ash samples were dissolved in 2(M) HCl following the method of Chapman and Pratt.<sup>17</sup> Zn content was estimated with the aid of an atomic absorption spectrophotometer (Varian SpectrAA 50B) using standard Zn solutions for calibration.

**k) Growth parameters**

To determine fresh weight, the harvested plants were rinsed with de-ionized water and blotted on paper towels before being weighed. Dry matter yields of the seedlings were determined after drying the seedlings in an oven at 80 °C to a constant weight. Relative water content (RWC) was measured according to the protocol described by Farooqui *et al.*<sup>18</sup>

**l) Copper tolerance index**

Copper tolerance index (TI) was calculated as the quotient of the dry weight of the plants grown under copper treated and control conditions according to the following formula:<sup>19</sup>

$$TI(\%) = \frac{\text{Dry weight of treated plants}}{\text{Dry weight of control plants}} \times 100$$

**m) Electrolyte leakage**

Ion leakage was measured as electrical conductivity (EC%) according to Yan *et al.*<sup>20</sup> The percentage of electrolyte leakage was calculated according to this formula:

$$EC(\%) = \frac{C_1}{C_2} \times 100$$

where, C<sub>1</sub> and C<sub>2</sub> are the electrolyte conductivities measured before and after boiling, respectively.

**n) Determination of free amino acids and proline**

Free amino acids were detected according to the method of Lee and Takahashi.<sup>21</sup> Proline content was determined by following the ninhydrin method.<sup>22</sup>

**o) Lipid peroxidation**

It was measured as the content of malonyldialdehyde (MDA) using the thiobarbituric method of Heath and Packer.<sup>23</sup>

**p) H<sub>2</sub>O<sub>2</sub> content**

H<sub>2</sub>O<sub>2</sub> levels in the leaves were estimated according to Jena and Choudhuri<sup>24</sup> with minor modifications. H<sub>2</sub>O<sub>2</sub> levels were calculated by using extinction coefficient 0.28  $\mu\text{mol}^{-1} \text{cm}^{-1}$ .

**q) Cell viability**

10 mm leaf disc from the control and the treated plants were kept in glass vials with 1% MTT (*i.e.*, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution in dark for 12 hours. Leaf samples were placed in 5% alcohol and kept for boiling till all the alcohol evaporated off. Thereafter the absorbance of the purple coloured extract was measured at 485 nm.<sup>25</sup>

**r) Leaf disc bioassay**

The fully expanded and fresh leaves from the plants were gently washed in deionized water and 1 cm diameter leaf discs were then floated in a 5 ml various concentration solutions of schiff base ligand (L7), Schiff base VO(II) complex (C7) and NH<sub>4</sub>VO<sub>3</sub> (AV) for 6 days. Leaf discs floated in sterile distilled H<sub>2</sub>O served as the experimental control. The effects of different complexes on leaf discs were assessed on the basis of the phenotypic alteration especially leaf colour.

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## **CHAPTER III**

### **Morphological and biochemical effects of some $N_2O_2$ donor Schiff bases and their $Zn^{2+}$ complexes on *Carica papaya* L. and *Cicer arietinum* L.\***

#### **3.1. Introduction**

Zinc is biologically one of the most important essential metals, because it is a constituent of over 200 metalloproteins and about 300 enzymes, *e.g.*, carboxypeptidase, carbonic anhydrase and alcohol dehydrogenase, *etc.*<sup>1</sup> It is also one of the most essential micronutrients for plant growth.<sup>2</sup> A large number of biochemical processes in plants require Zn.<sup>3,4</sup> Some biological processes, *viz.*, protein and carbohydrate metabolism, anti-oxidative defence and various enzymatic activities<sup>5</sup> involve Zn. It serves as structural and catalytic components of several proteins and enzymes required for growth and development. Zinc deficiency in soils has inevitably created a global micronutrient deficiency problem for most of the agricultural regions worldwide including India. In order to increase the Zn level in plants, fertilizers containing Zn are being used frequently. Most of the Zn contained fertilizers are ionic in nature with marked effects on pH of the medium and free electron activity<sup>6</sup> due to the presence of counter anions like sulphate and nitrate in most of the inorganic fertilizers. These counter anions can alter the pH of medium<sup>7</sup> when such fertilizers are applied excessively. However, such adverse effects of inorganic metal-fertilizers can be avoided if natural and synthetic metal chelates that remain in un-reactive form can be applied to reduce the micronutrient deficiency for a longer period of time.<sup>8,9</sup> Hence synthesis of such eco-friendly metal containing organic species is very promising and concern for the scientific world today.

Therefore in this chapter synthesis and physico-chemical characterization of four different Schiff bases with  $N_2O_2$  coordination sites and their  $Zn^{2+}$  complexes were synthesized. These compounds were characterized by a few analytical and spectroscopic methods and their morphological and biochemical effects on papaya and chickpea were thoroughly studied. Papaya and chickpea were selected for the study because they are very common plants and their crops are widely used in India as eatable species. Again the common factor amongst these ligands and complexes is the presence of azomethine group ( $RHC=N-R'$ , where R and R' are various alkyl or aryl groups). The N-atom of azomethine group is  $sp^2$  hybridized with a lone pair of

electrons, therefore it exhibit excellent chelating power especially when one or more donor atom is adjacent to this group. Also  $N_2O_2$  donor Schiff bases and their  $Zn^{2+}$  complexes show excellent effects on O-atom transfer reactions, enantioselective epoxidation, aziridination, mediating organic redox reactions and other oxidative processes.<sup>10-13</sup>

## **3.2. Materials and methods**

### **3.2.1. Synthesis of the ligands and their Zn(II) complexes**

The synthesis of four Schiff base ligands (**L1-L4**) and their Zn(II) complexes (**C1-C4**) are discussed in Chapter II These were synthesized by following literature method<sup>14</sup> with slight alteration as required.

### **3.2.2. Treatment of papaya seeds**

The seed germination and morphological changes of papaya were monitored at Sriniketan, India during April-May in 2014. Papaya (*C. papaya* L.) seeds were soaked with 10 ppm solutions of the ligands and their Zn complexes separately for approximately 12 hour. Bi-distilled de-ionized water as used as a control and so there were total 9 sets (*i.e.*, **L1-L4** for four ligands, **C1-C4** for four complexes and one control) of solutions. 100 seeds were used for each solution, so for each batch of experiment a total of 900 seeds were used and three replicas of such experiment was performed using a total of 2700 seeds. When the seeds were soaked, they were transformed to polythene container filled in with a growing media comprising of garden soil, sand and well-rotten cow dung in 1:1:1 ratio at a depth of 1 cm under controlled conditions in a greenhouse with 8 hour light period at 28-35 °C day/night temperature and 65-75% relative humidity. Two seeds were planted in each pot. The length of seedlings, root lengths and number of leaves were monitored at the intervals of 15 days and 30 days. The observations were analyzed in completely randomized design as suggested by Gomez and Gomez (1983).<sup>15</sup> Chlorophyll contents of the leaves were measured through chlorophyll meter (SPAD-502PLUS, Conica Minolta, Japan). After 15 days and 30 days, interval leaves were plucked off. The plant leaves were dried immediately in a forced-air oven at 70 °C to a constant weight and the dried leaves were grounded to a fine powder. Dry samples (1 g) were placed into ceramic vessels and combusted in a muffle furnace at 550 °C for 8 h. The ashed samples were removed from the muffle furnace, cooled and then dissolved in 2(M) HCl.<sup>16</sup> The final solution was diluted to meet the range requirements of the analytical

procedures. Zn contents were determined with the aid of an atomic absorption spectrophotometer (Varian, Spectra AA 50B).

#### **3.2.3. Treatment of chickpea seeds**

A local chickpea variety was selected as plant material for studying the morphological and biochemical effects of the selected Schiff bases and their Zn(II) complexes. The study was conducted in month of March, 2015 at University of North Bengal, India. Selected chickpea seeds surface sterilized with 5% Sodium hypochlorite (NaOCl) solution.<sup>17</sup> Chickpea seeds were soaked with 10 ppm solutions of the ligands and their Zn(II) complexes separately for approximately 12 hour in 9 different sets (*i.e.*, L1-L4 for four ligands, C1- C4 for four complexes and one control) with a control set made up with bi-distilled de-ionized water. Fifty seeds were used for each solution and each set consisted of three replicates. To measure different parameters, soaked chickpea seeds were then germinated on sterile petri plates. 5 mL of sterile distilled water was added to each petri dish every day. Twenty germinated seeds from each treatment were transferred to different pots containing garden soil, well rotten cow dung and sand in 1:1:1 ratio under controlled conditions with 8 hour light period at 28-35 °C day/night temperature and 65-75 % relative humidity. Radicle and seedling length were measured and different biochemical parameters were analyzed by taking one month old seedlings. Germination index, germination percentage, seedling vigour index, coefficient of velocity of germination, protein, sugar, oxidative enzyme, chlorophyll and carotenoid estimations were done as detailed earlier in chapter II.

### **3.3. Results and Discussion**

#### **3.3.1. Characterization of the ligands and their Zn(II) complexes**

The analytical and IR spectral data obtained for all four Schiff bases and their Zn<sup>2+</sup> complexes are listed in Tables 3.1-3.4 and such data were found to be very much similar to those reported in the literature.<sup>14</sup> Characteristic IR bands in the regions 1640-1610, 1400-1000 and 3100-2500 cm<sup>-1</sup> appeared due to  $\nu_{C=N}$ ,  $\nu_{O-H}$  and  $\nu_{N-H}$ , respectively for the ligands. Also the appearance of the  $\nu_{N-H}$  absorption bands signifies hydrogen bonding between the hydroxyl hydrogen and the N- atom (O-H...N=C) of the azomethine group. Characteristic IR absorption bands due to  $\nu_{C-H}$ ,  $\nu_{C=N}$ ,  $\nu_{C-O}$  stretching vibrations and the aromatic ring vibrations were observed for all the four synthesized Zn complexes as well.

**Table 3.1.** Analytical data of the prepared ligands (L1-L4).

Ligand	Molecular formula	IUPAC Name	Formula weight	Yield (%)	% Found (Calculated)		
					C	H	N
L1	C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	<i>N, N'</i> -ethylene bis(salicylidenediamine)	268.31	92.3	71.6 (71.3)	5.9 (5.9)	10.2 (10.5)
L2	C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	<i>N, N'</i> -( <i>o</i> -phenylene) bis(salicylidenediamine)	316.35	90.4	75.9 (75.8)	5.1 (5.1)	8.9 (9.0)
L3	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	<i>N, N'</i> -ethylene bis(3-methoxysalicylidenediamine)	328.36	91.2	65.9 (65.7)	6.1 (6.2)	8.5 (8.4)
L4	C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	<i>N, N'</i> -( <i>o</i> -phenylene) bis(3-methoxysalicylidenediamine)	376.41	89.5	70.2 (70.1)	5.3 (5.2)	7.5 (8.0)

**Table 3.2.** IR spectral data of the four ligands (L1-L4).

Ligand	$\nu_{C=N}$	$\delta_{O-H}$	Aromatic ring vibrations	$\nu_{C-H} + \nu_{N-H}$	$\nu_{C-O}$
L1	1635.7	1371.2	1577.8, 857.4	3048.3, 3007.6,	1199.4, 1151.1
		1284.2	773.1, 742.2	2955.4, 2930.1	1042.1, 1021.3
			647.2	2900.3, 2868.5	
L2	1613.5	1363.4	1562.3, 830.6	3054.4, 2987.1	1192.8, 1151.0
		1277.2	760.5, 639.1	2926.7, 2854.3	1044.3
			581.8, 512.1	2711.1	
L3	1632.7	1325.1	838.1, 790.2	3087.2, 3004.5	1190.5, 1169.3
		1251.8	733.1, 623.2	2930.2, 2913.4	1132.4
		1082.5		2839.2, 2584.7	
		1047.9			
		1009.2			
L4	1614.6	1401.8	735.1, 646.4	3464.2, 3367.3	1206.1, 1093.5
		1377.2	584.2, 538.2	3058.7, 3012.1	1040.1
		1364.8		2954.7, 2925.4	
		1324.2		2836.5	
		1245.4			

Band frequencies are given in  $\text{cm}^{-1}$ .

A broad O-H stretching absorption band in the region  $3500\text{-}3200\text{ cm}^{-1}$  was found for each of the complex and this signifies the presence of water molecules in these compounds.

### 3.3.2. Effects of the ligands and their complexes on papaya seeds

Table 3.5 reveals that lowest days (10.09) for the first 10% seed germination was recorded under C4, which was statistically at par with C2 (10.82 days). While the lowest period of germination was observed under C4 (16.67 days), the period of germination was maximum for the control. Significantly highest percentage of germination (80.83) was measured for C4, followed by C2 (78.32). Data in Table 3.5 thus indicate that the ligands and the control statistically imparted almost same result, but the complexes especially C2 and C4 are more efficient in seed germination.

**Table 3.3.** Analytical data of the prepared four complexes (C1-C4).

Complex	Molecular formula	IUPAC Name	Formula weight	Yield (%)	% Found (Calculated)		
					C	H	N
C1	(C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> Zn)	[ <i>N,N'</i> -ethylene bis(salicylidenediamine) zinc(II)] Monohydrate	349.7	82.0	54.9 (54.8)	4.6 (4.7)	8.0 (8.1)
C2	(C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> Zn)	[ <i>N,N'</i> -( <i>o</i> -phenylene) bis(salicylidenediamine) zinc(II)] Monohydrate	397.74	81.7	60.5 (60.2)	4.0 (4.2)	7.1 (7.1)
C3	(C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> Zn)	[ <i>N,N'</i> -ethylene bis(3methoxysalicylidenediamine) zinc(II)] Monohydrate	409.75	81.3	52.8 (52.5)	4.9 (5.0)	6.9 (6.8)
C4	(C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> Zn)	[ <i>N,N'</i> -( <i>o</i> -phenylene) bis(3-methoxysalicylidenediamine) zinc(II)] Monohydrate	457.79	79.6	57.8 (57.5)	4.4 (4.6)	6.1 (6.2)

**Table 3.4.** IR spectral data of the prepared four complexes (C1-C4).

Complex	$\nu_{\text{C=N}} + \delta_{(\text{O-H})\text{H}_2\text{O}}$	$\nu_{(\text{O-H})\text{H}_2\text{O}}$	Aromatic ring vibrations	$\nu_{\text{C-H}}$	$\nu_{\text{C-O}}$
C1	1653.2	3300-2900	1595.2, 1551.2	3046.4, 3019.2	1184.2
	1633.8		1514.2, 848.8	2950.7, 2928.1	1140.9
			608.3	2869.5	1124.5
					1090.7
C2	1615.5	3400-2800	1585.2, 1530.6	2921.1, 2821.5	1170.3
			854.3, 747.8	2896.2	1151.2
			601.5		1125.5
					1033.8
C3	1661.2	3400-3000	1599.5, 1546.2	3056.7, 3010.5	1169.8
	1642.8		856.4, 726.3	2947.8, 2897.3	1102.3
			584.2		1070.4
					1043.6
C4	1688.7	3600-3000	1586.2, 1538.7	3046.2, 2925.1	1192.6
	1614.4		862.1, 738.2	2809.7	1105.4
			559.5		1074.2
					1048.5

Band frequencies are given in  $\text{cm}^{-1}$ .

Perusal of data, presented in Table 3.6, reveal that Schiff bases (L1-L4) and their Zinc complexes (C1-C4) significantly affect the growth of young seedlings of papaya. Observations were taken at 15<sup>th</sup> and 30<sup>th</sup> days after germination. In both cases more or less similar trends have been observed for different parameters. Selected Schiff bases and their Zinc complexes showed varied range of measured data in seedling growth, root growth and highest number of leaves. In all cases C4 showed prominent and noticeably marked results. Although similar trends were observed for all the parameters for the ligands and the control, the effects of the ligands were statistically similar to those of the control.

**Table 3.5.** Germination data of papaya seeds after the treatment of all the ligands, their Zn(II) complexes and the control.

<b>Treatments</b>	<b>Days to first 10% seed germination</b>	<b>Period of germination (days)</b>	<b>Germination percentage</b>
<b>L1</b>	12.52	22.51	71.07
<b>L2</b>	12.01	20.73	73.40
<b>L3</b>	11.69	21.98	70.52
<b>L4</b>	12.13	20.23	74.44
<b>C1</b>	11.05	18.36	77.65
<b>C2</b>	10.82	17.92	78.32
<b>C3</b>	10.94	18.41	76.21
<b>C4</b>	10.09	16.67	80.83
<b>Control</b>	12.47	23.07	72.45
<b>CD<sub>0.05</sub></b>	0.74	1.03	2.06
<b>SE<math>\pm</math>m</b>	0.39	0.54	0.97

The statistical analysis of the observations on leaf chlorophyll content of papaya seedlings at 15<sup>th</sup> day and 30<sup>th</sup> day interval showed both non-significant and significant variations (shown in Table 3.7). On 15<sup>th</sup> day leaf chlorophyll content for all ligands and the complexes ranged from 28.33 to 34.54 mg /100 g. On 30<sup>th</sup> day maximum leaf chlorophyll content was observed for C4, while it was least for the control. A significant variation was also observed in leaf zinc content of papaya seedlings. At 15<sup>th</sup> day after germination it was highest for C4 and lowest for the control. Some sort of reduced range of leaf zinc content was observed in 30<sup>th</sup> day interval as the zinc, absorbed from the supplied complexes and growing media, further transported over the entire growing plant and thus such transportation ultimately reduced the zinc concentration with increase in dry weight of seedlings. So all the observations reveal that every complex has some effects on the morphological and biochemical parameters measured in this study and the ligands were seemed to be inert while the complexes are responsible for such changes.

**Table 3.6.** Length of seedlings, Root length, Number of leaves data of the papaya plants.

Treatments	Length of seedling (cm)		Root length (cm)		Number of leaves	
	15 days	30 days	15 days	30 days	15 days	30 days
<b>L1</b>	7.36	13.13	6.21	11.10	3.07	4.32
<b>L2</b>	7.02	13.22	6.42	11.34	3.11	5.09
<b>L3</b>	6.87	12.75	6.10	12.00	3.34	4.68
<b>L4</b>	6.98	13.34	7.34	11.56	3.23	4.92
<b>C1</b>	8.12	15.31	8.56	13.37	3.67	5.24
<b>C2</b>	8.23	14.86	9.29	13.25	3.56	5.41
<b>C3</b>	8.50	15.23	9.13	14.05	3.81	6.10
<b>C4</b>	9.23	16.31	10.18	14.84	3.98	6.79
<b>Control</b>	7.11	12.06	6.45	11.14	3.21	4.52
<b>CD<sub>0.05</sub></b>	0.62	0.92	0.97	0.72	NS	0.65
<b>SE<math>\pm</math>m</b>	0.29	0.43	0.50	0.39	NS	0.34

**Table 3.7.** Leaf chlorophyll content and Leaf Zn content of papaya plants after treatment.

Treatments	Leaf chlorophyll content (mg/100 g)		Leaf zinc content (ppm)	
	15 days	30 days	15 days	30 days
<b>L1</b>	31.51	41.65	12.42	12.00
<b>L2</b>	28.33	39.27	13.11	12.87
<b>L3</b>	30.52	39.45	12.52	12.36
<b>L4</b>	29.31	40.16	12.27	11.89
<b>C1</b>	32.64	45.40	17.12	16.75
<b>C2</b>	34.42	46.38	16.33	16.21
<b>C3</b>	33.33	44.79	16.54	16.30
<b>C4</b>	34.54	52.10	17.97	17.65
<b>Control</b>	29.87	40.56	12.20	11.99
<b>CD<sub>0.05</sub></b>	NS	3.43	0.98	0.92
<b>SE<sub>±m</sub></b>	NS	1.67	0.41	0.45

### 3.3.3. Effects of the ligands and their complexes on chickpea seeds

Zinc, as one of the essential micronutrients in plant system, plays an important role in the regulation of plant growth, enzyme activity, protein synthesis and structural stability of cell membrane, *etc.*<sup>18</sup> Plants coming out of their seeds grown in Zinc deficit soil have reduced seedling vigour and growth.<sup>19</sup> In barley and rice, seed priming with Zinc was very effective for seedling vigour and early seedling development.<sup>20,21</sup> Foliar application of Zinc in case of pepper increased the grain protein content and yield.<sup>22</sup> All the previous reports clearly indicate that Zinc is essentially associated with seedling growth.

Data obtained from this study (listed in Table 3.8) reveal that for all the germination parameters no significant differences ( $P \leq 0.05$ ) were noticed among the Schiff base ligand treated seeds in terms of coefficient of velocity of germination, germination percentage, germination index and seed vigour index. But the selected Zn(II) complexes significantly affect different germination parameters.

**Table 3.8.** Effects of the ligands (L1-L4) and their Zn(II) complexes (C1-C4) on various germination parameters (Mean  $\pm$  S.D).

Compounds	SVI	GP	GI	CVG
Control	1059.04 $\pm$ 60.530	73.33 $\pm$ 1.12	11.76 $\pm$ 1.20	0.294 $\pm$ 0.04
L1	1135.38 $\pm$ 174.40	76.66 $\pm$ 2.41	12.68 $\pm$ 1.16	0.385 $\pm$ 0.08
C1	1396.85 $\pm$ 151.46	80.00 $\pm$ 2.77	14.93 $\pm$ 0.92	0.315 $\pm$ 0.02
L2	1053.59 $\pm$ 37.116	73.33 $\pm$ 1.01	12.43 $\pm$ 1.87	0.299 $\pm$ 0.01
C2	1472.82 $\pm$ 105.33	83.33 $\pm$ 1.23	15.71 $\pm$ 1.81	0.308 $\pm$ 0.01
L3	1154.54 $\pm$ 151.32	66.66 $\pm$ 0.79	11.15 $\pm$ 1.03	0.301 $\pm$ 0.03
C3	1272.96 $\pm$ 202.60	76.66 $\pm$ 1.04	14.76 $\pm$ 2.20	0.293 $\pm$ 0.01
L4	1230.09 $\pm$ 114.46	76.66 $\pm$ 1.46	13.93 $\pm$ 2.07	0.309 $\pm$ 0.02
C4	1656.46 $\pm$ 122.92	86.66 $\pm$ 2.14	20.55 $\pm$ 1.99	0.317 $\pm$ 0.01
LSD (P $\leq$ 0.05)	252.33	4.06	3.28	0.066

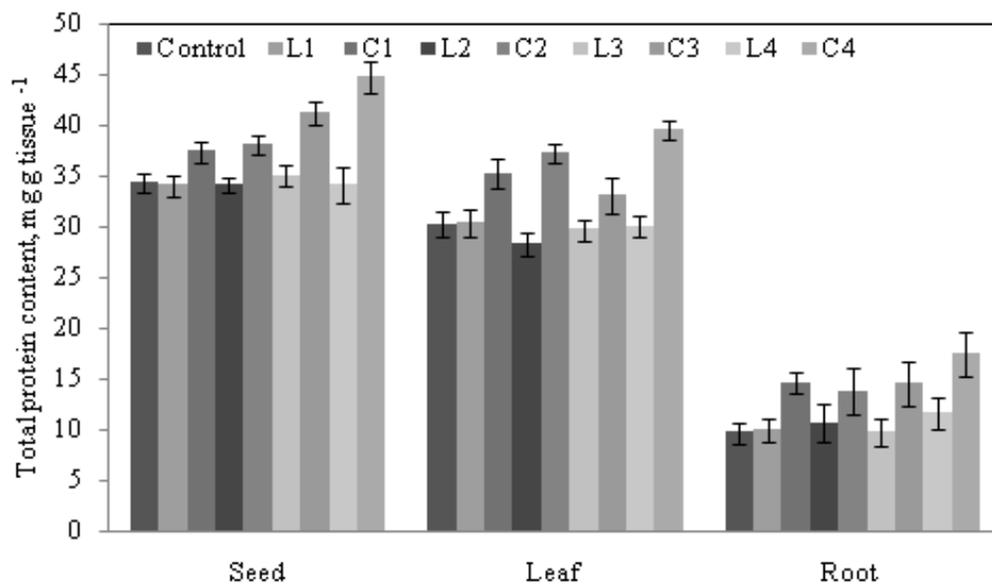
SVI = Seedling vigour index, GP = Germination percentage, GI = Germination index, CVG = Coefficient of velocity of germination. LSD values are computed (at P  $\leq$  0.05) to evaluate mean performances amongst different compounds.

Among the complexes C4 showed highest (P  $\leq$  0.05) and noticeably marked results for all the germination related parameters. SVI, GI, CVG and GP were 56.41%, 18.17%, 74.74%, 7.8% higher respectively in case of C4 in relation to control (Table 3.8). Similar trends were also found in hypocotyls and radicle length. Data concerned with seedling growth, hypocotyls and radicle length clearly indicated gradual and significant increase in all Zn(II) complex treated seedlings. Highest increase was observed for C4 complex treated seedlings. On the contrary growth parameters for ligands treated seedlings were almost identical to the control (listed in Table 3.9). These results are in accordance with our previous study with papaya described above.

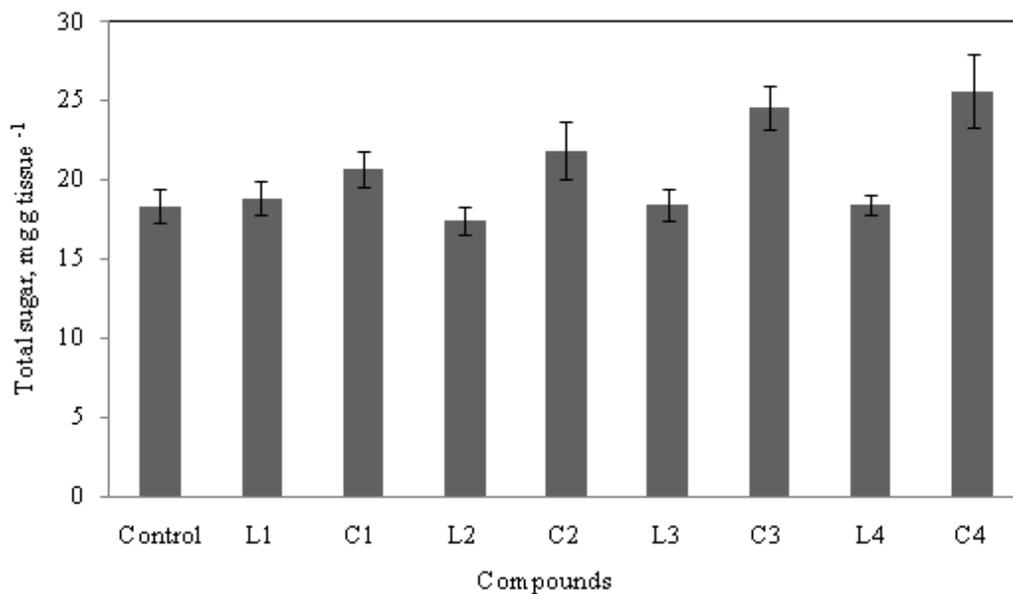
**Table 3.9.** Effects of the ligands, their Zn(II) complexes on various growth parameters (Mean  $\pm$  S.D). LSD values are computed (at  $P \leq 0.05$ ) to evaluate mean performances amongst different compounds.

<b>Compounds</b>	<b>Seedling length (cm)</b>	<b>Hypocotyl length (cm)</b>	<b>Radicle length (cm)</b>
Control	14.43 $\pm$ 1.01	8.23 $\pm$ 1.01	6.86 $\pm$ 1.55
L1	14.80 $\pm$ 1.80	7.70 $\pm$ 0.90	6.70 $\pm$ 1.30
C1	17.46 $\pm$ 1.20	9.53 $\pm$ 1.04	7.60 $\pm$ 1.04
L2	14.40 $\pm$ 0.70	8.56 $\pm$ 1.00	6.53 $\pm$ 0.96
C2	17.66 $\pm$ 1.01	9.00 $\pm$ 0.96	8.73 $\pm$ 1.46
L3	15.70 $\pm$ 0.95	8.66 $\pm$ 0.81	6.20 $\pm$ 0.51
C3	17.20 $\pm$ 1.21	9.06 $\pm$ 0.51	7.36 $\pm$ 1.20
L4	16.03 $\pm$ 1.25	8.73 $\pm$ 0.43	7.70 $\pm$ 1.15
C4	19.10 $\pm$ 0.99	10.16 $\pm$ 1.75	8.66 $\pm$ 0.50
LSD ( $P \leq 0.05$ )	1.09	2.06	1.88

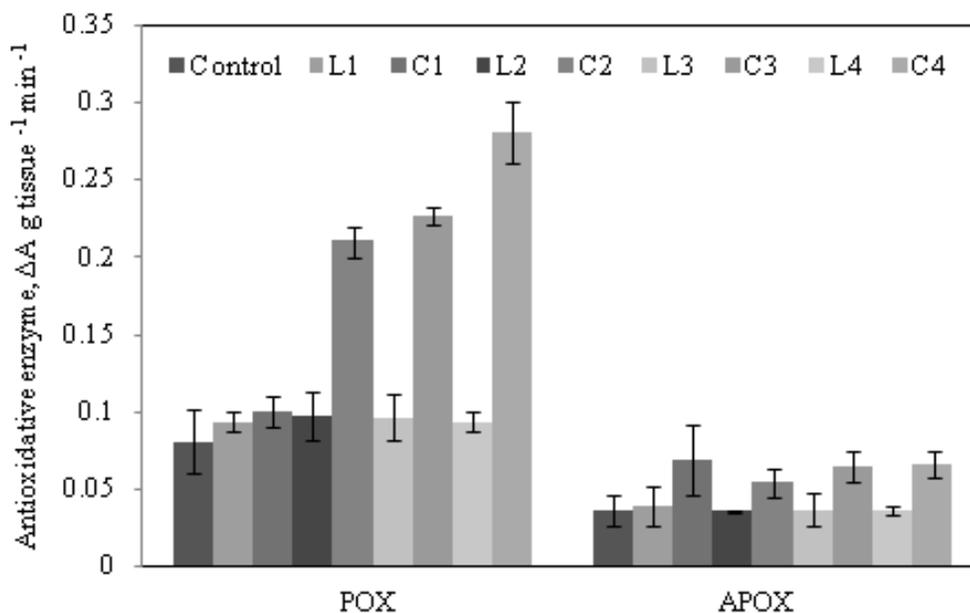
In plant system, Zn is present as cofactor of more than 300 enzymes.<sup>23</sup> based on the above biochemical parameters it is found that total soluble protein content gradually increases in seed, root and shoot after the treatment with Zn(II) complexes and these increments range from 9.07-30.50% in case of seed, 49.12- 79.99 % in case of root, 16.39-30.60% in case of shoot from C1 to C4 complexes as compared to the control (illustrated in Fig 3.1).



**Fig 3.1.** Effects of the ligands, their Zn(II) complexes and the control on seed, leaf and root total protein content of desi chickpea. Results are represented as mean  $\pm$  SD (n=3).

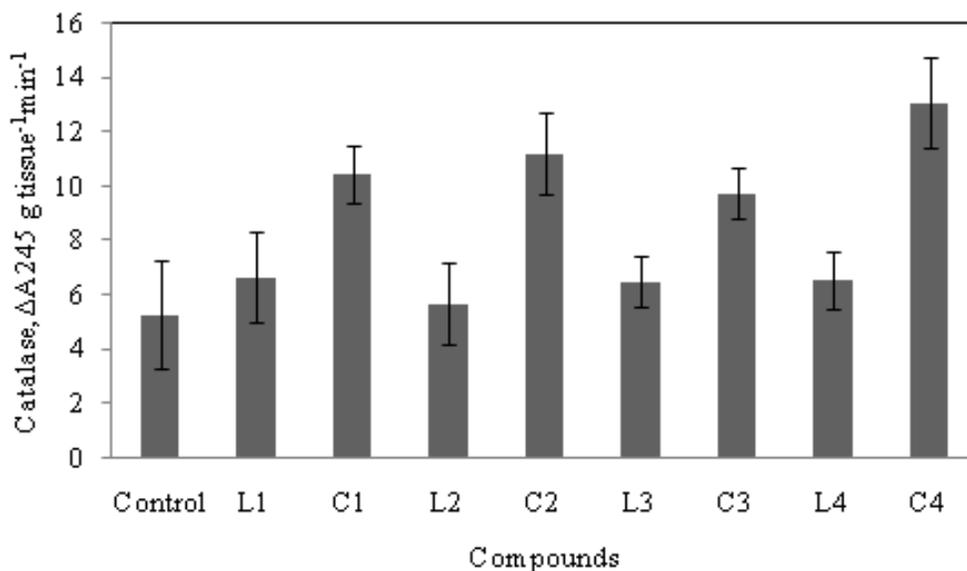


**Fig 3.2.** Total sugar estimation after the treatment with the ligands, their Zn(II) complexes and the control. Results are represented as mean  $\pm$  SD (n=3).



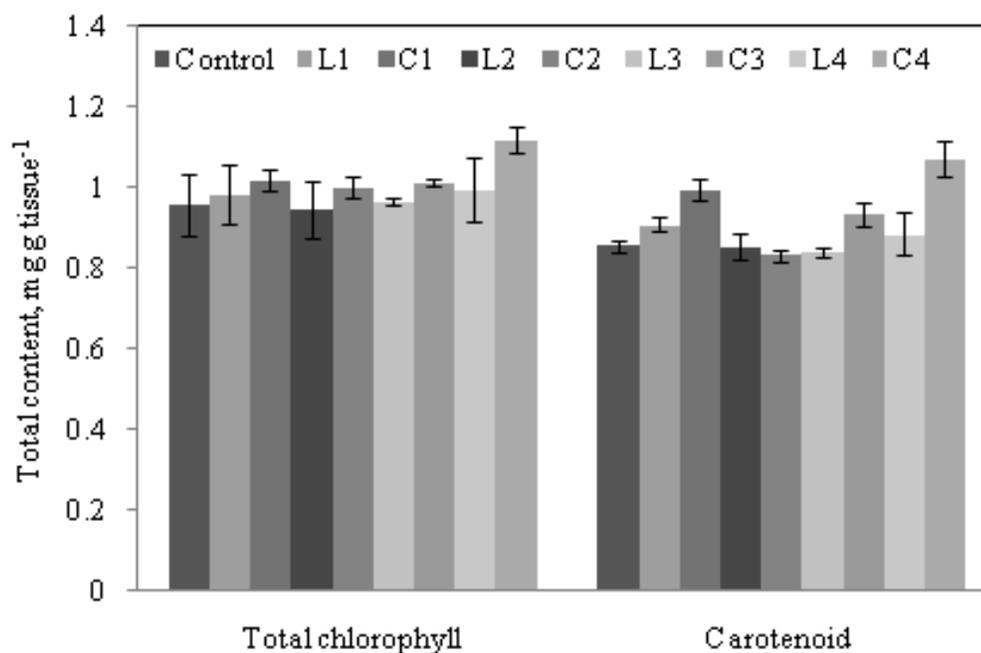
**Fig 3.3.** Effects of the ligands, their Zn(II) complexes and the control on antioxidative enzymes, Peroxidase ( POX) and Ascorbate Peroxidase (APOX) in leaves of desi chickpea. Results are represented as mean  $\pm$  SD (n=3).

After application of different Zn complexes total sugar content in leaf gradually increased in range of 0.124-0.395 folds from C1 to C4 complex (illustrated in Fig 3.2). Antioxidant enzymes expression get enhanced in presence of Zn and provide tolerance to oxidative damage.<sup>24,25</sup> Analysis of antioxidative enzymes clearly indicated a higher and gradual increase in activity of antioxidative enzymes POX, APOX and CAT for the Zn(II) complex treated plants than for the ligand treated plants and the control plants. Peroxidase (POX) activity and ascorbate peroxidase (APOX) activity are illustrated in Fig 3.3. Zn maintains ascorbate and thiol pool in ascorbate-glutathione cycle and then ascorbate reacts with H<sub>2</sub>O<sub>2</sub> in presence of ascorbate peroxidase.<sup>26</sup> Increase in APOX was also noticed in rye grass in presence of Zn.<sup>27</sup> In our observation ascorbate peroxidase (APOX) activity was maximum in case of C2 complex treated plants in relation to the control (illustrated in Fig 3.3). Among the four complexes used for treatment maximum activity of antioxidative enzymes except APOX was noticed in case of C4 complex indicating the higher efficiency for delivering Zn ion to the root. Catalase (CAT) expression (illustrated in Fig 3.4) increased in a range of 0.97 to 1.47 folds from C1 to C4 treated plants with respect to



**Fig 3.4.** Effects of the ligands, their Zn(II) complexes and the control on Catalase in leaves of desi chickpea. Results are represented as mean  $\pm$  SD (n=3).

control indicating high Zn(II) deliver efficiency of C4 among all Schiff base complexes. From this study better availability of Zn in form of Schiff base complex especially through C4 was clearly understood by its inducing effects on expression of different antioxidative enzymes. Zn attached to sulfhydryl group (-SH) of chlorophyll facilitates stability of the pigment.<sup>28,29</sup> Maximum and significant increase ( $P \leq 0.05$ ) of chlorophyll content (illustrated in Fig 3.5) was perceived for C3 and C4 complex treated seedlings in relation to the control clearly indicating positive involvement of Zn in chloroplast biosynthesis and rate of photosynthesis.<sup>30</sup> On the other hand, in ligand treated and control treated plants chlorophyll content remains almost identical. Carotenoid, a non-enzymatic antioxidant involved in quenching of oxidizing species participate in disrupting regular cellular functioning in *Artemisia annua*.<sup>31</sup> Carotenoid content of chickpea seedlings were also enhanced significantly after different Schiff base complex application in seedlings (illustrated in Fig 3.5). Maximum change was noticed in case of C4 but it was insignificant ( $P \leq 0.05$ ) with respect to C3. No marked changes were noticed for the ligand treated plants. Zn concentrations were measured by taking the leaves of 30<sup>th</sup> days old seedlings and the data as given in Table 3.9 manifested the efficacy of the C4 complex to supply Zn in the plant system.



**Fig 3.5.** Effects of the ligands, their Zn(II) complexes and the control on total chlorophyll and carotenoid content in the leaves of desi chickpea. Results are represented as mean  $\pm$  SD (n=3).

**Table 3.10.** Leaf Zinc concentration data of 30<sup>th</sup> days old seedlings after treatment with the ligands (L1-L4) and their Zn(II) complexes (C1-C4).

Compounds	Zinc concentration ( $\mu\text{g g}^{-1}$ )
Control	52.83 $\pm$ 1.25
L1	53.83 $\pm$ 0.76
C1	54.66 $\pm$ 1.52
L2	53.80 $\pm$ 0.64
C2	54.40 $\pm$ 0.69
L3	55.83 $\pm$ 0.58
C3	57.66 $\pm$ 1.12
L4	58.56 $\pm$ 0.98
C4	60.66 $\pm$ 1.15
LSD (P $\leq$ 0.05)	1.02

### **3.4. Conclusion**

Outcomes of the study revealed that the seed germination parameters, seedling growth parameter and biochemical parameters vary with variation of the treatments. Most of the parameters under control were least and all the four selected ligands failed to impart net statistical increment over the control. Thus, the control and the ligands may not serve as effective plant growth stimulator for papaya and chickpea. Thus, perusal of all the parameters undoubtedly signifies the Zn(II) complexes (C1-C4) as far better stimulators than the control and the ligands (L1-L4). The parity and correlation of all the parameters signified the better efficacy of the complex C4 amongst all the complexes and thus it can be used as a potential seed soaking chemical for both the papaya and chickpea plants. However these effects must be further enquired at multi-locations and varied environmental conditions to draw a direct conclusion about the superiority.

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## CHAPTER IV

### **Morphological and Biochemical Effects of two azo-functionalized Schiff base Cu(II) complexes on Rice plants\***

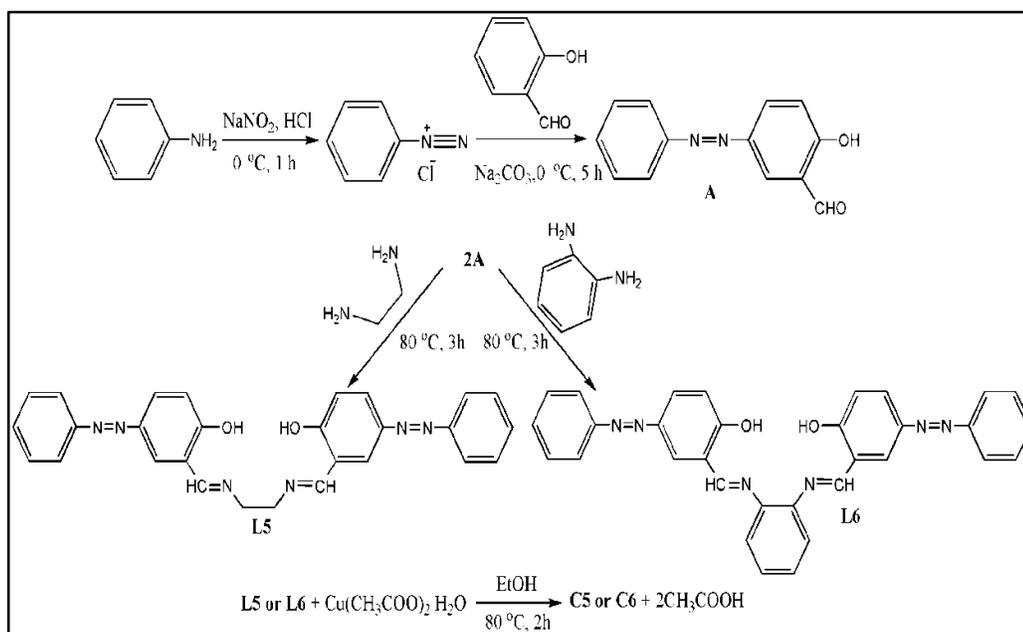
#### **4.1. Introduction**

Copper is one of the important redox active transition metal for plants.<sup>1</sup> The optimum content of Cu<sup>2+</sup> ion in plant tissue is around 10 µg per gram dry weight of the plant.<sup>2</sup> It serves as a component of different enzymes (plastocyanin, cytochrome oxidase, *etc.*) which are mainly associated in electron transfer chain. It acts as cofactors in several enzymes such as Cu/Zn superoxide dismutase, lactase, cytochrome C-oxidase.<sup>3</sup> It is required in the pathway of photosynthesis, respiration and is also associated with carbohydrate and protein metabolism.<sup>4</sup> In plants, copper is necessary for cytosol, endoplasmic reticulum, chloroplast stroma, thylakoid lumen, *etc.* To make copper available in plants generally copper containing fertilizers of inorganic origin (chiefly CuSO<sub>4</sub>) are used. These types of compounds being ionic in nature may alter the pH of the medium.<sup>5,6</sup> Therefore more emphasis is being given recently to the metal chelates that are less reactive but such compounds can solve the deficiency problem for longer period of time.<sup>7,8</sup> Inspired by these facts herein this chapter two azo-functionalized Schiff base ligands and their Cu(II) complexes were synthesized and applied as copper supplement to rice plants. The important feature of these types of ligands and their complexes is that they contain azomethine group (RHC=N-R', where R and R' stand for various alkyl or aryl groups) in which the N-atom is *sp*<sup>2</sup> hybridized and the presence of a lone pair of electrons makes it a good donor especially when one or more donor atoms are remain adjacent to it. Complexes of this type have draw much attention because of their diverse applications like their catalytic power in O-atom transfer reactions and organic redox reactions as well as their use as metallodrug.<sup>9-12</sup> Actually in this chapter the responses of rice seedlings to metal toxicity while being exposed to Copper Sulphate (CuSO<sub>4</sub>) and two synthesized Schiff base complexes (containing Cu<sup>2+</sup>ion) are thoroughly explored. The experimental results are compared in terms of pigments, biochemical components, osmolyte accumulation, oxidative stress markers and overall tolerance level of the rice plants (*Oryza sativa* L.).

## 4.2. Materials and methods

### 4.2.1. Synthesis of the ligands and their Cu(II) complexes

The syntheses of azo-functionalized Schiff base ligands (**L5** and **L6**) and their Cu(II) complexes (**C5** and **C6**) have been detailed in the chapter II. These compounds were prepared by following methods as described in the literature<sup>13</sup> with required alteration (illustrated in Scheme 4.1).



**Scheme 4.1.** Preparation of the ligands (**L5** and **L6**) and their Cu(II) complexes (**C5** and **C6**).

### 4.2.2. Treatment with the ligands and their Cu(II) complexes

To investigate the effects of prepared ligands (**L5** and **L6**), complexes (**C5** and **C6**), and  $\text{CuSO}_4$ , rice seeds were selected as plant material. Grains of rice (*Oryza sativa* L.) were surface sterilized with sodium hypochlorite (0.1%) for 1 minute and then they were washed thrice with de-ionized distilled water and soaked for 12 hours with various concentrations (10, 50, 100, 200 ppm) of  $\text{CuSO}_4$ , **L5**, **L6**, **C5** and **C6** along with a control (*i.e.*, no treatment with micronutrients). After soaking was done, the seeds were allowed to germinate on sterile petri plates. From each treatment twenty germinated seeds were selected and transferred to plastic pots containing garden soil, well rotten cow dung and sand in 1:1:1 ratio under controlled conditions with 8 hour light period at 28-35 °C day/night temperature and 65-75% relative humidity. 45 days old seedlings were then subjected to morphological and

biochemical analyses. Different growth and biochemical parameters as detailed in chapter II were subsequently monitored.

### 4.3. Results and Discussion

#### 4.3.1. Characterization of the prepared ligands and their Cu(II) complexes

The analytical data and corresponding Infrared spectra of the prepared ligands and their Cu(II) complexes are almost identical with those available in the literature<sup>13</sup> and some characteristic physico-chemical and spectral data are furnished in Tables 4.1-4.3. The two ligands have characteristic bands around 1280 cm<sup>-1</sup> due to H-bonded O-H in-plane bending vibrations, around 1610-1640 cm<sup>-1</sup> due to C=N bond stretching vibrations. Also a broad but weak band around 2800-2700 cm<sup>-1</sup> due to strong intra-molecular interactions. For the prepared Cu(II) complexes characteristic bands due to C=N, phenolic C-O, Cu-N and Cu-O stretching vibrations were observed (listed in Table 4.2). The ligand L5 shows mainly two peaks in its electronic spectrum at 270.5 and 362.5 nm, respectively for the  $\pi$ - $\pi^*$  and  $n$ - $\pi^*$  transitions. In C5 the peak for the  $n$ - $\pi^*$  transition shifted from 362.5 to 396 nm due to coordination bond formation. Similarly, The ligand L6 shows three peaks at 254.0, 318.0 and 368.5 nm in its electronic spectrum, respectively for benzene  $\pi$ - $\pi^*$ , imino  $\pi$ - $\pi^*$  and  $n$ - $\pi^*$  transitions. For C6 the peak due to the  $n$ - $\pi^*$  transition shifted from 368.5 to 398 nm indicating donation of nitrogen lone pair of electrons to the Cu<sup>2+</sup> ion.

**Table 4.1.** Analytical data and some physical properties of the Schiff bases and their complexes

	Molecular Formula	Colour	Yield (%)	Found (Calcd) %			
				C	H	N	M
L5	C <sub>28</sub> H <sub>24</sub> N <sub>6</sub> O <sub>2</sub>	Orange	84	70.60 (70.57)	5.20 (5.08)	17.58 (17.64)	
C5	C <sub>28</sub> H <sub>22</sub> N <sub>6</sub> O <sub>2</sub> Cu	Green	73	62.55 (62.50)	4.02 (4.12)	15.53 (15.62)	11.76 (11.81)
L6	C <sub>32</sub> H <sub>24</sub> N <sub>6</sub> O <sub>2</sub>	Orange	80	73.02 (73.26)	4.53 (4.61)	15.98 (16.03)	
C6	C <sub>32</sub> H <sub>22</sub> N <sub>6</sub> O <sub>2</sub> Cu	Red	75	65.64 (65.57)	3.69 (3.78)	14.42 (14.34)	10.78 (10.84)

Conductivity (ohm<sup>-1</sup>·cm<sup>2</sup>·mol<sup>-1</sup>): C5, 5.6 and C6, 5.1

**Table 4.2.** Some characteristic infrared (in  $\text{cm}^{-1}$ ) and electronic spectral (in nm) data of the Schiff bases (L5 and L6) and their Cu(II) complexes (C5 and C6).

Compound	$\nu_{\text{C=N}}$	$\nu_{\text{phenolic C-O}}$	$\nu_{\text{M-N}}$	$\nu_{\text{M-O}}$	$\lambda_{\text{max}}$
<b>L5</b>	1640	1588	1290		362, 270.5
<b>C5</b>	1627	1578	1257	514 449	396, 266
<b>L6</b>	1610	1578	1285		368.5, 318, 254
<b>C6</b>	1630	1592	1258	530 430	398, 318, 251

### 4.3.2. Application of the ligands and their Cu(II) complexes to the plant material

Copper (Cu) being an indispensable trace element for the plant development, plays an important role in the photosynthetic reactions and is required for cell signaling, transcription, protein trafficking, oxidative phosphorylation. But at the same time over indulgence of Cu negatively impacts plants growth, inhibits photo system, electron transport and generates oxidative stress within plant cells and diminishes the content of photosynthetic pigments. In the present chapter, the response of rice seedlings in presence of different concentrations of Copper Sulphate ( $\text{CuSO}_4$ ), the Schiff base ligands (**L5** and **L6**) and their Cu(II) complexes (**C5** and **C6**) are evaluated. The results were compared on the basis of various biochemical and growth parameters to see the efficacy of the copper complexes (**C5** and **C6**) over  $\text{CuSO}_4$ .

#### 4.3.2.1. Growth and tolerance to the copper complexes (C5 and C6)

$\text{Cu}^{2+}$  can hamper growth and development of seedlings by causing damage to epidermal cells and cell membranes.<sup>14</sup> The outcomes reveal that plants treated with C5 and C6 complexes were able to retain a higher percentage of fresh weight, dry weight and relative water content (RWC) than  $\text{CuSO}_4$  treated plants with increasing concentration suggesting less toxicity of Schiff base complexes (illustrated in Figs 4.1-4.3).

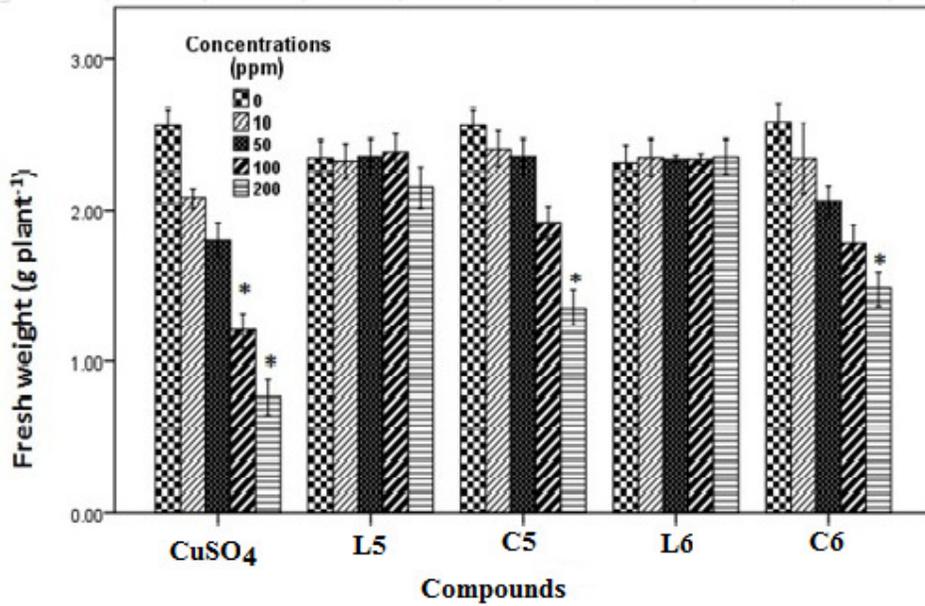


Fig 4.1. Fresh weight (g plant<sup>-1</sup>) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3.  
\*The mean difference is significant at the 0.05 level.

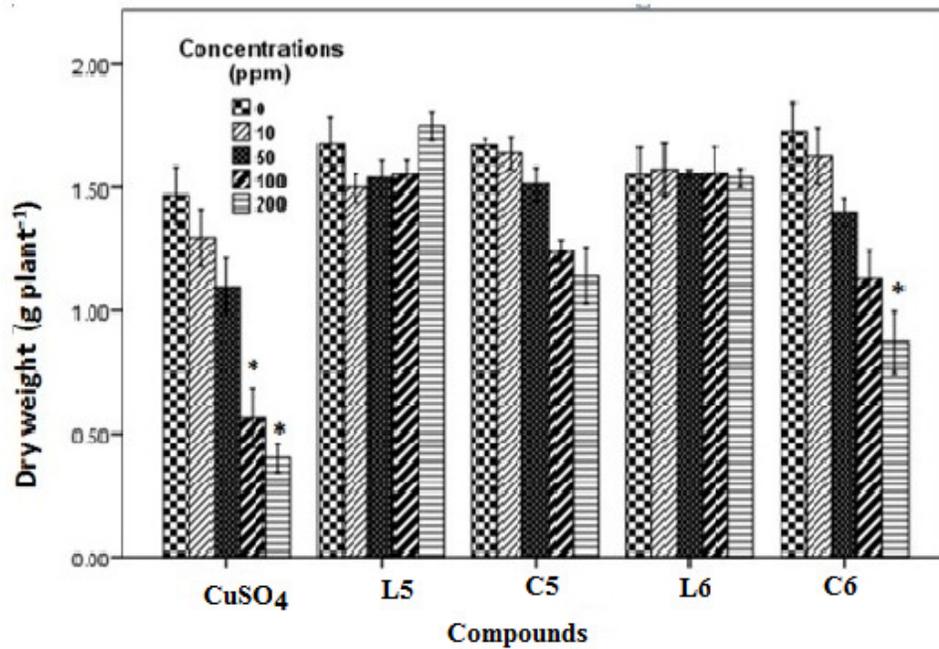
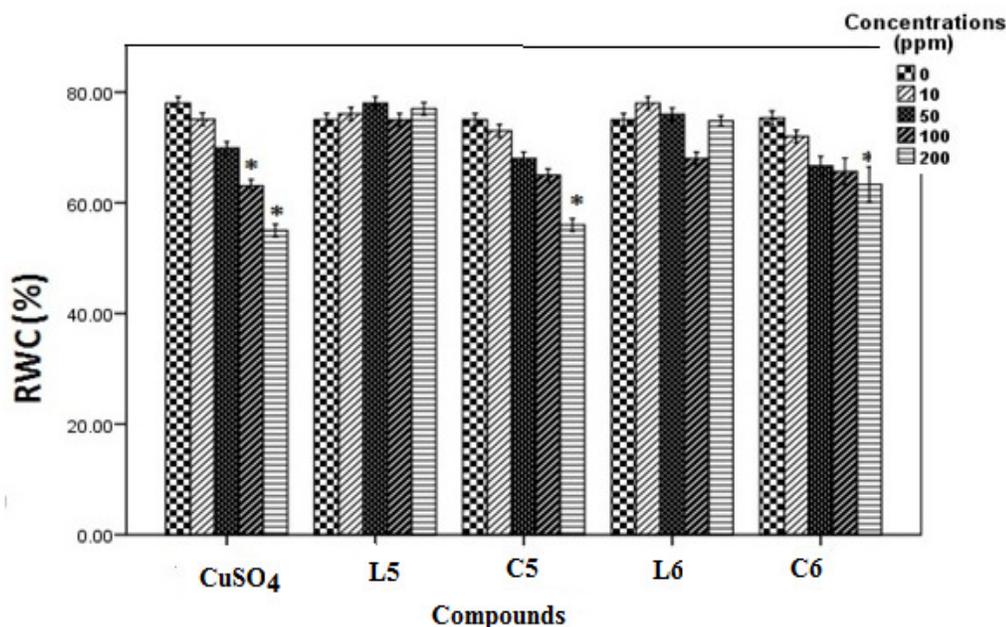
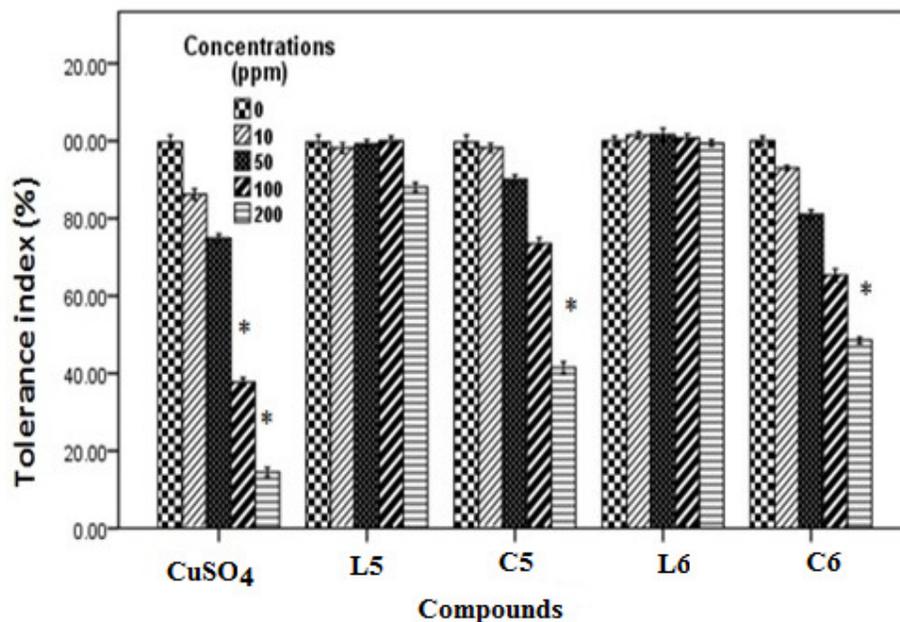


Fig 4.2. Dry weight (g plant<sup>-1</sup>) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3.  
\*The mean difference is significant at the 0.05 level.



**Fig 4.3.** Relative water content (RWC) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level.

For instance at 100 and 200 ppm CuSO<sub>4</sub> significantly reduces RWC by 22.99% where as C5 and C6 complex reduce only by 12.01% and by 12.34%, respectively over the control. Greater negative impact of CuSO<sub>4</sub> than the Schiff base complexes (C5 and C6) on seedlings was further approved by the Tolerance index (TI). TI for C5 and C6 complex treated plants appeared to be significantly higher than that of CuSO<sub>4</sub> treated plants (illustrated in Fig 4.4). Similar type of negative effect of CuSO<sub>4</sub> was also reported by *Azooz et al.*<sup>15</sup> in wheat seedlings. *Verma et al.*<sup>16</sup> also reported that the application of copper at lesser concentrations enhanced the plant's dry biomass, while excess use of copper reduced the biomass production of these plants.

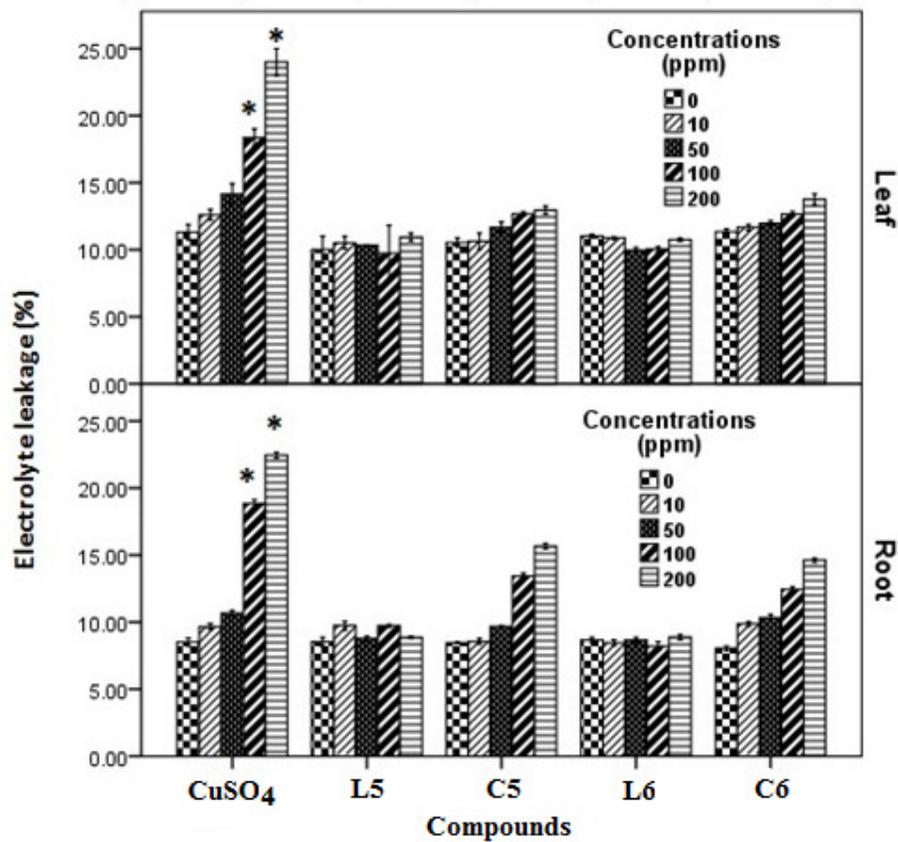


**Fig 4.4.** Tolerance index (TI) of 45days old rice seedlings. Mean  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.

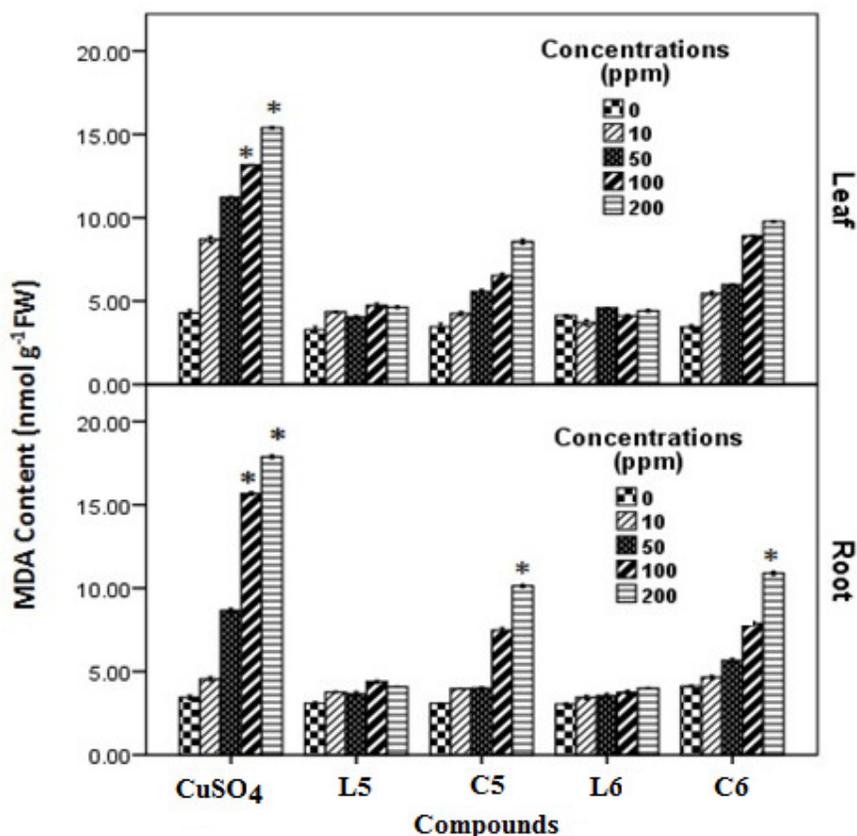
#### 4.3.2.2. Membrane damage and ion leakage

Copper can distress the membrane permeability by oxidation of membrane lipids and this effect can be accessed from the increase of malonyldialdehyde accumulation (MDA), one of the lipid peroxidation products. Data obtained from this study revealed copper induced membrane damage which can be expressed in terms of electrolyte leakage. Lipid peroxidation enhanced significantly in CuSO<sub>4</sub> treated plants with increasing concentration but in case of the Schiff base complex (C5 and C6) treated plants malonyldialdehyde accumulation as well as electrolyte leakage were less as compared to CuSO<sub>4</sub> treated plants with respect to the control indicating comparatively less membrane damage in the Schiff base complex (C5 and C6) treated plants.



**Fig 4.5.** Electrolyte leakage (%) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.



**Fig 4.6.** MDA content of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.

The outcomes showed that the MDA content in leaf (illustrated in Fig 4.6) and electrolyte leakage in leaf and root (illustrated in Fig 4.5) of the Schiff base complex (C5 and C6) treated plants increase with increasing dose but did not echo any significant changes. But in case of CuSO<sub>4</sub> treated plants ion leakage and oxidation of membrane lipid enhanced drastically at 100 and 200 ppm level with respect to the control plants. Reactive oxygen species (ROS) induced plasma membrane damage increase MDA and ion leakage at the higher levels of Cu(II) ion.<sup>17</sup> Hydrogen peroxide [one form of reactive oxygen species (ROS)] accumulation is minimum in case of the Schiff base Cu(II) complex (*i.e.*, C5 and C6) treated plants than in CuSO<sub>4</sub> treated plants (illustrated in Fig 4.7).

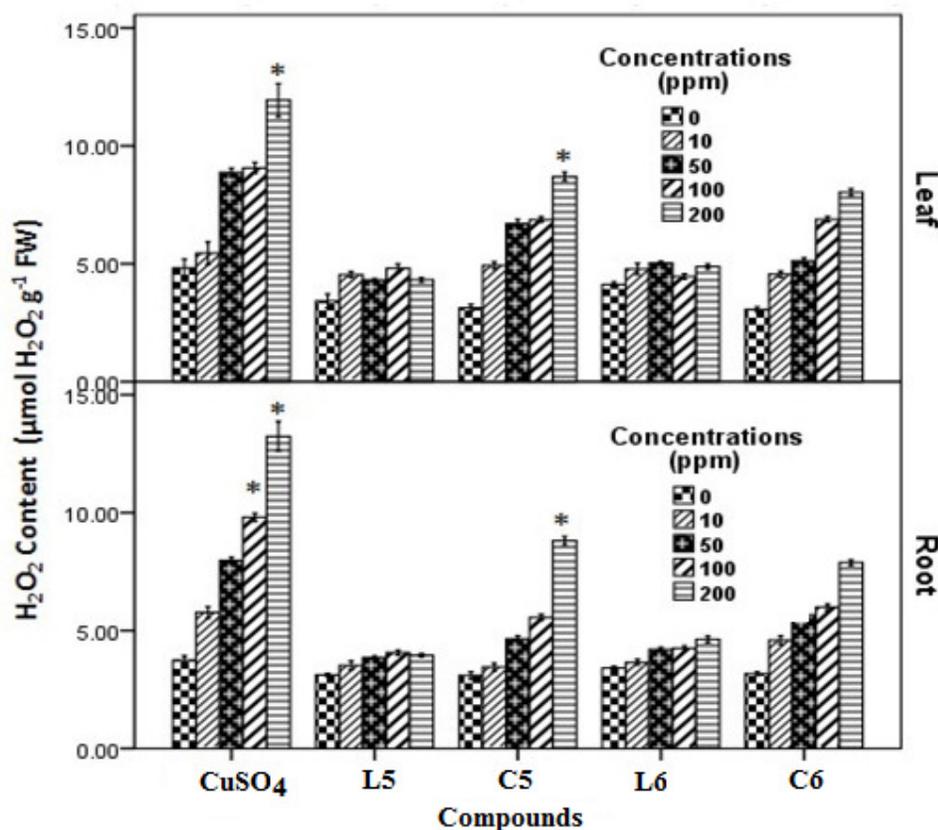


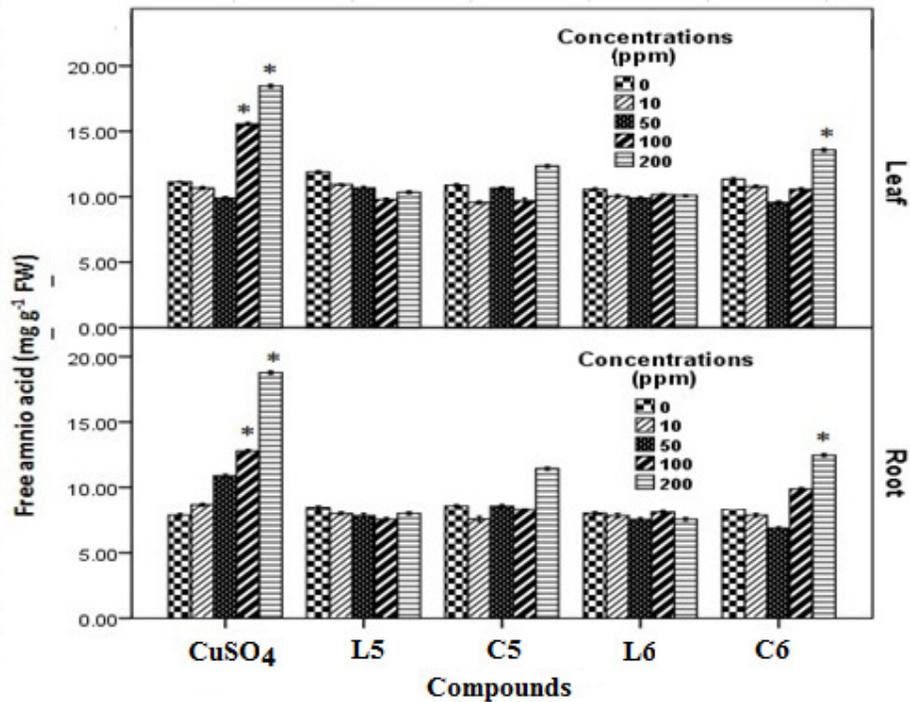
Fig 4.7. H<sub>2</sub>O<sub>2</sub> accumulation in 45 days old rice seedlings. Mean  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.

In copper sulfate treated plants H<sub>2</sub>O<sub>2</sub> increased 1.35 fold at 200 ppm whereas in Schiff base complex treated plants especially in case of C6 complex H<sub>2</sub>O<sub>2</sub> accumulation was less (0.62 fold) in relation to control. This observations appear to be due to the redox-active character of Cu<sup>2+</sup> ion resulting in creation of extremely reactive hydroxyl radicals.<sup>18</sup> These results suggest that the Schiff base Cu(II) treated plants had better ability to tolerate Cu<sup>2+</sup> ion stress. The significant increase of MDA in plants, exposed to CuSO<sub>4</sub> indicated that the increase of lipid peroxidation in Cu-treated plants led to disorder of plasma membranes, whereas plasma membrane damage was less in case of the Schiff base Cu (II) complex treated plants.

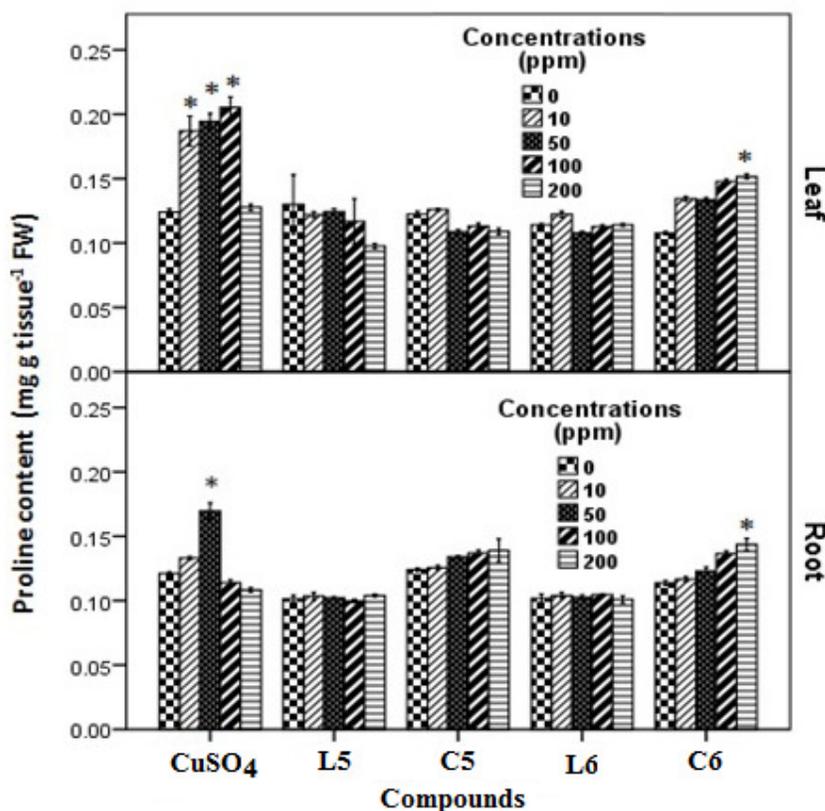
#### 4.3.2.3. Total free amino acids and proline

Accumulations of total free amino acids (illustrated in Fig 4.8) were significantly increased in root and leaf tissues at higher CuSO<sub>4</sub> concentrations. However, low CuSO<sub>4</sub> concentrations up to 50 ppm had non-significant enhancement



**Fig 4.8.** Free amino acid accumulation in 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level.

on the total free amino acids. The highest increase in free amino acids in case of CuSO<sub>4</sub> treatment was noticed at 100 and 200 ppm in both leaf and root tissues. On the contrary both the Schiff base Cu(II) complex treated plants showed insignificant enhancement of the amino acids. *Al-Hakimi and Hamada*<sup>19</sup>, in their study, suggested that free amino acids content enhances in plant tissues upon Cu<sup>2+</sup> ion exposure. In agreement the amino acids are looked upon as key player in metal chelation through which plant detoxify/alleviate heavy metal stresses.<sup>20</sup> Therefore, it might be suggested that the plants experiencing higher amount of copper induced oxidative stress can accumulate greater amount of free amino acids to alleviate oxidative stress. In that scenario the copper Schiff base complexes (C5 and C6) are proved to be less toxic than CuSO<sub>4</sub>.



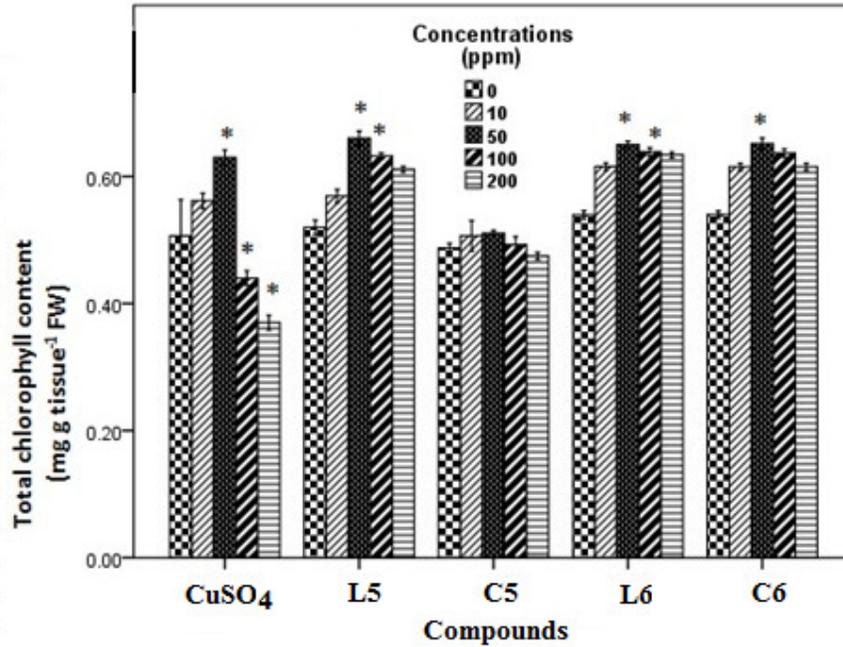
**Fig 4.9.** Proline level in 45 days old rice seedlings. Means  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.

Proline accumulation in plant tissues, an indicator of oxidative stress,<sup>21</sup> increased in all the treatments. In CuSO<sub>4</sub> treated plants proline accumulation was maximum at 100 ppm and at 50 ppm in leaf and root tissues, respectively. Beyond this concentration proline accumulation decreased. Decline in proline accumulation may be attributed to reduction competence of the plants to withstand oxidative stress.<sup>22</sup> Conversely, in the Schiff base Cu(II) complex treated plants, proline content continued to be increased up to 200 ppm signifying lesser oxidative stress in those plants (illustrated in Fig 4.9). Proline accumulation indeed stabilizes plasma membrane free radical scavenger and some macro molecules and thus facilitates rapid recovery from heavy metal stress.<sup>23</sup>

#### 4.3.2.4. Photosynthetic pigments

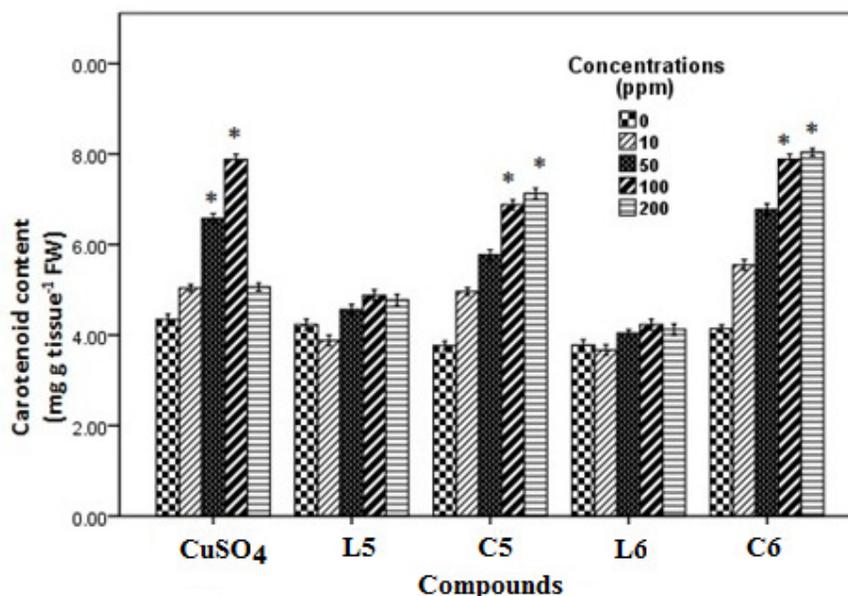
Copper facilitates in the utilization of iron during chlorophyll synthesis and enhances photosynthesis at low concentration. But at higher concentration copper reduces photosynthetic competence, low quantum efficiency of Photosystem-II and reduced cell elongation.<sup>24</sup> The present study revealed that total chlorophyll content



**Fig 4.10:** Total chlorophyll content in 45 days old rice seedlings. Mean  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.

increased in case of all the treatments up to 50 ppm. Beyond this concentration total chlorophyll content reduced, whereas the reduction was maximum in case of CuSO<sub>4</sub> treated plants and minimum in case of C6 treated plants (illustrated in Fig 4.10). Less reduction of chlorophyll may be attributed to less copper toxicity of the Schiff base complexes (C5 and C6) to plants. Carotenoid, a non enzymatic antioxidant, involved in quenching of oxidizing species participates in disrupting regular cellular functioning. In CuSO<sub>4</sub> treated plants carotenoid content increased gradually up to 100 ppm. At 200 ppm carotenoid content reduced drastically signifying the huge toxicity of CuSO<sub>4</sub> but on the contrary for C5 and C6 treated plants the carotenoid content continued to increase up to 200 ppm (illustrated in Fig 4.11).



**Fig 4.11.** Carotenoid content in 45 days old rice seedlings. Means  $\pm$  SE, n = 3.

\*The mean difference is significant at the 0.05 level.

#### 4.4. Conclusions

The present study reveals that Schiff base Cu(II) complexes (C5 and C6) have less toxic effects than copper sulphate on rice seedlings and provide better tolerance to copper toxicity than copper sulphate. Maximum positive impact of the Schiff base complexes was found mostly at 50 ppm concentration. Though different stress marker and reactive oxygen species accumulation were less and minimum pigment damage was noticed in the Schiff base Cu(II) complex treated seedlings but the optimum positive impact of these Schiff base Cu(II) complexes largely depends on the dose and beyond certain concentration these complexes may have inhibitory effects on rice plants. The Schiff base Cu(II) complexes (C5 and C6) can thus be used as a potential supplement to meet up micronutrient deficiency and at the same time such complexes can minimize the toxicity generated by application of different ionic form of Copper ( $\text{Cu}^{2+}$ ) ion.

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## **CHAPTER V**

### **Efficacy of a multi-dentate Schiff base and its vanadyl complex on various morphological and biochemical parameters of *Vigna radiata L.***

#### **5.1. Introduction**

Vanadium, one of the important trace elements for plants, is found scattered in the environment through the leaching of rocks, combustion of coal or petroleum products and residual slag from the steel industry.<sup>1</sup> Although Vanadium was discovered in early eighteenth century, very little work was done on Vanadium before the 1950s.<sup>2</sup> Initial studies showed that Vanadium is toxic for most of the plant species and that is why there was very little interest in evaluating its effects on plants.<sup>3</sup> However, interest intensified when Arnon and Wessel (1953) concluded that Vanadium is essential for some plants.<sup>4</sup> Subsequent studies showed that Vanadium is generally toxic to terrestrial plants when applied in amounts greater than pico-molar levels.<sup>5</sup> But it has been found to be beneficial for plant growth and development when applied in trace amount.<sup>6,7</sup> Further studies indicated that for Vanadium due to its various oxidation states (-I to +V) toxicity varies. It was also found that the pentavalent state ( $V^{5+}$ ) is more toxic than the corresponding tetravalent state ( $V^{4+}$ ).<sup>8</sup> Moreover  $V^{4+}$ , predominantly found in the soil, is responsible for the development of the plants.<sup>9</sup> Study revealed that Vanadium acts as constituent of the cofactors in vanadate-dependent haloperoxidases and vanadium nitrogenase.<sup>10</sup> Many important electron transfer processes and plasma membrane hydrogen ( $H^+$ )-translocation ATPase are largely dependent upon vanadium.<sup>11</sup> The monomeric form of vanadate is both structurally and electronically identical with phosphate. This facilitates vanadate to inhibit or to activate the enzymes which interact with phosphorylated species.<sup>12</sup>

Generally Vanadium contained fertilizers (*e.g.*,  $NH_4VO_3$ ) are used to provide vanadium to plants in optimum level. These types of fertilizers, being ionic in nature, are responsible for the alteration of pH of the soil.<sup>13</sup> So now-a-days, more emphasis is given to metal chelates, being less reactive, can solve the vanadium deficiency for longer period of time without making the medium toxic.<sup>14,15</sup> Inspired by these facts, a polydentate ( $N_2O_2$  donor type) Schiff base ligand (**L7**) and its vanadyl complex (**C7**) was synthesized and their effects on Mung bean (*Vigna radiata L.*) were thoroughly monitored. Mung bean was chosen as plant material because of its global importance

as a pulse. It is grown in South, East and Southeast Asia where 90% of global production currently occurs.<sup>16</sup> Mung bean provides significant amounts of protein, carbohydrates and a range of micronutrients in diets. Its cultivation is also important as it maintains the soil fertility through nitrogen fixation. Therefore herein this chapter the responses of vigna seedlings to vanadium toxicity when exposed to  $\text{NH}_4\text{VO}_3$  and the vanadyl complex were thoroughly studied in terms of relative water content, biochemical components, oxidative stress markers and overall tolerance level, *etc.*

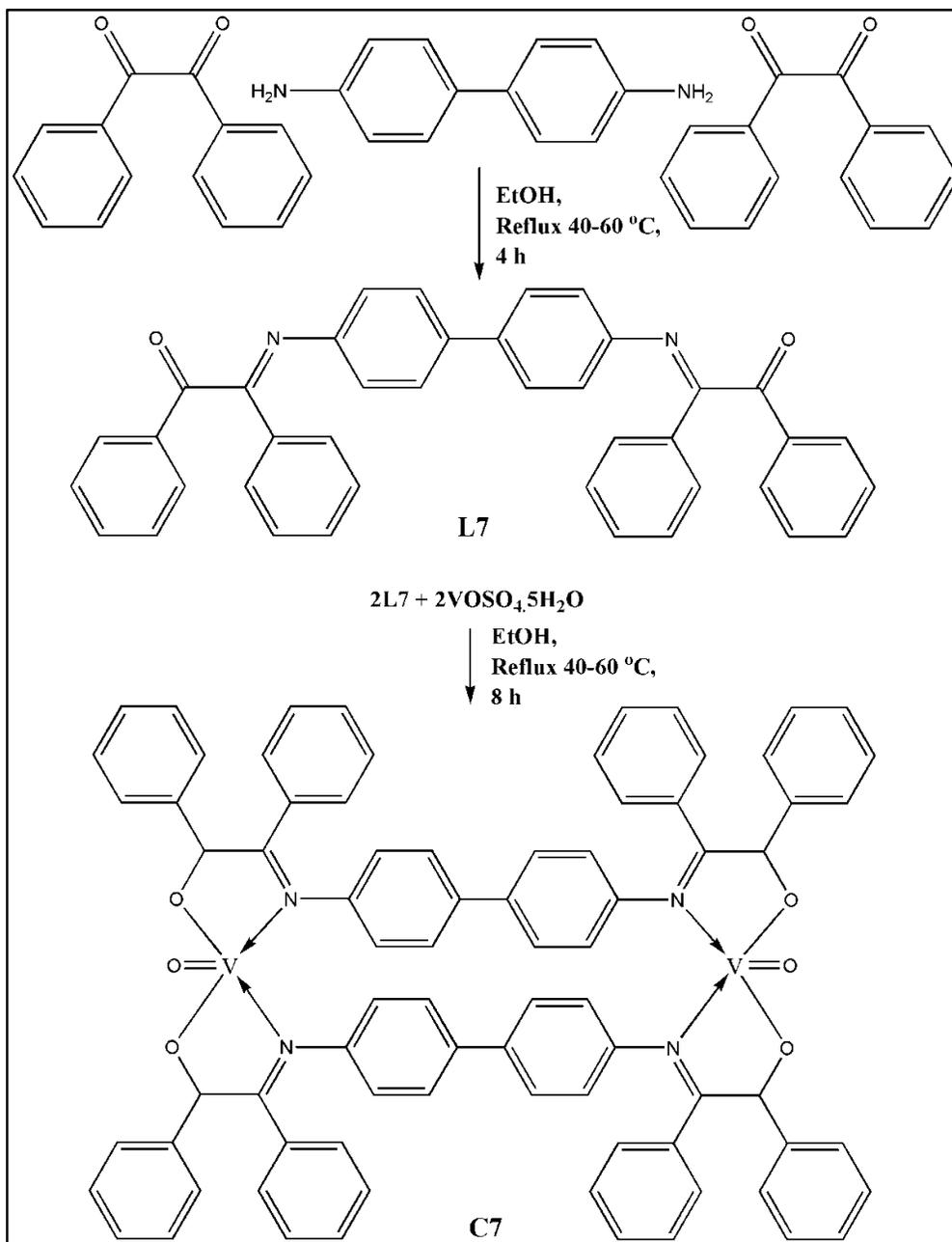
### **5.2. Materials and methods**

#### **5.2.1. Synthesis of the ligands and its vanadyl complex**

The synthesis of a polydentate Schiff base ligand (**L7**) and its vanadyl ( $\text{VO}^{2+}$ ) complex (**C7**) are discussed in Chapter II. These compounds were prepared by following literature method<sup>17</sup> (illustrated in Scheme 5.1) with slight alteration as required.

#### **5.2.2. Maintenance of the plants**

Vigna seeds were surface sterilized using 1% (w/v) sodium hypochlorite solution and rinsed with double distilled water. Seeds were then transferred to plastic pots (Diameter 11cm) containing sterile soil. Each pot contained five seeds and the pots were kept at the temperature of  $25 \pm 2$  °C for a photoperiod of 8 hour with 65-70% relative humidity regime. Seedlings were watered regularly every alternate day and one month plants were utilized for further experiments. After a growth period of 30 days, the plants in their vegetative phase were taken, roots were gently washed with sterile  $\text{H}_2\text{O}$  and transferred to 20% Steiner nutrient solution (1.8 mM  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 0.8 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 mM  $\text{KH}_2\text{PO}_4$ , 0.6 mM  $\text{KNO}_3$ , 0.6 mM  $\text{K}_2\text{SO}_4$ , 89.31  $\mu\text{M}$  Fe, 42.37  $\mu\text{M}$  Mn, 7.12  $\mu\text{M}$  Zn, 39.98  $\mu\text{M}$  B, 2.93  $\mu\text{M}$  Cu, 1.80  $\mu\text{M}$  Mo). The plants were then allowed to acclimatize in this solution for 48 h. After 48 h of acclimation, this nutrient solution was entirely replaced and treatments were applied in the renewed nutrient solution with different concentrations (5, 10, and 20  $\mu\text{M}$ ) of the Schiff base ligand (**L7**), its vanadyl complex (**C7**) and  $\text{NH}_4\text{VO}_3$  (**AV**) along with a control (no treatment of nutrient solution the ligand, the complex and  $\text{NH}_4\text{VO}_3$ ) separately for 7 days. Each treatment had three replicate sets and experiment was conducted in randomized design method. After 7 days leaf samples were collected, freeze in liquid nitrogen and subsequently used for biochemical tests.



**Scheme 5.1.** Preparation of the ligand (L7) and its vanadyl complex (C7).

The fresh weight of seedlings was taken immediately after sampling to avoid any water loss from leaf samples. Different growth and biochemical parameters were monitored as detailed in Chapter II.

### 5.3. Results and Discussion

#### 5.3.1. Characterization of the ligand and its vanadyl complex

The analytical and spectral data recorded for the Schiff base (L7) and its vanadyl complex (C7) were found to be almost as same as reported in the literature.<sup>17</sup> Some of the characteristic analytical and spectral data are listed in Tables 5.1 and 5.2. Characteristic IR bands at 1620, 1731 and 3375  $\text{cm}^{-1}$  appeared due to  $\nu_{\text{C=N}}$ ,  $\nu_{\text{C=O}}$  and  $\nu_{\text{O-H}}$  vibrations, respectively for the ligand (L7). After complexation the  $\nu_{\text{C=N}}$  band shifted to 1624  $\text{cm}^{-1}$  due to coordination bond formation. For the complex two new bands appeared at 982 and 491  $\text{cm}^{-1}$  due to  $\nu_{\text{V-O}}$  and  $\nu_{\text{V-N}}$  vibrations, respectively. The electronic spectra were measured with  $5 \times 10^{-4}$  molar solutions in dimethylformamide for both the compounds. The ligand (C7) has a characteristic  $\lambda_{\text{max}}$  at 263.1 nm due to  $\pi-\pi^*$  transition and its vanadyl complex (C7) manifested three peaks at 844.7, 445.1 and 367.8 nm, respectively due to characteristic transitions as reported earlier in the literature.<sup>17</sup>

**Table 5.1.** Analytical data of the prepared the ligand and its vanadyl complex.

	Molecular Formula	Colour	Formula Weight	m. p ( $^{\circ}\text{C}$ )	% Found (Calculated)		
					C	H	N
L7	$\text{C}_{40}\text{H}_{28}\text{N}_2\text{O}_2 \cdot 2\text{H}_2\text{O}$	Olive green	605.67	230	78.75 (79.45)	4.96 (5.3)	5.28 (4.6)
C7	$\text{C}_{80}\text{H}_{56}\text{N}_4\text{O}_6\text{V}_2 \cdot 5\text{H}_2\text{O}$	Pale green	1361.28	> 280	70.01 (70.58)	4.59 (4.8)	5.33 (4.12)

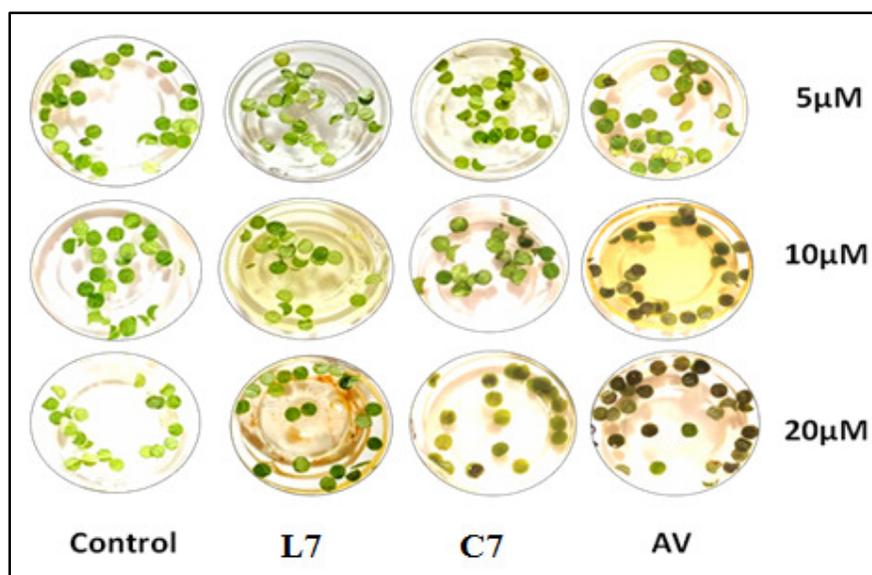
**Table 5.2.** Some characteristic infrared (in  $\text{cm}^{-1}$ ) and electronic spectral (in nm) data of the ligand (L7) and its vanadyl complex (C7).

	$\nu_{\text{C=N}}$	$\nu_{\text{O-H}}$	$\nu_{\text{C=O}}$	$\nu_{\text{V-O}}$	$\nu_{\text{V-N}}$	$\lambda_{\text{max}}$
L7	1620	3375	1731	-	-	263.1
C7	1624	3223	1731	982	491	844.7, 445.1, 367.8

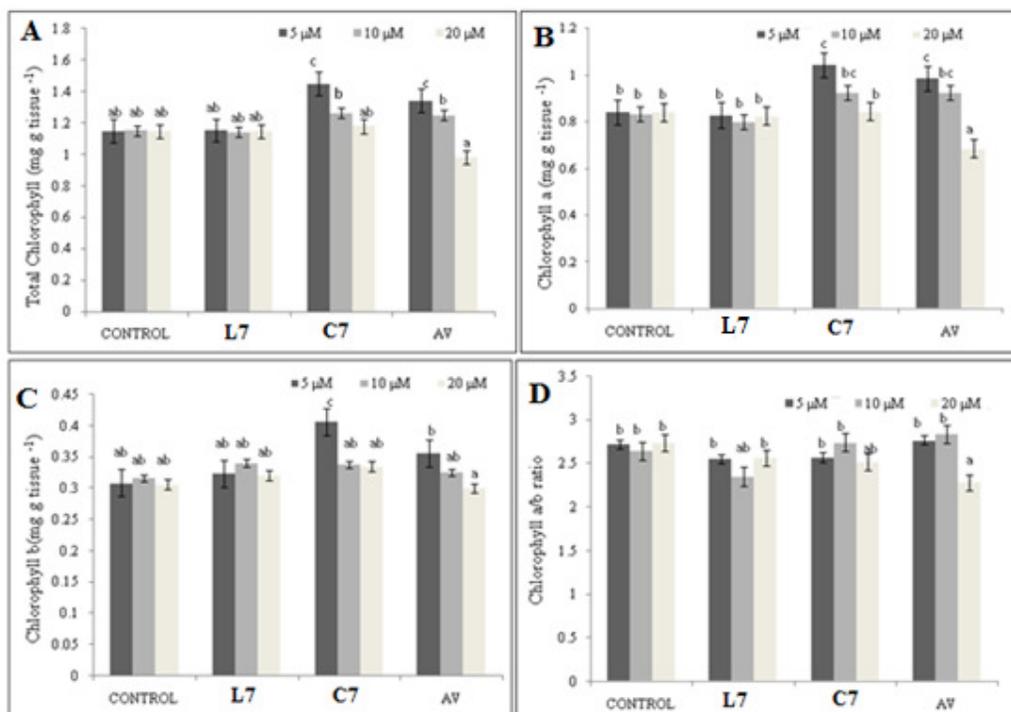
### 5.3.2. Effects of the ligand and its vanadyl complex on vigna seedlings

#### 5.3.2.1. Leaf disc bioassay

Vanadium sensitivity of mung leaf was determined by leaf disc senescence bioassay. It is represented in terms of degree of leaf decolouration and percentage(%) decrease of the chlorophyll content of the detached leaves at the concentration range 5 $\mu$ M, 10 $\mu$ M and 20 $\mu$ M of L7, C7 and NH<sub>4</sub>VO<sub>3</sub> in comparison to the detached leaves kept in sterile distilled water. For control and L7 treated *Vigna* seedlings, the leaf colour and chlorophyll content were almost alike after 7 days of treatment indicating that L7 ligand don't have negative impact on seedlings (as illustrated in Figs 5.1 and 5.2). The leaf discs turned slightly blackish when kept at 20 $\mu$ M concentration of NH<sub>4</sub>VO<sub>3</sub> for 7 days. On the contrary, leaf decolouration was found to be least for C7 treated leaf discs indicating less negative impact of the Schiff base complex (C7) on seedlings chlorophyll.



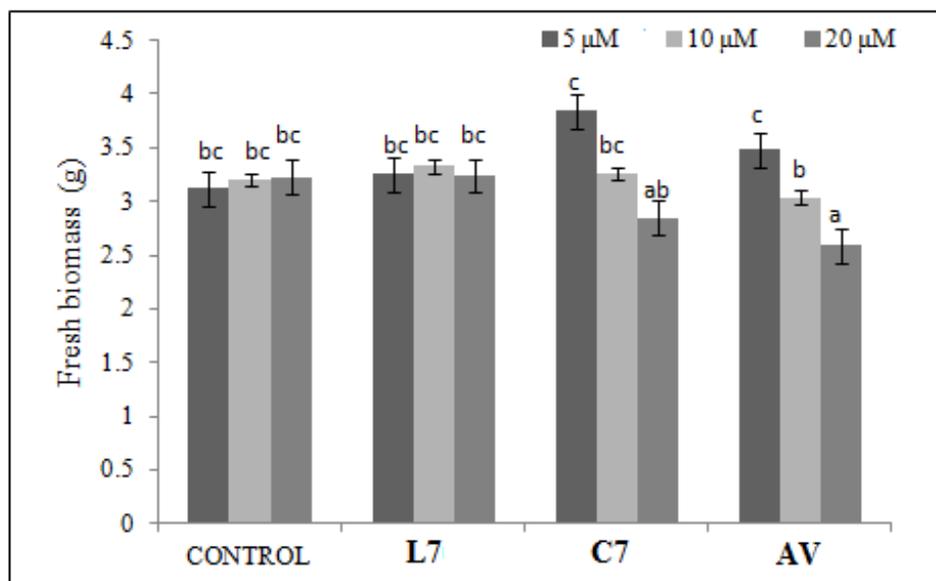
**Fig 5.1.** Leaf senescence assay: phenotypic changes observed as chlorophyll bleaching in response to 5 $\mu$ M, 10 $\mu$ M and 20 $\mu$ M of L7, C7 and NH<sub>4</sub>VO<sub>3</sub> for 7 days.



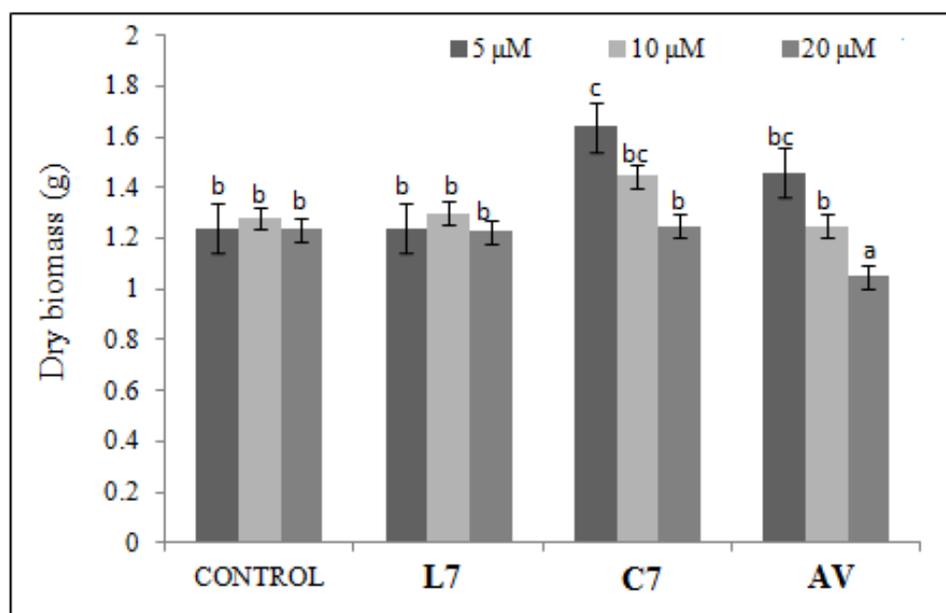
**Fig 5.2.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on: A, total chlorophyll content; B, chlorophyll a content; C, chlorophyll b content and D, chlorophyll a/b ratio of mung bean at various concentrations. Values are represented as mean  $\pm$  SD (n=3). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischers LSD set.

### 5.3.2.2. Fresh biomass, dry biomass and relative water content

Plants from different treatment sets were harvested and weighed to get fresh biomass. Subsequently to get dry biomass the samples were parched in a hot air oven at  $70^\circ\text{C}$  for 48 h. The outcomes reveal that plants treated with the complex (C7) were able to retain higher percentage of fresh mass and dry mass over the period of time than  $\text{NH}_4\text{VO}_3$  treated plants with increasing concentration suggesting less toxicity of the Schiff base complex (C7) (as illustrated in Figs 5.3 and 5.4). Same trend has been observed for relative water content (RWC). Drastic decrease in relative water content at higher concentration for  $\text{NH}_4\text{VO}_3$  treated plants signified higher stress in cells (shown in Table 5.3).



**Fig 5.3.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on fresh biomass of mung bean at various concentrations. Values are represented as mean  $\pm$  SD (n=3). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischers LSD set.



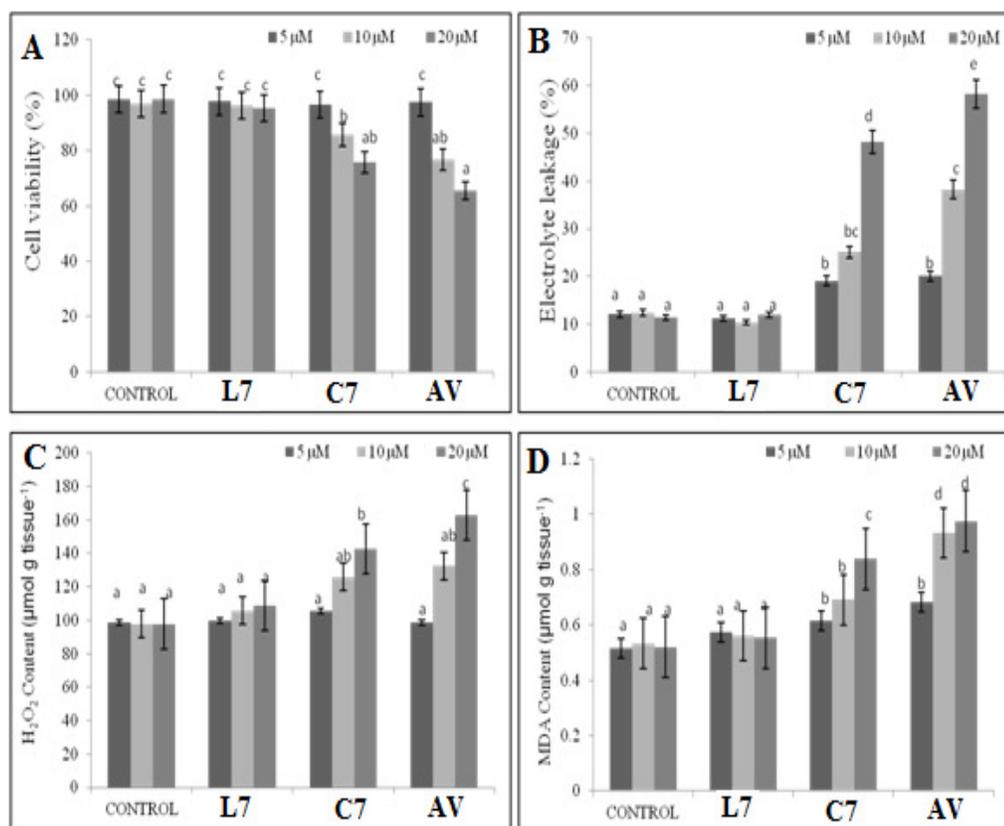
**Fig 5.4.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on dry biomass of mung bean at various concentrations. Values are represented as mean  $\pm$  SD (n=3). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischers LSD set.

**Table 5.3.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on relative water content (RWC) of mung bean at various concentrations. Values are represented as mean  $\pm$  SD (n=3). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischers LSD set.

Concentration	RWC (%)			
	CONTROL	L7	C7	AV
5 $\mu\text{M}$	81.29 $\pm$ 2.12c	81.16 $\pm$ 1.95	81.53 $\pm$ 1.45c	80.75 $\pm$ 2.01c
10 $\mu\text{M}$	81.60 $\pm$ 2.45c	81.29 $\pm$ 1.56	76.63 $\pm$ 1.58c	68.29 $\pm$ 1.92b
20 $\mu\text{M}$	81.53 $\pm$ 2.74c	79.92 $\pm$ 1.87c	68.93 $\pm$ 1.94b	59.75 $\pm$ 1.86a

### 5.3.2.3. Oxidative stress

Plants facing adverse conditions produce reactive oxygen species at vital cell organelles like chloroplast, mitochondria and peroxisomes. These reactive oxygen species (ROS) are formed as a byproduct of plant aerobic metabolism.<sup>18-20</sup> Amongst a variety of ROS,  $\text{H}_2\text{O}_2$  is highly stable and can remain in cell causing damage to cell viability and induce senescence. ROS also increases lipid peroxidation in both cellular and organelle membranes and thus further induce membrane injury, protein degradation and thereby affects photosynthesis.<sup>21,22</sup> Lipid peroxidation produces malonaldehyde as the end product in a chain of reactions with membrane phospholipid molecules.<sup>23</sup> Application of beneficial elements in a dose dependent manner can modulate metabolic functions positively to favor plant vigour development. These effects can further vary depending on dose frequency, chemical form and genotypes. Previous reports have suggested many basic elements including vanadium salt (mainly  $\text{NH}_4\text{VO}_3$ ) when applied in low concentrations can positively stimulate various plant functions and increase plant vigour and biomass but these elements may induce cellular toxicity and stress at high conc.<sup>24-26</sup> To have a better understanding of the adverse impact of different concentrations of the Schiff base  $\text{VO}^{2+}$  complex(C7) and  $\text{NH}_4\text{VO}_3$  (AV) on plants; stress markers such as electrolyte leakage (EL), Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and peroxidation of membrane lipids (MDA) and cell viability were assessed (illustrated Fig 5.5).



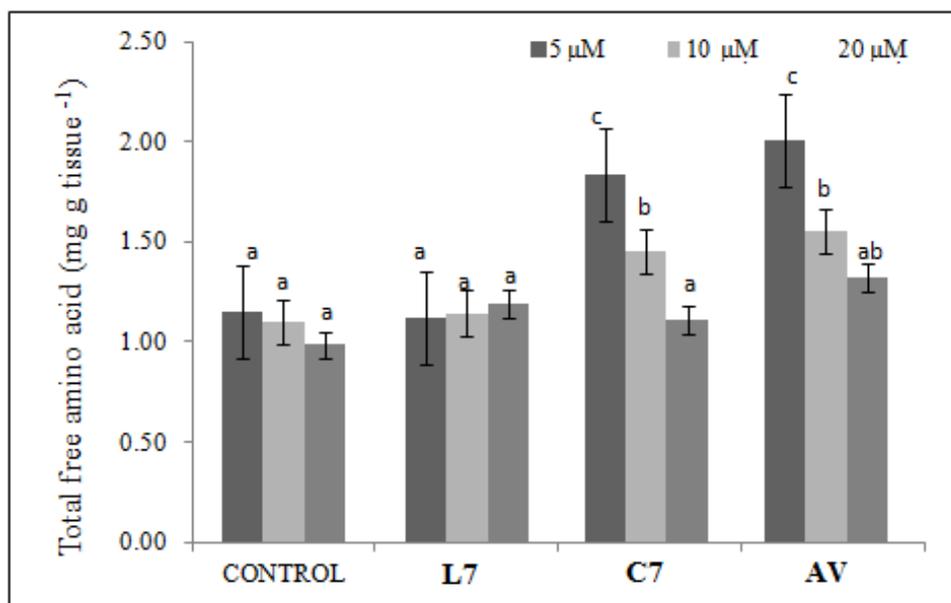
**Fig 5.5.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on: A, Cell viability; B, Electrolyte leakage; C,  $\text{H}_2\text{O}_2$  content and D, MDA content respectively of mung bean at various concentrations. Values are represented as mean  $\pm$  SD ( $n=3$ ). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischer's LSD set.

In all the plants, electrolyte seepage from the membranes increased gradually with the increasing concentration of the Schiff base complex (C7) and  $\text{NH}_4\text{VO}_3$  (AV) whereas in L7 there were no such changes in these parameters in relation to control. At 5  $\mu\text{M}$  level electrolyte leakage (EL) remained similar for both C7 and AV whereas 10  $\mu\text{M}$  and 20  $\mu\text{M}$  of AV caused considerable membrane leakage as compared to C7 treated plants.  $\text{H}_2\text{O}_2$  accumulation was pretty similar in the Schiff base complex (C7) and AV treated plant leaves up to 10  $\mu\text{M}$  while at 20  $\mu\text{M}$ , there was greater  $\text{H}_2\text{O}_2$  accumulation in AV treated plants as compared to those treated with C7. Similar trends were also noticed for membrane lipids peroxidation (MDA). Greater impact of the Schiff base complex (C7) treatment on MDA accumulation was observed at 20  $\mu\text{M}$  whereas in AV treated plants significant induction was observed from 20  $\mu\text{M}$ . Survival prospect of plants was measured in terms of cell viability. There was no significant

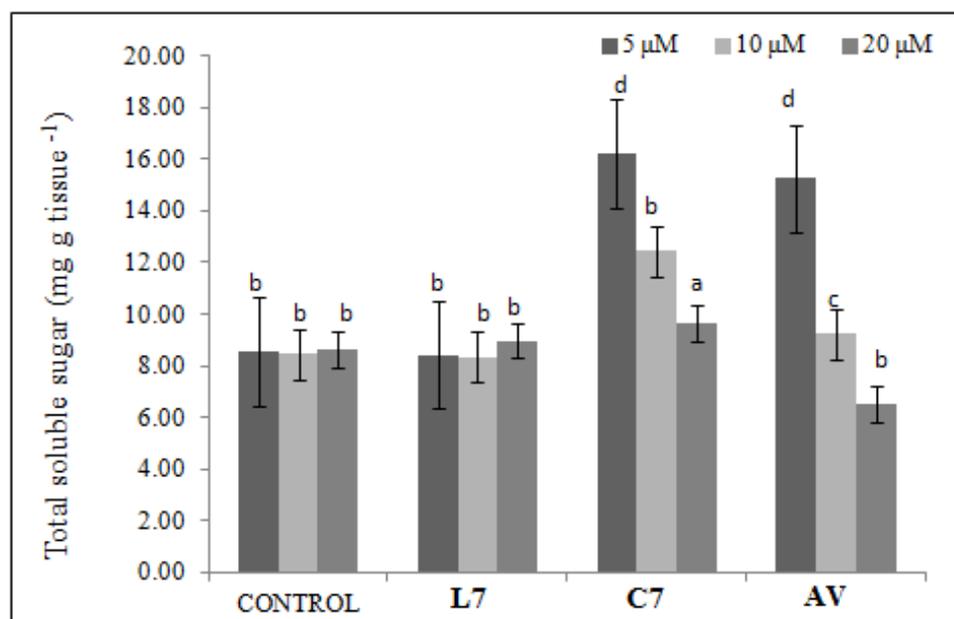
changes in cell viability across all concentrations in L7 treated plants and even in C7 and AV treated plants when given at 5 $\mu$ M. But beyond that there was gradual drop of cell viability in both the Schiff base complex (C7) and AV treated plants and this drop was found be slightly lesser in Schiff base complex treated plants. Findings of the present study suggest that the ligand (L7) has neither any of positive or negative impact on the accumulation of stress indicators. So far the Schiff base complex (C7) and AV both elicited certain level of membrane injury and ROS accumulation. While at low concentrations (5 $\mu$ M) both C7 and AV have similar impacts but at higher concentrations harmful effects of the Schiff base complex (C7) was comparatively lesser compared to those with AV regarding membrane injury and oxidative stress, *i.e.*, it can impart comparatively better cell survival.

#### **5.3.2.4. Total free amino acid and total soluble sugar**

Amino acids play as key player in metal chelation by which plant detoxify or alleviate heavy metal stress.<sup>27</sup> Therefore, it can be suggested that plants experiencing higher amount of vanadium induced stress can accumulate more amount of free amino acid. Results revealed that although both the C7 and AV are responsible for alleviating free amino level in vigna seedlings but the accumulation is much higher in AV treated plants. This signifies the more toxicity of AV than C7 for vigna plants (illustrated in Fig 5.6). Total soluble sugar content was also estimated. Higher sugar content in cell symbolizes less stress. Results show that both the C7 and AV are responsible for the increase of soluble sugar content at low concentrations (5 $\mu$ M). But at higher concentration (20 $\mu$ M) the soluble sugar content decrease drastically for AV than C7 treated plants (illustrated in Fig 5.7). This justifies that both the C7 and AV are beneficial for vigna plants at low concentrations but at higher concentrations AV become more toxic than C7.



**Fig 5.6.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on total free amino acid content of mung bean at various concentrations. Values are represented as mean  $\pm$  SD (n=3). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischer's LSD set.



**Fig 5.7.** Effects of the control, L7, C7 and  $\text{NH}_4\text{VO}_3$  (AV) on total soluble sugar content of mung bean at various concentrations. Values are represented as mean  $\pm$  SD (n=3). Bars with different letters are significantly different at  $P \leq 0.05$  according to Fischer's LSD set.

## **5.4. Conclusions**

Outcomes of the present experiment reveal that the Schiff base complex (C7) has less toxic effects than  $\text{NH}_4\text{VO}_3$  on mung bean seedlings and it also provide better tolerance to vanadium toxicity. Though different stress marker and reactive oxygen species (ROS) accumulation were less and minimum pigment damage was noticed in the Schiff base complex (C7) treated seedlings but the optimum positive impact largely depends on the dose. Beyond certain concentration the complex (C7) may show inhibitory effects on the plants. Therefore the present study revealed that heavy metal Schiff base complexes can be used as potential supplement to meet up micronutrient deficiency and at the same time such complexes can minimize the toxicity generated by application of different heavy metals.

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## **CHAPTER VI**

### **Concluding Remarks**

In the present dissertation various polydentate ligand systems and their transition metal complexes are synthesized and their effects on various morphological and biochemical parameters of selected plant systems are thoroughly studied. Three first row transition elements, viz., Zn, Cu and V have been taken for complexation and four plants system, viz., papaya, chickpea, rice and mung bean were selected to monitor the effects of above selected polydentate ligand systems and their metal complexes on various morphological and biochemical parameters of the selected plant systems.

In Chapter I a general introduction about micronutrients, their role in plants body and causes of micronutrient deficiencies have been given with special focus on how to solve the micronutrient deficiencies in an alternative environment friendly way. It also includes the driving force behind the synthesis of the Schiff base polydentate ligand systems with transition metal complexes to solve the micronutrient deficiencies in plants body.

Chapter II contains the experimental section briefly describing the chemicals and materials used for various experiments. The details of the instruments used for the physico-chemical characterization of the synthesized complexes and the protocols for measuring the various morphological and biochemical parameters were discussed briefly. This chapter also describes the procedures for synthesizing various Schiff base ligand systems and their transition metal complexes consisting of Zn, Cu and V, respectively.

In chapter III, morphological and biochemical effects of some  $N_2O_2$  donor Schiff bases and their  $Zn^{2+}$  complexes on *Carica papaya* L. and *Cicer arietinum* L. were presented. Schiff base polydentate ligands of types like salen and salophen can form stable complexes with  $Zn^{2+}$  and these metal complexes can act as a source of zinc to plant's body if they are used as micronutrient supplements. Inspired by these facts, four different Schiff base ligands and their  $Zn^{2+}$  complexes were first synthesized and then characterized by different analytical and spectroscopic techniques. To investigate their effects papaya and local chickpea seeds were treated with each ligands and their complexes and different morphological and biochemical parameters were monitored. Among all the ligands (L1-L4) and their complexes (C1-

## Chapter VI

C4) it was found that complex (C4) showed the maximum efficacy when treated as a micronutrient supplement for *Carica papaya* L. and *Cicer arietinum* L. The parity and correlation of all the parameters signified the better efficacy of the complex (C4) amongst all the complexes and thus it can be used as a potential seed soaking chemical for both the papaya and chickpea plants. Therefore these complexes specially the complex C4 can act as a potential source of Zn for plants.

In Chapter IV, morphological and biochemical effects of some azo functionalized  $N_2O_2$  donor Schiff bases and their  $Cu^{2+}$  complexes on rice plants (*Oryza sativa* L.) were presented. Azo fictionalized Schiff base ligands having  $N_2O_2$  donor binding sides are capable of forming stable metal complexes and such complexes can be used as potential plant micronutrient supplier. Two different ligands and their copper complexes were synthesized by conventional protocols and then characterized by both spectroscopic and elemental analyses. Investigations were done by taking rice seeds as plant material. Various growth and biochemical parameters were monitored by taking different concentrations of  $CuSO_4$ , the prepared ligands (L5 and L6) and their Cu(II) complexes (C5 and C6). Analysis of various biochemical results reveal that the Schiff base Cu(II) complexes (C5 and C6) have less toxic effects than copper sulphate on rice seedlings and thus facilitates better tolerance to copper toxicity than copper sulphate.

In Chapter V, efficacy of a  $N_2O_2$  donor Schiff base (L7) and its vanadyl complex (C7) on various morphological and biochemical parameters of mung bean (*Vigna radiata* L.) were presented. The Schiff base polydentate ligand was synthesized by the condensation of benzidine and benzil in ethanolic medium. The formed ligand was condensed further with  $VOSO_4 \cdot xH_2O$  to get the corresponding vanadyl complex. Both the ligand and the complex were characterized by spectroscopic and elemental analyses. Mung bean was selected as a plant material. Various morphological and biochemical parameters, *e.g.*, leaf senescence assay, chlorophyll content, different reactive oxygen species (ROS) were estimated and were compared to those with ammonium vanadate. Outcomes of the experiments revealed that the Schiff base complex has less toxic effects than ammonium vanadate on mung bean seedlings and provide better tolerance to vanadium toxicity. Though different stress marker and reactive oxygen species accumulation were less and minimum

pigment damage was noticed in the Schiff base complex (C7) treated seedlings but the optimum positive impact largely depends on the dose. Beyond certain concentration the complex may show inhibitory effects on the plants. Therefore the present study revealed that heavy metal Schiff base complexes can be used as potential supplement to meet up micronutrient deficiency and at the same time such complexes can minimize the toxicity generated by application of different heavy metals.

Deficiency of micronutrients in soil is a global problem. It is always challenging to solve the micronutrient deficiency by adding supplements without hampering the environment. Conventional micronutrient supplements, being mostly ionic in form, in most of the cases are responsible for the alteration of the pH of the medium. Such challenges always bring the opportunities to develop unique alternative methods having synergistic behavior. The synergistic behavior leads to improve performance of materials and substantially make the environment sustainable.

In the present dissertation, an attempt has been undertaken to synthesize very well known Schiff base transition metal complexes and to compare their efficacy over conventional micro nutrients supplements when applied in plants material. Although these complexes are well known but literature survey revealed that very little works wherein such complexes are used as micronutrient supplements were performed. It has been found that these Schiff base transition metal complexes are often superior to conventional micronutrient supplements in various concentration ranges. The procedures of preparations of these compounds are very simple and thus can be synthesized in large scale. Therefore heavy metal Schiff base complexes can be used as a supplement to meet up micronutrient deficiency and at the same time they can minimize the toxicity generated by application of different heavy metals. However these aspects must be further enquired at multi-locations and varied environmental conditions to draw a direct conclusion about the superiority. Anyway, this simple field of research may stand valuable as far as environmental issues are concerned in agricultural sector worldwide and thus such research can open up a new dimension of research in chemical sciences in conjunction with horticulture.

# APPENDIX I

## List of Publications

1. Efficacy of N<sub>2</sub>O<sub>2</sub> donor Schiff bases and their Zn<sup>2+</sup> complexes on various morphological and biochemical parameters of *Cicer arietinum* L. *Journal of Plant Nutrition*, 2018, 41(4), 487-496. DOI:10.1080/01904167.2017.1385810
2. Responses of *Oryza sativa* L. towards Azo Functionalised Schiff base Cu(II) Complexes and CuSO<sub>4</sub>: A Comparative Biochemical Study *Oriental Journal of Chemistry*, 2018, 34(4), 1991-2001 DOI:10.13005/ojc/3404037
3. A partial derivatives approach for estimation of the viscosity Arrhenius temperature in N,N-dimethylformamide + 1,4-dioxane binary fluid mixtures at temperatures from 298.15 K to 318.15 K *Physics and Chemistry of Liquids*, 2016, 54(5), 17 DOI:10.1080/00319104.2016.1139707
4. Synthesis, characterization and antibacterial studies of Mn(II) and Co(II) complexes of an ionic liquid tagged Schiff base, *J. Serb. Chem. Soc.*, 2016, 81(10), 1151-115, DOI:10.2298/JSC160425065S
5. Effect of some Schiff base ligands and their Zn (II) complexes on germination and seedling growth of papaya (*Carica papaya* L.) *Indian Journal of Advances in Chemical Science*, 2015, 3(2), 141-146.
6. Efficacy of a multi-dentate Schiff base and its vanadyl complex on various morphological and biochemical parameters of *Vigna radiata* L. (*Communicated*)

## APPENDIX III

### **Seminar, Symposium & Conventions Attended**

- **16<sup>th</sup> CRSI National Symposium in Chemistry**, organized by Indian Institute of Technology Bombay Powai, Mumbai, India during February 7-9, 2014 (**Presented a Poster**).
- **National Seminar on Frontiers in Chemistry 2014** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on March 11<sup>th</sup> & 12<sup>th</sup>, 2014 (**Presented an Oral Presentation**).
- **17<sup>th</sup> CRSI National Symposium in Chemistry**, organized at CSIR- National Chemical Laboratory, IISER, Pune (India) held from 7-9 February, 2015 (**Presented a Poster**).
- **National Seminar on Frontiers in Chemistry 2015** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on February 17<sup>th</sup> & 18<sup>th</sup>, 2015 (**Presented an Oral Presentation**).
- 
- **International Symposium on Sustainable Horticulture**, organized by Mizoram University, Aizawl, India during 14<sup>th</sup> -16<sup>th</sup> March, 2016 (**Presented an Oral Presentation**).
- **53<sup>rd</sup> Annual Convention of Chemists 2016 (National Conference)** organized by the Indian Chemical Society at Gitam University, Vishakhapatnam, Andhra Pradesh, India held during December 27-29, 2016 (**Young Scientist Award for Oral Presentation**).
- **19<sup>th</sup> CRSI National Symposium in Chemistry**, organized by Department of Chemistry, University of North Bengal, Darjeeling, India held from July 14<sup>th</sup>-16<sup>th</sup> 2017 (**Presented a Poster**).
- **54<sup>th</sup> Annual Convention of Chemists 2017 (National Conference)** organized by the Indian Chemical Society at Uka Tarsadia University, Bardoli, Surat, Gujrat, India during December 23-25, 2017 (**Presented an Oral Presentation**).
- **National Seminar on Frontiers in Chemistry 2017-18** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on September 14<sup>th</sup> 2017 (**Presented an Oral Presentation**).

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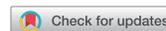
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## Efficacy of N<sub>2</sub>O<sub>2</sub> donor schiff bases and their Zn<sup>2+</sup> complexes on various morphological and biochemical parameters of *Cicer arietinum* L.

Kaushik Acharjee<sup>a</sup>, Jayanwita Sarkar<sup>b</sup>, Prahlad Deb<sup>c</sup>, Usha Chackraborty<sup>b</sup>, and Biswajit Sinha <sup>a</sup>

<sup>a</sup>Department of Chemistry, University of North Bengal, Darjeeling, West Bengal, India; <sup>b</sup>Department of Botany, University of North Bengal, Darjeeling, West Bengal, India; <sup>c</sup>Division of Horticulture, Institute of Agriculture, Visva-Bharati University, Sriniketan, West Bengal, India

### ABSTRACT

Schiff-base polydentate ligands types of salen and salophen can form stable complexes with Zn<sup>2+</sup> and these metal complexes can act as a source of zinc (Zn) to plant's body if they are used as micronutrient supplements. Inspired by these facts, four different Schiff-base ligands and their Zn<sup>2+</sup> complexes were first synthesized and then characterized by different analytical and spectroscopic techniques. To investigate their effects local chickpea seeds were treated with each ligands and complexes and different morphological and biochemical parameters were monitored. Among all the complexes and ligands it was found that the C4 complex, that is, [N,N'-(o-phenylene)bis-(3-methoxysalicylidenediamine)] monohydrate showed the maximum efficacy when treated as a micronutrient supplement for *Cicer arietinum* L. So that these complexes especially the C4 can act as an potential source of Zn.

### ARTICLE HISTORY

Received 24 August 2016  
Accepted 4 December 2016

### KEYWORDS

antioxidative enzymes;  
azomethine group; chickpea;  
germination parameters;  
metal chelates; plant growth

### Introduction

Zinc (Zn) is one of the most essential micronutrients for plant growth (Camp and Fudge 1945). A large number of biochemical processes in plants require Zn. It plays an important role in cellular metabolism (Rout, Samantaray, and Das 1999; Aravind, Narasimba, and Prasad 2005). Some biological processes, such as protein and carbohydrate metabolism, anti-oxidative defense, and various enzyme activities are largely dependent upon Zn (Vallee and Auld 1990). It serves as structural and catalytic components of several proteins and enzymes, which are required for growth and development. In order to increase the Zn level in plants, fertilizers containing Zn are being used frequently. Most of the Zn contained fertilizers are ionic in nature and have marked effect on pH of the medium and free electron activity (Thind and Rowell 1999; Toker, Canci, and Siddique 2010), as oxides, sulphates, and nitrates are present as counter ions in most fertilizers and these counter anions are responsible for the alteration of pH of medium (Brennan and Bolland 2006). Due to such adverse effects of inorganic metal – fertilizers more emphasis is being given to natural and synthetic metal chelates, which remain in un-reactive form and is quite capable of solving the micronutrient deficiency for a longer period of time (Khoshgoftarmanesh et al. 2010; Wallace and Wallace 1982). So synthesis of such eco-friendly metal containing organic species is the area of major concern for the scientific world.

**CONTACT** Biswajit Sinha, Assistant Professor  biswachem@gmail.com  Department of Chemistry, University of North Bengal, North Bengal, Darjeeling, 734013, India.

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Inspired by this, we have synthesized four different  $N_2O_2$  (that means two nitrogen and two oxygen atoms are there for chelation purpose) donor Schiff bases and their  $Zn^{2+}$  complexes and have monitored their effect on chickpea (*Cicer arietinum* L.), which is a common legume used widely in India as a pulse. The common factor among these ligands and complexes is the presence of azomethine group ( $RHC = N-R'$ , The R and R' are various alkyl or aryl groups). The nitrogen (N) atom of azomethine group is  $sp^2$  hybridized and has a lone pair of electrons, therefore, exhibit excellent chelating power especially when one or more donor atom is adjacent to this group. Also  $N_2O_2$  donor Schiff bases and their  $Zn^{2+}$  complexes show excellent effect on oxygen atom transfer reactions, enantioselective epoxidation, aziridination, mediating organic redox reactions and other oxidative processes (Butler and Carrano 1991; Katsuki 1995; Boghaei and Mohebbi 2002; Mohebbi et al. 2005). The present study has been focused on the morphological and biochemical effects of these compounds on chickpea.

## Materials and methods

### Synthesis of ligands and complexes

Two different aldehydes, viz; salicylaldehyde (A. R grade, S. D. Fine Chemicals, India) and o-vanillin (A.R grade Sigma Aldrich, Germany), each of purity level >99% were used without any additional purification.  $Zn(AcO)_2 \cdot 2H_2O$ , ethylenediamine, and o-phenylene diamine (A.R grade, purity level >99%) were purchased from S. D. Fine Chemicals, India. Spectroscopic grade methanol (minimum assay, GC >99.8% with 0.05% of water, procured from Merck) was used as solvent for the synthesis of the ligands and complexes. Slight alterations from standard procedures were done to prepare both complexes and ligands (OConner and West 1967; Bately and Graddon 1967; Kotova et al. 2008). Two aldehydes (Salicylaldehyde/o- vanillin) were alternatively treated in 2:1 molar ratio in MeOH with two corresponding amines (ethylenediamine/o-phenylene diamine) to prepare four ligands [L1 – N,N'- Ethylene bis- (salicylidenediamine), L2 – N,N'- (o-Phenylene)bis-(salicylidenediamine), L3 – Ethylene bis-(3-methoxysalicylidenediamine), L4 – N,N'-(o-Phenylene)bis-(3-methoxy salicylidenediamine)] with yield 90–95%. The four prepared ligands were individually treated with  $Zn(AcO)_2 \cdot 2H_2O$  in 1:1 molar ratio in MeOH under refluxed condition at temperature of 40–60 °C to obtained four  $Zn^{2+}$  complexes {C1 – [N,N'- Ethylene bis-(salicylidenediamine) Zn (II)] monohydrate, C2 – [N,N'- (o- Phenylene) bis-(salicylidenediamine) Zn (II)] monohydrate, C3 – [Ethylene bis- (3-methoxy salicylidenediamine) Zn (II)] monohydrate, C4 – [N,N'- (o- Phenylene) bis – (3- methoxy salicylidenediamine) Zn (II)] monohydrate}. Characterization of these ligands and complexes were done by elemental microanalysis (Perkin Elmer 2400 CHN analyzer) and IR data (PerkinElmer FT/IR-RX 1 spectrometer) and the results were found to be more or less identical with the literature (Kotova et al. 2008).

### Treatment with ligands and complexes

To analyze the effects of different Schiff-base ligands and their Zn complexes on various morphological and biochemical parameters, a local chickpea variety was selected as the subject plant. The study was conducted in month of March at University of North Bengal, India. Seeds of chickpea were surface sterilized using 5% sodium hypochlorite (NaOCl) solution (Sauer and Burroughs 1986). Seeds were then treated with 10 ppm solutions of prepared complexes and equal concentration of ligands differently in nine different set (L1–L4 for four ligands, C1–C4 for four complexes, and one control) for 12 hrs along with a control set made up with pure distilled water. Fifty seeds were used for every solution and each set consisted of three replicates. To measure different parameters, soaked chickpea seeds were then germinated on sterile petri plates. 5 ml of sterile distilled water was added to each petri dish every day. Twenty germinated seeds from each treatment were transferred to different pots containing garden soil, well rotten cow dung, and sand in 1:1:1 ratio under controlled conditions with 8 hrs light period at 28–35°C day/night temperature, and 65–75% relative humidity. Radicle and seedling length were measured and different biochemical parameters were analyzed by taking one month old seedlings.

**Germination index (GI)**

Germination Index (GI) was calculated using following formula given by AOSA (1983).

$$GI = \left[ \frac{a_1}{t_1} \right] + \dots + \left[ \frac{a_2}{t_2} \right]$$

where  $a_1$  = No. of seed germinated in first count,  $a_2$  = No. of seed germinated in final count,  $t_1$  = Days of first count and  $t_2$  = Days of final count.

**Germination percentage (GP)**

Germination percentage (GP) was calculated ten days after germination by dividing the number of germinated seeds by total number of seeds in each pot, multiplied by 100 (Cokkizgin and Cokkizgin 2010; Tanveer et al. 2010).

$$GP = \frac{x}{y} \times 100$$

where  $x$  = total number of germinated seeds in each pot and  $y$  = total number of seeds in each pot.

**Seedling vigour index (SVI)**

Seedling vigour index (SVI) was calculated according to (Baki and anderson 1973) as follows:

$$SVI = [L \times P]$$

where L is seedling length in cm and P is GP.

**Coefficient of velocity of germination (CVG)**

Formula described by Maguire (1962) was followed to calculate coefficient of velocity of germination (CVG).

$$CVG = \frac{(G_1 + G_2 + \dots G_n)}{(1 \times G_1 + 2 \times G_2 + \dots + n \times G_n)}$$

G and n are the number of germinated seeds and the last day of germination, respectively.

**Extraction and estimation of total soluble proteins**

Ice cold 0.05 M sodium phosphate buffer (pH 7.2) was used for extraction of total soluble protein from seed, leaf, and root tissue by following the method of Chakraborty, Chakraborty, and Kapoor (1993) Estimation was done by the method of Lowry, Rosebrough, and Randall (1951).

**Extraction and estimation of chlorophyll**

Chlorophyll from the leaves was extracted in 80% acetone in dark by following the method of Harborne (1973) by taking the absorbance of the filtrate at 663 nm and 645 nm, respectively in a Uv-vis spectrophotometer.

**Extraction and estimation of carotenoids**

Carotenoids from leaves were extracted in 100% methanol following method of Lichtenthaler (1987) and absorbance of the filtrate was immediately observed at 663, 645, and 480 nm in a VIS spectrophotometer.

**Extraction and estimation of antioxidative enzymes**

For the extraction of antioxidative enzymes [peroxidase (POX, EC 1.11.1.7), ascorbate peroxidase (APOX, EC 1.11.1.11), and catalase (CAT, EC 1.11.1.6)] leaf tissue was homogenized in ice cold 50 mM sodium phosphate buffer, pH 7.2 with 1% (w/v) poly vinyl pyrrolidone and homogenate was

centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used as crude enzyme extract. POX activity was assayed spectro-photometrically, where the oxidation of O-dianisidine was observed following the method described by Chakraborty, Chakraborty, and Kapoor (1993) with some modifications using 4802 Uv-vis spectrophotometer (Cole Parmer, USA). Method described by Asada and Takahashi (1987) was followed for assay of APOX activity, where quantity of ascorbate oxidized was measured spectro-photometrically. CAT activity was measured by quantifying H<sub>2</sub>O<sub>2</sub> broken down at 240 nm as described by Beers and Sizer (1952). One enzyme unit was interpreted as 1Δ absorbance per min.

#### **Extraction and estimation of total sugar**

Total sugar from leaf was extracted in 95% ethanol using method of Harborne (1973) with minor modifications and Anthrone's method given by Plummer (1978) was followed for estimation.

#### **Extraction and estimation of Leaf Zn content**

The chickpea leaves were plucked off after 30 days and were dried in a forced air oven at 70°C to a constant weight and grounded to a fine powder. The formed dry samples (1 g) were combusted in a muffle furnace at 550°C for 8 hrs. The ashed samples were dissolved in 2 (M) HCl according to the method described by Chapman and Pratt (1961). Estimation of Zn was done by an atomic absorption spectrophotometer (Varian, Spectr AA 50B).

#### **Statistical analysis**

All experiments were conducted in three replicates and results were subjected to analysis of variance (ANOVA). LSD test was performed for statistical investigation at  $p \leq 0.05$  level by SAS software (SAS Inc., Carey, NC).

### **Results and discussion**

The analytical data and IR values obtained for all four Schiff bases and their Zn<sup>2+</sup> complexes are found to be almost same with literature (Kotova et al. 2008) and are given in Tables (SI–SIV). For the synthesized four ligands there are certain characteristic IR bands which are supposed to be for  $\nu_{C=N}$  (in the region 1640–1610 cm<sup>-1</sup>),  $\nu_{O-H}$  (in the region 1400–1000 cm<sup>-1</sup>) and  $\nu_{N-H}$  (in the region 3100–2500 cm<sup>-1</sup>). There exists a significant hydrogen bonding between the hydroxyl hydrogen and the N atom (O-H...N = C), which is supported by the appearance of the  $\nu_{N-H}$  absorption bands in the IR spectra. Characteristic IR absorption bands of the  $\nu_{C-H}$ ,  $\nu_{C=N}$ ,  $\nu_{C-O}$  stretching vibrations, and the aromatic ring vibrations are observed for all the four synthesised Zn complexes. A broad O–H stretching absorption band found in the region 3500–3200 cm<sup>-1</sup> for every complex, which signifies the existence of water molecule in the compounds.

Zn as one of the essential micronutrients in plant system, plays an important role in regulation of plant growth, enzyme activity, protein synthesis, and retain structural stability of cell membrane (Broadley et al. 2007). Plants coming out of seeds grown in Zn deficit soil have reduced seedling vigour and growth (Yilmaz et al. 1998). In barley and rice, seed priming with Zn was very effective for seedling vigour and early seedling development (Ajouri, Asgedom, and Becker 2004). Foliar application of Zn in case of pepper increased grain protein content and yield (Ebrahim and Aly 2005). All these previous reports clearly indicate that Zn is positively associated with seedling growth.

Data obtained from this study reveal that for all the germination parameters no significant differences ( $p \leq 0.05$ ) were noticed among the Schiff-base ligands treated seeds in terms of CVG, GP, GI, and seed vigour index. But Zn(II) complexes significantly affect different germination parameters. Among the complexes C4 showed highest ( $p \leq 0.05$ ) and positively marked results for all the germination related parameters. SVI, GI, CVG, and GP were 56.41%, 18.17%, 74.74%, and 7.8% higher, respectively, in case of C4 in relation to control (Table 1).

Similar trends were also found in hypocotyls and radicle length. Data concerned with seedling growth, hypocotyls, and radicle length clearly indicated gradual and significant increase in all Zn

**Table 1.** Effects of ligands, Zn complexes on various germination parameters (Mean  $\pm$  S.D). LSD values are computed (at  $p \leq 0.05$ ) to evaluate mean performances amongst different compounds.

Compounds	SVI	GP	GI	CVG
Control	1059.04 $\pm$ 60.530	73.33 $\pm$ 1.12	11.76 $\pm$ 1.20	0.294 $\pm$ 0.04
L1	1135.38 $\pm$ 174.40	76.66 $\pm$ 2.41	12.68 $\pm$ 1.16	0.385 $\pm$ 0.08
C1	1396.85 $\pm$ 151.46	80.00 $\pm$ 2.77	14.93 $\pm$ 0.92	0.315 $\pm$ 0.02
L2	1053.59 $\pm$ 37.116	73.33 $\pm$ 1.01	12.43 $\pm$ 1.87	0.299 $\pm$ 0.01
C2	1472.82 $\pm$ 105.33	83.33 $\pm$ 1.23	15.71 $\pm$ 1.81	0.308 $\pm$ 0.01
L3	1154.54 $\pm$ 151.32	66.66 $\pm$ 0.79	11.15 $\pm$ 1.03	0.301 $\pm$ 0.03
C3	1272.96 $\pm$ 202.60	76.66 $\pm$ 1.04	14.76 $\pm$ 2.20	0.293 $\pm$ 0.01
L4	1230.09 $\pm$ 114.46	76.66 $\pm$ 1.46	13.93 $\pm$ 2.07	0.309 $\pm$ 0.02
C4	1656.46 $\pm$ 122.92	86.66 $\pm$ 2.14	20.55 $\pm$ 1.99	0.317 $\pm$ 0.01
LSD ( $p \leq 0.05$ )	252.33	4.06	3.28	0.066

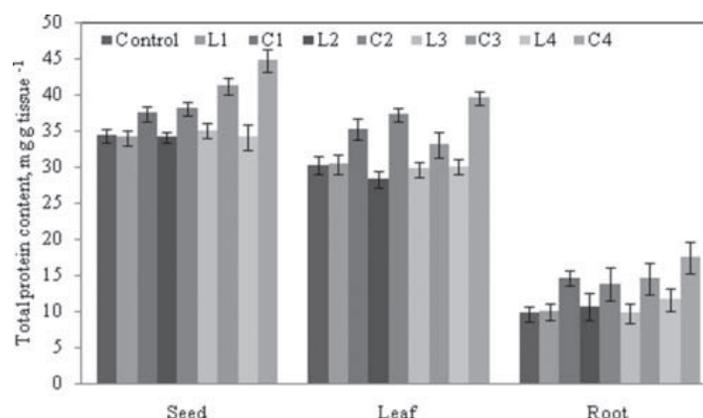
SVI = Seedling vigour index, GP = Germination percentage, GI = Germination index, CVG = Coefficient of velocity of germination

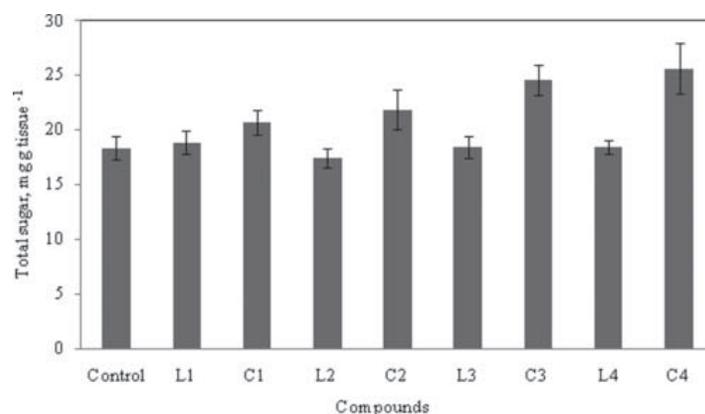
**Table 2.** Effects of ligands, Zn complexes on various growth parameters (Mean  $\pm$  S.D). LSD values are computed (at  $p \leq 0.05$ ) to evaluate mean performances amongst different compounds.

Compounds	Seedling Length (cm)	Hypocotyl Length (cm)	Radicle Length (cm)
Control	14.43 $\pm$ 1.01	8.23 $\pm$ 1.01	6.86 $\pm$ 1.55
L1	14.80 $\pm$ 1.80	7.70 $\pm$ 0.90	6.70 $\pm$ 1.30
C1	17.46 $\pm$ 1.20	9.53 $\pm$ 1.04	7.60 $\pm$ 1.04
L2	14.40 $\pm$ 0.70	8.56 $\pm$ 1.00	6.53 $\pm$ 0.96
C2	17.66 $\pm$ 1.01	9.00 $\pm$ 0.96	8.73 $\pm$ 1.46
L3	15.70 $\pm$ 0.95	8.66 $\pm$ 0.81	6.20 $\pm$ 0.51
C3	17.20 $\pm$ 1.21	9.06 $\pm$ 0.51	7.36 $\pm$ 1.20
L4	16.03 $\pm$ 1.25	8.73 $\pm$ 0.43	7.70 $\pm$ 1.15
C4	19.10 $\pm$ 0.99	10.16 $\pm$ 1.75	8.66 $\pm$ 0.50
LSD ( $p \leq 0.05$ )	1.09	2.06	1.88

complex treated seedlings. Highest increase was observed in C4 complex treated seedlings. On contrary growth parameters in ligands treated seedlings were almost identical to control (Table 2). Our results are in accordance with previous study in papaya by Acharjee and others (2015), where chelated Zn compound clearly increased root and shoot length.

In plant system, Zn is present as cofactor of more than 300 enzymes (Henriques, Chalfun, and Aarts 2012). If we see biochemical parameters, then it is found that total soluble protein content gradually increases in seed, root, and shoot after the treatment with Zn complexes and these enhancements ranges from 9.07% to 30.50% in case of seed, 49.12% to 79.99% in case of root, 16.39% to 30.60% in case of shoot from C1 to C4 complexes pertaining to control (Figure 1).

**Figure 1.** Effects of ligands, control and Zn complexes on seed, leaf and root total protein content of desi chickpea. Results are represented as mean  $\pm$  SD ( $n = 3$ ).



**Figure 2.** Total sugar estimation after the treatment with ligands and Zn(II) complexes. Results are represented as mean  $\pm$  SD ( $n = 3$ ).

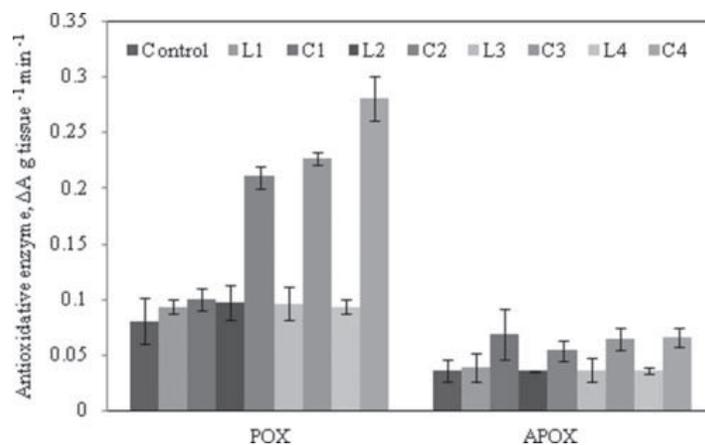
After application of different Zn complexes total sugar content in leaf gradually increased in range of 0.124–0.395 fold from C1 to C4 complex (Figure 2).

Antioxidant enzymes expression get enhanced in presence of Zn and provide tolerance to oxidative damage (Cakmak 2000; Miszalski et al. 1998) Analysis of antioxidative enzymes clearly indicated a higher and gradual increase in activity of antioxidative enzymes POX, APOX, and CAT in Zn(II) complex treated plants than in ligand treated plants and control plants. POX activity and APOX activity are shown in Figure 3.

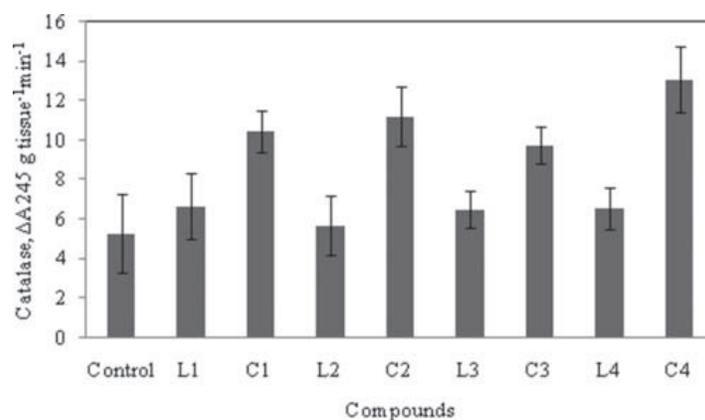
Zn maintains ascorbate and thiol pool in ascorbate–glutathione cycle and then ascorbate reacts with  $H_2O_2$  in presence of APOX (Smirnoff, Conklin, and Loewus 2001). Increase in APOX was also noticed in rye grass in presence of Zn (Bonnet, Camares, and Veisseire 2000). In our observation APOX activity was maximum in case of C2 complex treated plants in relation to control (Figure 3). Among the four complexes used for treatment maximum activity of antioxidative enzymes except APOX was noticed in case of C4 complex indicating the higher efficiency for delivering Zn ion to root.

CAT expression (Figure 4) increased in a range of 0.97–1.47 folds from C1 to C4 treated plants with respect to control indicating high Zn(II) deliver efficiency of C4 among all Schiff-base complexes.

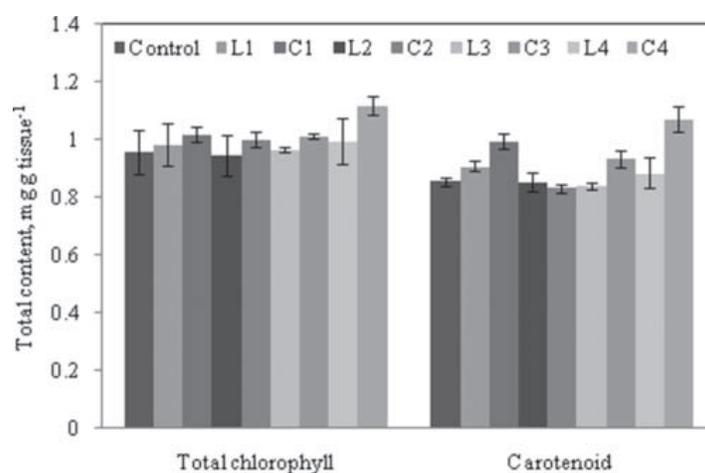
From this study, better availability of Zn in form of Schiff-base complex especially through C4 was clearly understood by its inducing effect on expression of different antioxidative enzymes. Zn attached to sulfhydryl group ( $-SH$ ) of chlorophyll facilitates stability of the pigment (Chvapil 1973; Kösesakal and Unal 2012). Maximum and significant increase ( $p \leq 0.05$ ) of chlorophyll content (Figure 5) was perceived in C3 and C4 complex treated seedlings in relation to control clearly



**Figure 3.** Effect of ligands, control and Zn complexes on antioxidative enzymes, Peroxidase (POX) and Ascorbate Peroxidase (APOX) in leaves of desi chickpea. Results are represented as mean  $\pm$  SD ( $n = 3$ ).



**Figure 4.** Effect of ligands, control and Zn complexes on Catalase in leaves of desi chickpea. Results are represented as mean  $\pm$  SD ( $n = 3$ ).



**Figure 5.** Effect of ligands, control and Zn complexes on total chlorophyll and carotenoid content in leaves of desi chickpea. Results are represented as mean  $\pm$  SD ( $n = 3$ ).

indicating positive involvement of Zn in chloroplast biosynthesis and rate of photosynthesis (Aslam et al. 2014). On the other hand, in ligand treated and control treated plants chlorophyll content remains almost identical.

Carotenoid, a non-enzymatic antioxidant involved in quenching of oxidizing species participates in disrupting regular cellular functioning in *Artemisia annua* (Khudsar, Mahmooduzzafar, and Sairam 2014). Carotenoid content of chickpea seedlings were also enhanced significantly after different Schiff-base complex application in seedlings (Figure 5). Maximum change was noticed in case of C4 but it was insignificant ( $p \leq 0.05$ ) with respect C3. No marked changes were noticed in ligand-treated plants.

**Table 3.** Leaf Zinc concentration data of 30 th day old seedlings after treatment with ligands and complexes.

Compounds	Zinc Concentration ( $\mu\text{g g}^{-1}$ )
Control	52.83 $\pm$ 1.25
L1	53.83 $\pm$ 0.76
C1	54.66 $\pm$ 1.52
L2	53.80 $\pm$ 0.64
C2	54.40 $\pm$ 0.69
L3	55.83 $\pm$ 0.58
C3	57.66 $\pm$ 1.12
L4	58.56 $\pm$ 0.98
C4	60.66 $\pm$ 1.15
LSD ( $p \leq 0.05$ )	1.02

Zn concentrations were measured by taking the leaves of 30th day old seedlings and the data are given in Table 3 showing the efficacy of the C4 complex to supply Zn in plant system.

## Conclusion

Outcome of the present experiment reveals that different seed germination parameters, seedling growth parameter, and biochemical parameters vary with variation of treatments. Most of the parameters under control (no treatment) are least. All four used ligands failed to impart net statistical increment from the control. Thus, control and ligands may not serve as effective plant growth stimulator for chickpea. Perusal of all the parameters undoubtedly signifies Zn complexes are far better stimulator than the control and ligands. The parity and correlation of all the parameters signify the better efficacy of C4 complex, which can be used as seed soaking chemical for chickpea. However, these effects must be further observed at multi-locations and varied environmental conditions to draw a direct conclusion about the superiority.

## Acknowledgments

The authors (K. Acharjee and J. Sarkar) are thankful to UGC, India and Department of science and technology, Govt. of India, respectively, for providing fellowships.

## Funding

The authors are grateful to the Departmental Special Assistance Scheme under the University Grants Commission, New Delhi (SAP-DRS-III, No. 540/12/DRS/2013) for financial support.

## ORCID

Biswajit Sinha  <http://orcid.org/0000-0003-0468-4035>

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## Effect of some Schiff base ligands and their Zn (II) complexes on germination and seedling growth of papaya (*Carica papaya* L.)

K. Acharjee<sup>1</sup>, D. K. Sangma<sup>2</sup>, D. K. Mishra<sup>1</sup>, P. Deb<sup>2\*</sup>, B. Sinha<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, University of North Bengal, Darjeeling - 734 013, West Bengal, India, <sup>2</sup>Division of Horticulture, Institute of Agriculture, Visva-Bharati University, Sriniketan - 731 236, West Bengal, India.

Received 18<sup>th</sup> November 2014; Revised 10<sup>th</sup> March 2015; Accepted 18<sup>th</sup> March 2015

### ABSTRACT

Salen and salophen type of ligands have the ability to form stable complexes with Zn<sup>2+</sup> and thereby can increase the availability of Zn<sup>2+</sup> for plants when used as growth promoters in ppm level. Four Schiff base ligands and their Zn<sup>2+</sup> complexes were synthesized and characterized by different analytical and spectroscopic techniques. These Schiff bases and their Zn<sup>2+</sup> complexes were applied to the seeds of papaya (*Carica papaya* L.). The efficacy of the complexes was monitored through the parameters like germination, root growth, seedling growth, leaf-chlorophyll content, leaf-zinc content, etc. Among the complexes it was found that [N, N-(*o*-phenylene)-bis-(3-methoxysalicylidenediamine)] monohydrate is more efficient for different horticultural traits when used in papaya seeds and can be used as eco-friendly plant growth promoter and source of zinc to plants like papaya.

**Key words:** Zinc complex, Ligand, Plant growth stimulator, Salen, Salophen.

### 1. INTRODUCTION

Zinc deficiency in soils and plants is a global micronutrient deficiency problem in most agricultural regions of the world as well as in India [1]. The easiest and most straight forward practice to correct micronutrient deficiency is to apply zinc fertilizers. Although there is evidence to suggest that some chemical properties of soils, such as pH and pe (pe=Eh [mV]/59.2) (being pe the negative logarithm of the free electron activity and Eh the redox potential), can be affected by the addition of fertilizers to soils [2]. Most of the zinc fertilizers contain sulfates or oxides as counter ions [3,4]. These counter ions are responsible to alter the pH of the soil, and continuous use of these inorganic fertilizers can make the soil infertile. In comparison with inorganic fertilizers, synthetic and natural chelates have the advantages of keeping the applied nutrient in a less reactive form [5]. In contrast with inorganic salts, synthetic metal chelates are more useful to correct the metal deficiency [6] for a longer period.

A recent development is carried out by taking more stable but biodegradable chelating ligands. Here we have hypothesized the possibility of synthesis and use of Zn- Salen, Zn- Salophen types of complexes as a plant growth stimulator and Zn source in agricultural systems. We have chosen Salen, salophen types of

ligands because these ligands provide strong donor sites for transition metal ions. The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R', where R and R' may be alkyl, aryl, cycloalkyl or heterocyclic groups and may be variously substituted. Presence of a lone pair of electrons in a sp<sup>2</sup>-hybridized orbital of N- atom of the azomethine group is of considerable chemical importance and imparts excellent chelating ability especially when used in combination with one or more donor atoms close to the azomethine group. Several reviews [7-11] have shown that the salen type Schiff base complexes possess excellent properties in many fields like oxygen atom transfer reaction, enantioselective epoxidation, aziridination, mediating organic redox reactions and other oxidative processes. Due to these versatility offered by the salen type ligands in this work, we have synthesized four different Zn complexes and investigated their effect on germination and seedling growth of papaya (*Carica papaya* L.).

### 2. MATERIALS AND METHODS

Analytical grade Zn (AcO)<sub>2</sub>·2H<sub>2</sub>O, ethylene diamine, *o*-phenylene diamine, salicylaldehyde each of purity >99% were procured from S. D. Fine Chemicals, India. These chemicals were used without additional purification. Spectroscopic grade methanol (minimum

\*Corresponding Author:

E-mail: biswachem@gmail.com, debprld@yahoo.com

assay, GC >99.8% with 0.05% of water) was procured from Merck, India and used as received. Analytical grade o-vanillin of purity >99% were procured from Sigma Aldrich, Germany.

The Schiff base ligands were synthesized according to standard procedures [12] with slight modifications. The four ligands were prepared by the reactions of aldehyde (Salicylaldehyde/*o*-vanillin) and the corresponding diamine (ethylene diamine/*o*-phenylene diamine) in MeOH in 2:1 ratio. The yields were ~90-95%. Reactions of solutions of Zinc (II) acetate and a Schiff base in MeOH in 1:1 ratio at ambient temperature of 40°-60°C and under reflux led to precipitation of corresponding zinc (II) complexes [13,14]. The precipitates were isolated by filtration. The yields were ~70-75%.

Elemental microanalysis (C, H, N) were done by a Perkin Elmer 2400 CHN analyzer and IR spectra of the Schiff bases and complexes were recorded on a PerkinElmer FT-IR-(RX 1) spectrometer in the region 4000-400  $\text{cm}^{-1}$ .

The seed germination and morphological changes of papaya were monitored at Sriniketan, in India during April-May of 2014. Seeds of papaya (*C. papaya* L.) were soaked with 10 ppm solutions of prepared Zn complexes and equal concentration of ligands differently for 12 h. A control was made up with pure distilled water so that there were total of 9 sets ( $L_1$ - $L_4$  for four ligands,  $C_1$ - $C_4$  for four complexes, and one control). 100 seeds were used for every solution. So per set of investigation took total of 900 seeds. We had performed three replicas of each set, making a total of 2700 seeds which were under observations. The seeds after soaking were transformed to polythene container containing a growing media consisting of garden soil, sand, and well-rotten cow dung in 1:1:1 ratio at a depth of 1 cm under controlled conditions in a greenhouse with a 8 h light period at 28°-35°C day/night temperature, and 65%-75 % relative humidity. Two seeds were planted in each pot. The length of seedlings, root lengths and number of leaves were monitored at intervals of 15 days and 30 days respectively. The observations were analyzed in completely randomized design as suggested by Gomez and Gomez (1983) [15]. The chlorophyll contents of leaves were measured through chlorophyll meter (SPAD-502PLUS, Conica Minolta, Japan). After 15 days and 30 days, interval leaves were plucked off. The plant leaves were dried immediately in a forced-air oven at 70°C to a constant weight and grounded to a fine powder. Dry samples (1 g) were placed into ceramic vessels and combusted in a muffle furnace at 550°C for 8 h. The ashed samples were removed from the muffle furnace, cooled, and then dissolved in 2 (M) HCl (Chapman and Pratt 1961) [16]. The final solution was diluted to meet the range requirements of the analytical procedures. Analyses of Zn were carried out

with an atomic absorption spectrophotometer (Varian, Spectr AA 50B).

### 3. RESULTS AND DISCUSSION

The ligands studied in the present work were prepared as standard procedures [12-14] and the yields were found to be very high and about 90-95%. The structures of the ligands were confirmed by different analytical and spectroscopic methods. The analytical data of the four prepared ligands and selected IR bands which are both found to be identical with reference [17] are depicted in Tables 1 and 2, respectively.

Zinc (II) complexes were obtained by reactions of zinc (II) acetate solution with corresponding Schiff bases in methanol in 1:1 ratio at ambient temperature of 40-60°C and under refluxed condition. The zinc (II) complexes were also identified using different analytical and spectroscopic techniques. The analytical data of the prepared four zinc complexes and selected IR bands which are both found to be identical with reference [17] are depicted in Tables 3 and 4, respectively.

The synthesized four ligands ( $L_1$ - $L_4$ ) exhibit the characteristic IR bands of the  $\nu(\text{C}=\text{N})$  (in the region 1640  $\text{cm}^{-1}$ -1610  $\text{cm}^{-1}$ ),  $\nu(\text{O}-\text{H})$  (in the region 1400  $\text{cm}^{-1}$ -1000  $\text{cm}^{-1}$ ) for aromatic ring,  $\nu(\text{C}-\text{H})$  and  $\nu(\text{N}-\text{H})$  (in the region 3100  $\text{cm}^{-1}$ -2500  $\text{cm}^{-1}$ ). The appearance of the  $\nu(\text{N}-\text{H})$  absorption bands in the IR spectra confirm the formation of hydrogen bonds O-H...N=C between the hydroxyl hydrogen and the nitrogen atom [18,19].

The IR spectra of the zinc complexes exhibit the characteristic absorption bands of the  $\nu(\text{C}-\text{H})$ ,  $\nu(\text{C}=\text{N})$ ,  $\nu(\text{C}-\text{O})$  stretching vibrations, and the aromatic ring vibrations. The presence of water molecules in the compounds is indicated by a broad O-H stretching absorption band in the region 3500  $\text{cm}^{-1}$ -3200  $\text{cm}^{-1}$ .

Discussions reveal that both Schiff bases and their Zn (II) complexes were formed rightly, and their formations are found to be justified by reference [17]. The structures of the synthesized ligands ( $L_1$ - $L_4$ ) and complexes ( $C_1$ - $C_4$ ).

Significant differences have been observed in the present experiment in different parameters. The data presented in Table 5 reveal lowest days (10.09), recorded for first 10% seed germination under  $C_4$ , which was statistically at par with  $C_2$  (10.82 days). Lowest period of germination was observed under  $C_4$  (16.67 days) which was maximum for control. Significantly highest percentage of germination (80.83) was measured for  $C_4$  followed by  $C_2$  (78.32). Data indicates that ligands and control statistically imparted an almost same result, but the complexes especially  $C_2$  and  $C_4$  are more efficient in seed germination.

**Table 1:** Analytical data of the prepared four ligands.

Ligand	Formula weight	Yield (%)	% found, (calculated)		
			C	H	N
L <sub>1</sub> (C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub> ) <i>N,N'</i> -Ethylene bis (salicylidenediamine)	268.31	92.27	71.6 (71.3)	5.9 (5.9)	10.2 (10.5)
L <sub>2</sub> (C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub> ) <i>N,N'</i> - ( <i>o</i> -Phenylene) bis (salicylidenediamine)	316.35	90.39	75.9 (75.8)	5.1 (5.1)	8.9 (9.0)
L <sub>3</sub> (C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub> ) <i>N,N'</i> -Ethylene bis (3-methoxysalicylidenediamine)	328.36	91.21	65.9 (65.7)	6.1 (6.2)	8.5 (8.4)
L <sub>4</sub> (C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub> ) <i>N,N'</i> ( <i>o</i> -Phenylene) bis (3-methoxysalicylidenediamine)	376.41	89.46	70.2 (70.1)	5.3 (5.2)	7.5 (8.0)

**Table 2:** Selected IR bands (cm<sup>-1</sup>) of the prepared four ligands.

Ligand	$\nu$ (C=N)	$\delta$ (O-H)	Aromatic ring vibrations	N (C-H)+ $\nu$ (N-H)	$\nu$ (C-O)
L <sub>1</sub>	1635.7	1371.2, 1284.2	1577.8, 857.4, 773.1, 742.2, 647.2	3048.3, 3007.6, 2955.4, 2930.1, 2900.3, 2868.5	1199.4, 1151.1, 1042.1, 1021.3.
L <sub>2</sub>	1613.5	1363.4, 1277.2	1562.3, 830.6, 760.5, 639.1, 581.8, 512.1	3054.4, 2987.1, 2926.7, 2854.3, 2711.1	1192.8, 1151.0, 1044.3
L <sub>3</sub>	1632.7	1325.1, 1251.8, 1082.5, 1047.9, 1009.2	838.1, 790.2, 733.1, 623.2	3087.2, 3004.5, 2930.2, 2913.4, 2839.2, 2584.7	1190.5, 1169.3, 1132.4
L <sub>4</sub>	1614.6	1401.8, 1377.2, 1364.96, 1324.2, 1245.4	735.1, 646.4, 584.2, 538.2	3464.2, 3367.3, 3058.7, 3012.1, 2954.7, 2925.4, 2836.5	1206.1, 1093.5, 1040.1

**Table 3:** Analytical data of the prepared four zinc (II) complexes.

Complex	Formula weight	Yield (%)	% found, (calculated)		
			C	H	N
C <sub>1</sub> (C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> Zn) [ <i>N,N'</i> ethylenebis (salicylidenediamine)] monohydrate	349.7	82.04	54.9 (54.8)	4.6 (4.7)	8.0 (8.1)
C <sub>2</sub> (C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> Zn) [ <i>N,N'</i> ( <i>o</i> -phenylene) bis (salicylidenediamine)] monohydrate	397.74	81.71	60.5 (60.2)	4.0 (4.2)	7.1 (7.1)
C <sub>3</sub> (C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> Zn) [ <i>N,N'</i> ethylene bis (3-methoxysalicylidenediamine)] monohydrate	409.75	81.28	52.8 (52.5)	4.9 (5.0)	6.9 (6.8)
C <sub>4</sub> (C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> Zn) [ <i>N,N'</i> ( <i>o</i> -phenylene) bis (3 methoxysalicylidenediamine)] monohydrate	457.79	79.63	57.8 (57.5)	4.4 (4.6)	6.1 (6.2)

**Table 4:** Selected IR bands (cm<sup>-1</sup>) of the prepared four zinc (II) complexes.

Complex	$\nu$ (C=N)+ $\delta$ (O-H) H <sub>2</sub> O	$\nu$ (O-H) H <sub>2</sub> O	Aromatic ring vibrations	$\nu$ (C-H)	$\nu$ (C-O)
C <sub>1</sub>	1653.2, 1633.8	3300-2900	1595.2, 1551.2, 1514.2, 848.8, 608.3	3046.4, 3019.2, 2950.7, 2928.1, 2869.5	1184.2, 1140.9, 1124.5, 1090.7
C <sub>2</sub>	1615.5	3400-2800	1585.2, 1530.6, 854.3, 747.8, 601.5	2921.1, 2821.5, 2896.2	1170.3, 1151.2, 1125.5, 1033.8
C <sub>3</sub>	1661.2, 1642.8	3400-3000	1599.5, 1546.2, 856.4, 726.3, 584.2	3056.7, 3010.5, 2947.8, 2897.3	1169.8, 1102.3, 1070.4, 1043.6
C <sub>4</sub>	1688.7, 1614.4	3600-3000	1586.2, 1538.7, 862.1, 738.2, 559.5	3046.2, 2925.1, 2809.7	1192.6, 1105.4, 1074.2, 1048.5

IR: Infrared

Perusal of data, presented in Table 6, reveals Schiff bases and their zinc complexes significantly affect the growth of young seedlings of papaya. Observations

were taken at 15<sup>th</sup> and 30<sup>th</sup> days after germination. In both cases, more or less similar trend has been observed under different parameters. Schiff bases and their zinc

complexes showed varied range of measurements in seedling growth, root growth, and the highest number of leaves. In all cases, C<sub>4</sub> showed prominent and positively marked results. A similar trend was also observed in all these cases, that was the effect of ligands were statistically identical with the control, but all the complexes were showing satisfactory results specifically the C<sub>4</sub> one. When it was subjected to a number of leaves, again C<sub>4</sub> complex showed the best result. The trend of ligands and complexes imparted identical trajectory at both the 15<sup>th</sup> day and 30<sup>th</sup> day interval after the seed germination.

The statistical analysis of the observations on leaf chlorophyll content of papaya seedlings at 15<sup>th</sup> day and 30<sup>th</sup> day interval showed both non significant and

**Table 5:** Germination data of papaya seeds after the treatment of all the ligands, Zn - complexes and control.

Treatments	Days to first 10% seed germination	Period of germination (days)	Germination percentage
L <sub>1</sub>	12.52	22.51	71.07
L <sub>2</sub>	12.01	20.73	73.40
L <sub>3</sub>	11.69	21.98	70.52
L <sub>4</sub>	12.13	20.23	74.44
C <sub>1</sub>	11.05	18.36	77.65
C <sub>2</sub>	10.82	17.92	78.32
C <sub>3</sub>	10.94	18.41	76.21
C <sub>4</sub>	10.09	16.67	80.83
Control	12.47	23.07	72.45
CD <sub>0.05</sub>	0.74	1.03	2.06
SE±m	0.39	0.54	0.97

SE: Standard error

**Table 6:** Length of seedlings, root length, number of leaves data of the papaya plants.

Treatments	Length of seedling (cm)		Root length (cm)		Number of leaves	
	15 days	30 days	15 days	30 days	15 days	30 days
L <sub>1</sub>	7.36	13.13	6.21	11.10	3.07	4.32
L <sub>2</sub>	7.02	13.22	6.42	11.34	3.11	5.09
L <sub>3</sub>	6.87	12.75	6.10	12.00	3.34	4.68
L <sub>4</sub>	6.98	13.34	7.34	11.56	3.23	4.92
C <sub>1</sub>	8.12	15.31	8.56	13.37	3.67	5.24
C <sub>2</sub>	8.23	14.86	9.29	13.25	3.56	5.41
C <sub>3</sub>	8.50	15.23	9.13	14.05	3.81	6.10
C <sub>4</sub>	9.23	16.31	10.18	14.84	3.98	6.79
Control	7.11	12.06	6.45	11.14	3.21	4.52
CD <sub>0.05</sub>	0.62	0.92	0.97	0.72	NS	0.65
SE±m	0.29	0.43	0.50	0.39	NS	0.34

SE: Standard error

significant variations which is revealed in Table 7. On 15<sup>th</sup> day leaf, chlorophyll content for all ligands and complexes ranged from 28.33 mg to 34.54 mg/100 g. On 30<sup>th</sup> day, maximum leaf chlorophyll content was observed in C<sub>4</sub>, which was least in control. A significant variation was also observed in leaf zinc content of papaya seedlings. At 15<sup>th</sup> day after germination, it was highest for C<sub>4</sub> and lowest for the control. Some sort of reduced range of leaf zinc content was observed in 30<sup>th</sup> day interval as the zinc, absorbed from the supplied complexes and growing media, further spread on the entire growing plant which ultimately reduced the concentration with increase in dry weight of seedlings.

Hence, all observations reveal that every complex has some effects on the parameters measured in this discussion whereas the ligands have not imparted such drastic effects and the results were more or less identical with the control. From that we can come to the conclusion that the ligands are stayed to be inert, and it is the Zn<sup>2+</sup> part which is responsible for such changes.

#### 4. CONCLUSION

From the findings of the present experiment, it is clear that seed germination parameters, seedling growth parameters, and leaf chlorophyll and zinc content varied with variation of treatments as it most of the parameters under control (no treatment) was lowest or minimum and the ligands used showed not such level of statistical increment from the control. Thus, control and ligands may not be suggested as plant growth stimulator for papaya. Perusal of the seed germination, seedling growth, and other parameters reveal the better effect of complexes than the ligands and control. The parity and correlation of leaf zinc content with the seed germination parameters and seedling growth parameters en campus the direct relation of Zn on those parameters. Use of different complexes in the present experiment

**Table 7:** Leaf chlorophyll content and leaf Zn content of papaya plants after treatment.

Treatments	Leaf chlorophyll content (mg/100 g)		Leaf zinc content (ppm)	
	15 days	30 days	15 days	30 days
L <sub>1</sub>	31.51	41.65	12.42	12.00
L <sub>2</sub>	28.33	39.27	13.11	12.87
L <sub>3</sub>	30.52	39.45	12.52	12.36
L <sub>4</sub>	29.31	40.16	12.27	11.89
C <sub>1</sub>	32.64	45.40	17.12	16.75
C <sub>2</sub>	34.42	46.38	16.33	16.21
C <sub>3</sub>	33.33	44.79	16.54	16.30
C <sub>4</sub>	34.54	52.10	17.97	17.65
Control	29.87	40.56	12.20	11.99
CD <sub>0.05</sub>	NS	3.43	0.98	0.92
SE±m	NS	1.67	0.41	0.45

SE: Standard error

clearly shows that C<sub>4</sub> has the maximum beneficial effect on germination and seedling growth of papaya. Hence, C<sub>4</sub> may be used as an effective seed soaking chemical for papaya. However, C<sub>4</sub> would be further studied in order to confirm its superiority in multi locations.

## 5. ACKNOWLEDGMENT

The authors are grateful to the Departmental Special Assistance Scheme under the University Grants Commission, New Delhi (No. F540/27/DRS/2007, SAP-1) for financial support and also to the University of North Bengal, India and VisvaVarati University for providing the infrastructures.

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## Responses of *Oryza sativa* L. towards Azo Functionalised Schiff base Cu(II) Complexes and $\text{CuSO}_4$ : A Comparative Biochemical Study

KAUSHIK ACHARJEE<sup>1</sup>, JAYANWITA SARKAR<sup>2</sup>, PRAHLAD DEB<sup>3</sup>,  
USHA CHACKRABORTY<sup>2\*</sup> and BISWAJIT SINHA<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, University of North Bengal, Darjeeling, West Bengal, India-734 013.

<sup>2</sup>Department of Botany, University of North Bengal, Darjeeling, West Bengal, India- 734013.

<sup>3</sup>Division of Horticulture, Institute of Agriculture, Visva-Bharati Univrsity, Sriniketan,  
West Bengal, India-731236.

\*Corresponding author E-mail: kashacharjee@gmail.com

<http://dx.doi.org/10.13005/ojc/3404037>

(Received: May 20, 2018; Accepted: July 17, 2018)

### ABSTRACT

Azo functionalised Schiff base ligands having  $\text{N}_2\text{O}_2$  donor binding sites are capable of forming metal complexes and can be used as potential plant micronutrient supplier. Two different ligands and their copper complexes were synthesised by conventional protocols and then characterised by both spectroscopic and elemental analysis. Investigations were done by taking rice seeds as plant material. Various growth and biochemical parameters were monitored by taking different concentrations of  $\text{CuSO}_4$ , prepared ligands and their Cu(II) complexes. Analysis of various biochemical results reveal that Schiff base Cu(II) complexes have less toxic effects than copper sulphate on rice seedlings and thus facilitates better tolerance to copper toxicity than copper sulphate.

**Keywords:** Plant growth, Cu(II) chelates, copper tolerance, electrolyte leakage, rice seeds, azomethine group.

### INTRODUCTION

Copper having the most stable oxidation state of +2, is one of the important redox active transition metal for plants<sup>1</sup>. The optimum content of  $\text{Cu}^{2+}$  in plant tissue is around  $10\mu\text{g g}^{-1}$  dry weight as reported by Baker and Senef in 1995.<sup>2</sup> It serves as a component of different enzymes (plastocyanin, cytochrome oxidase etc.) which

are mainly associated in electron transfer chain. It acts as cofactors in several enzymes such as Cu/Zn superoxide dismutase, laccase, cytochrome C oxidase<sup>3</sup>. It is required in the pathway of photosynthesis, respiration and associated in carbohydrate and protein metabolism<sup>4</sup>. In plants, Cu is necessary for cytosol, endoplasmic reticulum, chloroplast stroma, thylakoid lumen etc. To make Cu available in plants generally Cu contained



fertilisers of inorganic origin (mainly  $\text{CuSO}_4$ ) are used. These types of compounds being ionic in nature are responsible for the alteration of the pH of the medium<sup>5,6</sup>. Owing to that more emphasis is given to metal chelates which are less reactive but can solve the deficiency problem for longer period of time<sup>7,8</sup>. Inspired by the facts we have synthesised two azo functionalised Schiff base ligands and their  $\text{Cu}^{2+}$  complexes. The important feature of these ligands and complexes is that they contain azomethine group ( $\text{RHC}=\text{N-R/}$ , The R and R/ are various alkyl or aryl groups) in which the nitrogen atom is  $\text{sp}^2$  hybridised and the presence of a lone pair make it a good donor especially when one or more donor atoms are present adjacent to it. Complexes of these types draw the attention because of their catalytic power in oxygen atom transfer reaction, mediating organic redox reactions<sup>9-12</sup>. In our study, we have evaluated the responses of rice seedlings to metal toxicity while being exposed to Copper Sulphate ( $\text{CuSO}_4$ ) and two Copper Schiff base complexes and the results are compared in terms of pigments, biochemical components, osmolyte accumulation, oxidative stress markers and overall tolerance level of plants.

## MATERIALS AND METHODS

### Synthesis of ligands and complexes

0.1 mol of aniline (A.R grade, procured from S. D. Fine Chemicals, India) was dissolved first in concentrated HCl without any further purification. 8g of analytical grade  $\text{NaNO}_2$  (in water) was then added to aniline solution drop wise with constant stirring for 1 h keeping the reaction temperature throughout  $0^\circ\text{C}$ . A solution of 0.1 mol salicylaldehyde (A.R grade, procured from S. D. Fine Chemicals, India of purity level > 99%), sodium carbonate (36 g) and water was added drop wise in the above mixture with stirring. The reagents were allowed to react for 5 h at  $0^\circ\text{C}$ . After the completion of reaction the light red precipitate of 4-(Benzeneazo) salicylaldehyde is filtered off and recrystallised in ethanol. The melting point was  $118^\circ\text{C}$  with yield of 80%. Two different diamines (ethylenediamine and o-phenylene diamine) were treated with the azo compound synthesized earlier in 1:2 ratios in ethanol medium for 3 h at ambient temperature resulting two ligands. Ligands were washed and recrystallised in ethanol medium having yield 70%.

The prepared two ligands were alternatively refluxed with  $\text{Cu}(\text{AcO})_2 \cdot \text{H}_2\text{O}$  (A.R grade, purity level > 99%) in ethanol medium in 1:1 ratio for 2 h at ambient temperature resulting two Cu complexes. They were filtered, washed and recrystallised and the yield was 70% almost. The two ligands L1 and L2 { [N,N/-bis[4-(benzeneazo) salicylaldehyde]ethylenediamine and [N,N/-bis[4-(benzeneazo) salicylaldehyde]-o-phenylenediamine respectively} and two complexes C1 and C2 {[N,N/-bis[4-(benzeneazo) salicylaldehyde]ethylenediamine Copper(II) and [N,N/-bis[4-(benzeneazo) salicylaldehyde]-o-phenylenediamine Copper(II) respectively} were characterised by elemental analysis (Perkin Elmer 2400 CHN analyzer), IR spectroscopy (Perkin Elmer FT/IR-RX 1 spectrometer), conductance measurement (Shanghai DDS-11A conductivity apparatus at  $25^\circ\text{C}$ ) and electronic spectra (Perkin-Elmer Lambda 7 spectrophotometer). All results are quite identical with the literature<sup>13</sup>.

### Treatment with ligands and complexes

To investigate the effects of prepared complexes, ligands and  $\text{CuSO}_4$ , rice seeds were selected as plant material. Grains of rice (*Oryza sativa* L.) were surface sterilized with sodium hypochlorite (0.1%) for 1 min. and then they were washed 3 times with distilled water<sup>14</sup> and soaked for 12 h with various concentrations (10, 50, 100, 200 ppm) of  $\text{CuSO}_4$ , L1, L2, C1 and C2 along with a control (no treatment of micronutrients). After soaking was done the seeds were allowed to germinate on sterile petri plates. From each treatment we had chosen twenty germinated seeds which were transferred to plastic pots containing garden soil, well rotten cow dung and sand in 1:1:1 ratio under controlled conditions with a 8 h light period at  $28\text{-}35^\circ\text{C}$  day/night temperature and 65-75 % relative humidity. 45 days old seedlings were subjected to biochemical analysis.

### Growth parameters

To determine fresh weight, the harvested plants were rinsed with distilled water and blotted on paper towels before being weighed. Dry matter yields of the seedlings were determined after drying the seedlings in an oven at  $80^\circ\text{C}$  to a constant weight. Relative water content (RWC) was measured according to the protocol described by Farooqui *et al.*,<sup>15</sup>

**Copper tolerance index**

Copper tolerance index (TI) was calculated as the quotient of the dry weight of plants grown under copper treated and control conditions<sup>16</sup> according to this formula:

$$\text{Tolerance index (\%)} = \frac{\text{Dry weight of treated plants} \times 100}{\text{Dry weight of control plants}}$$

**Electrolyte leakage**

Ion leakage was measured as electrical conductivity (EC %) according to Yan *et al.*,<sup>17</sup> The percentage of electrolyte leakage was calculated according to this formula:

$$EC(\%) = \frac{A1}{A2} \times 100$$

Where A1 and A2 are the electrolyte conductivities measured before and after boiling, respectively.

**Determination of free amino acids and proline**

Free amino acids were detected according to the method of Lee and Takahashi<sup>18</sup>. Proline content was determined by ninhydrin method<sup>19</sup>.

**Lipid peroxidation**

It was measured as the content of malonyldialdehyde (MDA) using the thiobarbituric method of Heath and Packer<sup>20</sup>.

**H<sub>2</sub>O<sub>2</sub> content**

H<sub>2</sub>O<sub>2</sub> levels in the leaves were estimated according to Jena and Choudhuri<sup>21</sup> with minor modifications. H<sub>2</sub>O<sub>2</sub> levels were calculated using extinction coefficient 0.28 μmol<sup>-1</sup> cm<sup>-1</sup>.

**Chlorophyll content**

Chlorophyll was estimated according to the method of Harborne<sup>22</sup>. Estimation of chlorophyll was done by measuring the absorbance at 645 nm and 663 nm respectively in a UV-Vis spectrophotometer against a blank of 80% acetone and calculated using the formula as given by Arnon<sup>23</sup>.

$$\text{Total chlorophyll} = (20.2 A_{645} + 8.02 A_{663}) \text{ mg (g tissue)}^{-1} \text{ F.W}$$

**Carotenoid content**

The extraction and quantification of carotenoids was completed by following the method of Litchenthaler<sup>24</sup>. The absorbance was then assessed at 480 nm, 645 nm and 663 nm in UV-Vis

spectrophotometer and the carotenoid content was estimated using standard formula:

$$A_{480} - (0.114 \times A_{663}) - 0.638 (A_{645}) \mu\text{g (g tissue)}^{-1} \text{ F.W}$$

**Statistical analysis**

Data were analysed by using Standard Error and LSD tests at P ≤ 0.05 probability level using IBM SPSS statistics 21 software.

**RESULTS AND DISCUSSION****Characterisation of the prepared ligands and Cu (II) complexes**

The analytical data and corresponding Infrared spectra of the prepared ligands and the corresponding Cu(II) complexes are almost identical with the literature<sup>13</sup> and are provided in the supplementary (Table 1, Table 2, Table 3 and Scheme of reaction). The prepared two ligands show characteristic bands in the region of 1280 cm<sup>-1</sup> (for O-H bon), 1605-1635 cm<sup>-1</sup>(for C=N bond vibration). A broad band in the region of 2800-2700 cm<sup>-1</sup> signifies a strong hydrogen bonding between the hydroxyl hydrogen and nitrogen atom. For the prepared Cu complexes characteristic ν(C=N), ν(phenolic C-O), ν(Cu-N), ν(Cu-O) were observed. All Infrared spectra exhibit parity with literature.

The ligand L2 shows 3 main peaks 254.0, 318.0 and 368.5 nm in the electronic spectrum.

The first and second peaks are due to benzene π-π\* and imino π-π\* transitions where as the third peak is due to π-π\* transition. For C2 the third peak is shifted towards longer wave length due to donation of lone pair to Cu. The ligand L1 shows mainly two peaks and they are 270.5 and 362.5 nm (first one for π-π\* and second one for π-π\*). In C1 the π-π\* peak shifted to 396 nm.

**Growth and tolerance to different copper complexes**

Cu<sup>2+</sup> can hamper growth and development of seedlings by causing damage to epidermal cells and cell membranes.<sup>25</sup> The outcomes reveal that plants treated with C1 and C2 complex were able to retain a higher percentage of fresh weight, dry weight and relative water content (RWC) than CuSO<sub>4</sub> treated plants with increasing concentration suggesting less toxicity of Schiff base complexes (Figure 1, Figure 2 and Figure 3).

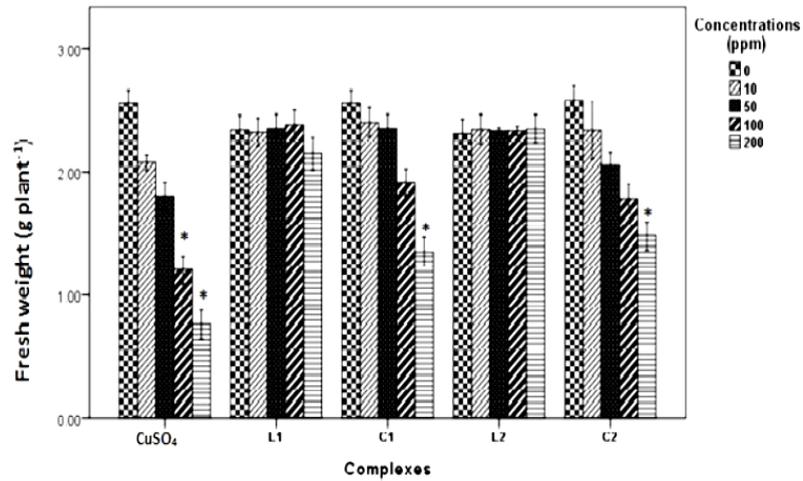


Fig. 1. Fresh weight (g plant<sup>-1</sup>) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

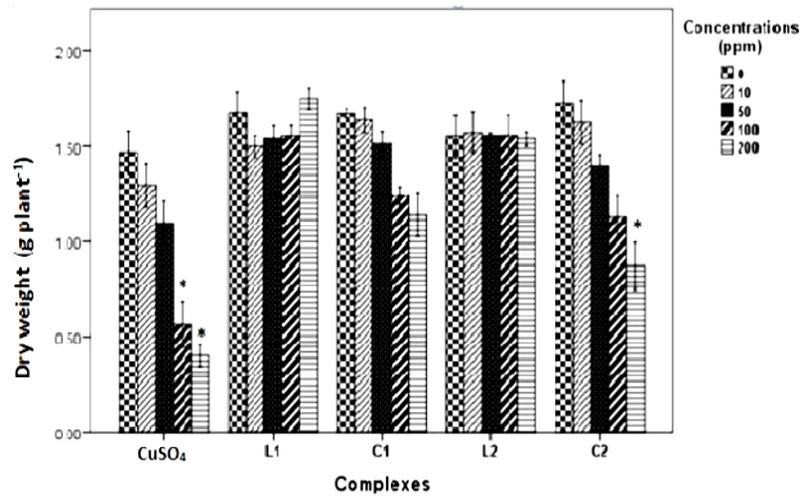


Fig. 2. Dry weight (g plant<sup>-1</sup>) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

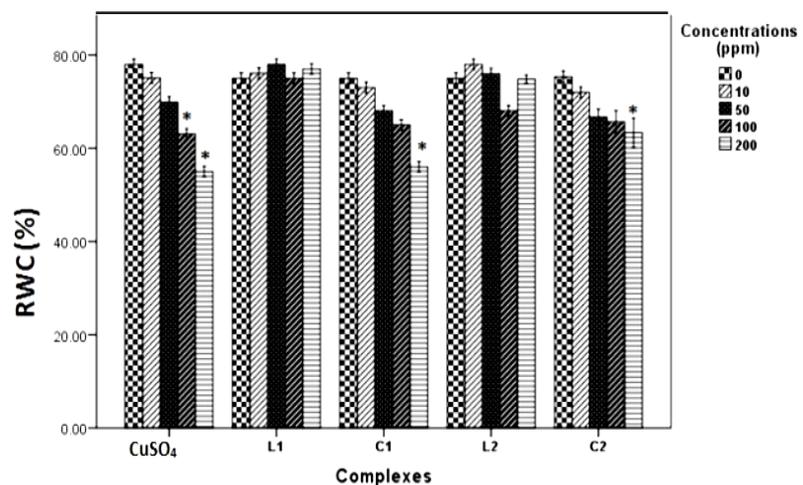


Fig. 3. Relative water content (RWC) of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

For instance at 100 and 200 ppm CuSO<sub>4</sub> significantly reduces RWC by 22.99% where as C1 and C2 complex reduce only by 12.01% and by 12.34% respectively over control. Greater negative impact of

CuSO<sub>4</sub> than the schiff base complexes on seedlings was further approved by Tolerance index. TI for C1 and C2 complex treated plants appeared to be significantly higher than that of CuSO<sub>4</sub> treated plants (Figure 4).

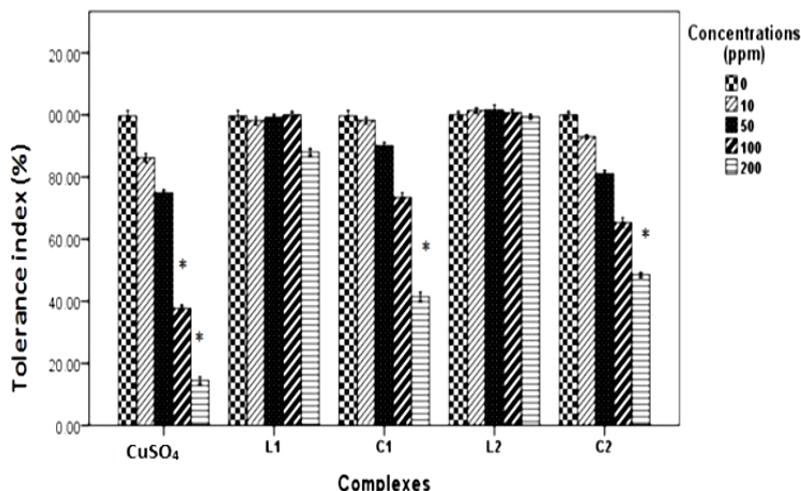


Fig. 4. Tolerance index (TI) of 45days old rice seedlings. Mean ± SE, n = 3. \* The mean difference is significant at the 0.05 level

Negative effect of CuSO<sub>4</sub> was also reported by Azooz *et al.*,<sup>26</sup> in wheat seedlings. Verma *et al.*,<sup>27</sup> reported application of copper at lesser concentrations enhanced the plant's dry biomass, while excess of copper reduced the biomass production of these plants.

**Membrane damage and ion leakage**

Copper can distress the membrane permeability by oxidation of membrane lipids which can be accessed from the increase of

MDA, one of the lipid peroxidation products. Data obtained from this study revealed copper induced membrane damage which is expressed in terms of electrolyte leakage, lipid peroxidation enhanced significantly in CuSO<sub>4</sub> treated plant with rising concentration but in case of Schiff base complex treated plants malonyldialdehyde accumulation as well as electrolyte leakage were less as compared to CuSO<sub>4</sub> treated plants in relation to control indicating comparatively less membrane damage in Schiff base complex treated plants.

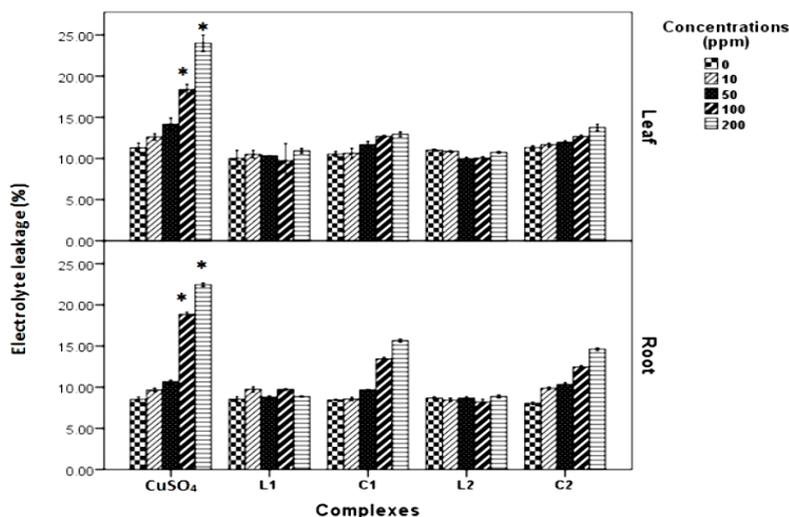


Fig. 5. Electrolyte leakage (%) Of 45 days old rice seedlings. Mean ± SE, n = 3. \* The mean difference is significant at the 0.05 level

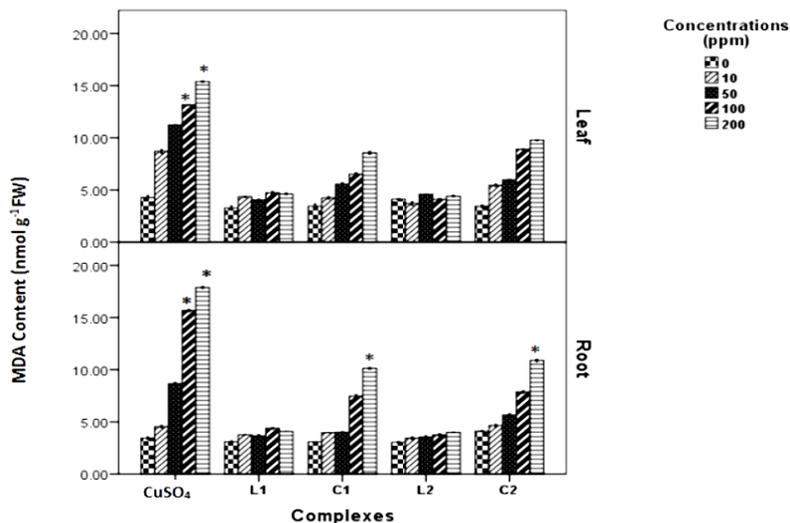


Fig. 6. MDA content of 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \* The mean difference is significant at the 0.05 level

The outcomes showed that the MDA content in leaf (Fig. 6) and electrolyte leakage in leaf and root (Fig. 5) of Schiff base Cu(II) complex treated plants increase with increasing dose but did not echo any significant changes. But in case of CuSO<sub>4</sub> treated plants ion leakage and oxidation of membrane lipid enhanced drastically at 100 and 200 ppm level in relation to control plants.

Reactive oxygen species induced plasma membrane damage increase MDA and ion leakage at the higher levels of Cu<sup>2+</sup>.<sup>28</sup> Hydrogen peroxide [one form of reactive oxygen species (ROS)] accumulation is minimum in case of Schiff base Cu(II) complex treated plants than in CuSO<sub>4</sub> treated plants (Figure 7).

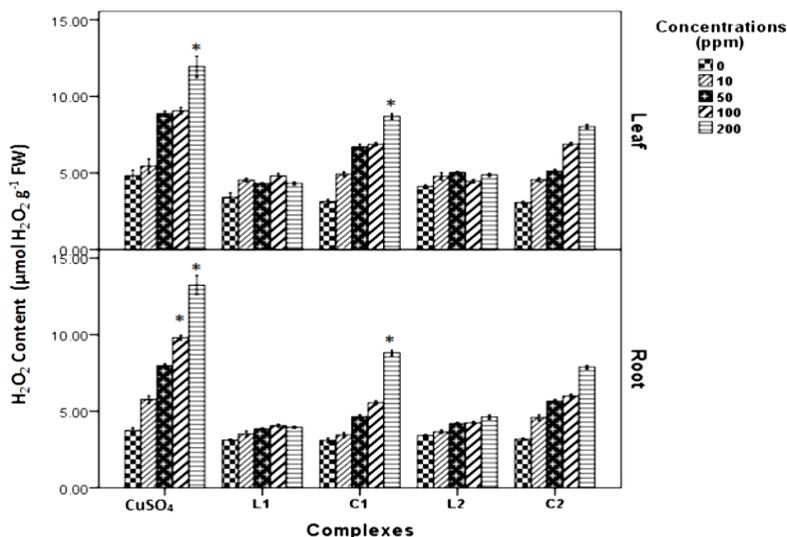


Fig. 7. H<sub>2</sub>O<sub>2</sub> accumulation in 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \* The mean difference is significant at the 0.05

In copper sulphate treated plants H<sub>2</sub>O<sub>2</sub> increased 1.35 fold at 200 ppm where in Schiff base complex treated plants especially in case of C2 complex H<sub>2</sub>O<sub>2</sub> accumulation was less (0.62 fold) in relation to control. This observation appears to be narrated the redox-active character

of Cu<sup>2+</sup> resulting in creation of extremely reactive hydroxyl radicals.<sup>29</sup> These results suggest that Schiff base Cu(II) treated plants had better ability to tolerate Cu<sup>2+</sup> stress. The significant increase of MDA in plants, exposed to CuSO<sub>4</sub> indicated that increase of lipid peroxidation in Cu-treated plants

led to disorder of plasma membranes on contrary plasma membrane damage was less in case of Schiff base Cu (II) complexes treated plants.

#### Total free amino acids and proline

Accumulations of total free amino acids (Fig. 8) were significantly increased in root and leaf tissue at higher  $\text{CuSO}_4$  concentrations. However,

low  $\text{CuSO}_4$  up to 50 ppm had non-significant enhancement on total free amino acids. The highest increase in free amino acids in case of  $\text{CuSO}_4$  treatment was noticed at 100 and 200 ppm in both leaf and root tissue. On contrary both the Schiff base Cu(II) complex treated plants showed insignificant enhancement of amino acids.

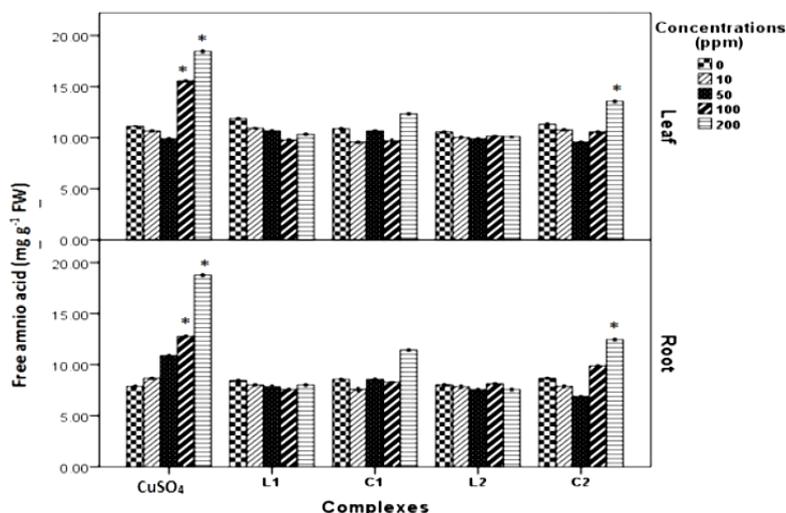


Fig. 8. Free amino acid accumulation in 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

Al-Hakimi and Hamada<sup>30</sup>, in their study, suggested that free amino acids content enhances in plant tissues upon  $\text{Cu}^{2+}$  exposure. In agreement amino acids are looked upon as key player in metal chelation through which plant detoxify alleviate heavy metal stress.<sup>31</sup> Therefore, it might be suggested that plants experiencing higher amount of copper induced oxidative stress can accumulate greater amount of free amino acid to alleviate oxidative stress. In that scenario copper Schiff base complexes are proved to be less toxic than  $\text{CuSO}_4$ .

Proline accumulation in plant tissue which is an indicator of oxidative stress<sup>32</sup> increased in all treatments. In  $\text{CuSO}_4$  treated plants proline accumulation was maximum at 100 ppm and at 50 ppm in leaf and root tissue respectively. Beyond this concentration proline accumulation decreased. Decline in proline accumulation may be attributed to reduction competence of plants to withstand oxidative stress.<sup>33</sup> Conversely, in Schiff base Cu(II) complex treated plants, proline content continued to be increased up to 200 ppm signifying lesser oxidative stress in those plants (Figure 9).

Proline accumulation indeed stabilizes plasma membrane free radical scavenger and some

macro molecules and facilitates rapid recovery from heavy metal stress.<sup>34</sup>

#### Photosynthetic pigments

Copper facilitates in the utilization of iron during chlorophyll synthesis and enhances photosynthesis at low concentration. But at higher concentration copper reduces photosynthetic competence, low quantum efficiency of Photosystem (II) and reduced cell elongation<sup>35</sup>. Our study revealed that total chlorophyll content increased in case of all treatments up to 50 ppm. Beyond this concentration total chlorophyll content reduced, but the reduction was maximum in case of  $\text{CuSO}_4$  treated plants and minimum for C2 treated plants (Fig. 10). Less reduction of chlorophyll may be attributed to less copper toxicity of Schiff base complexes to plants.

Carotenoid, a non enzymatic antioxidant involved in quenching of oxidizing species, participate in disrupting regular cellular functioning. In  $\text{CuSO}_4$  treated plants carotenoid content increased gradually upto 100 ppm. At 200 ppm carotenoid content reduced drastically signifying the huge toxicity of  $\text{CuSO}_4$  but on contrary in C1 and C2 treated plants carotenoid content continued to increase up to 200 ppm (Figure 11).

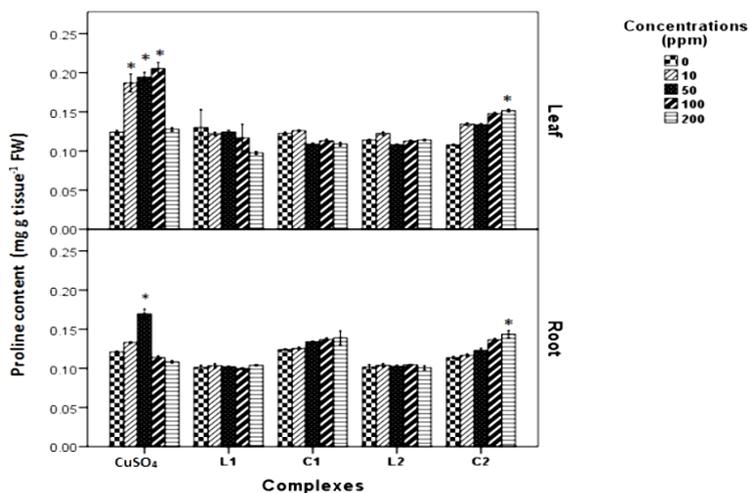


Fig. 9. Proline level in 45 days old rice seedlings. Means  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

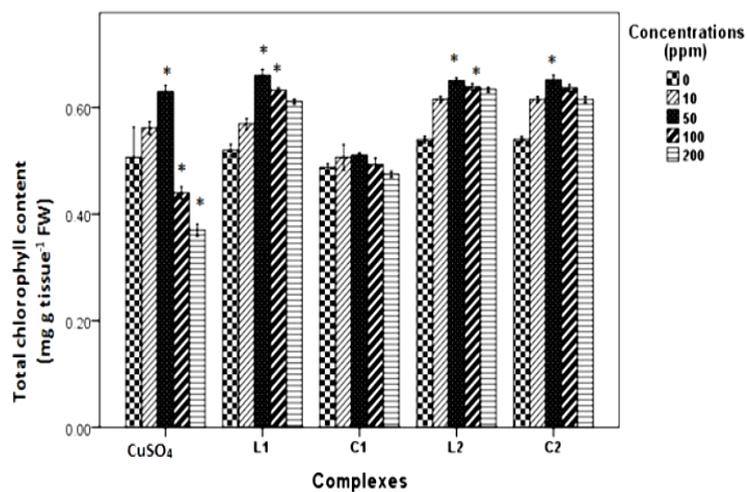


Fig. 10. Total chlorophyll content in 45 days old rice seedlings. Mean  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

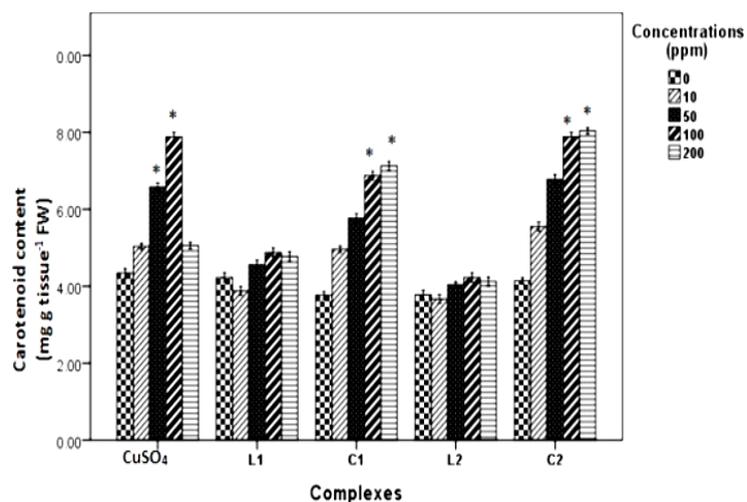


Fig. 11: Carotenoid content in 45 days old rice seedlings. Means  $\pm$  SE, n = 3. \*The mean difference is significant at the 0.05 level

**Table 1: Elemental analysis data and some physical properties of the Schiff bases and their complexes**

Formula	Colour	Yield (%)	Found (Calculated)%			Conductivity	
			C	H	N	M	C ohm <sup>-1</sup> .cm <sup>2</sup> . mol <sup>-1</sup>
L2	C <sub>32</sub> H <sub>24</sub> N <sub>6</sub> O <sub>2</sub>	Orange	80	73.02 (73.26)	4.53 (4.61)	15.98 (16.03)	
C2	C <sub>32</sub> H <sub>22</sub> N <sub>6</sub> O <sub>2</sub> Cu	Red	75	65.64 (65.57)	3.69 (3.78)	14.42 (14.34)	10.78 (10.84)
L1	C <sub>28</sub> H <sub>24</sub> N <sub>6</sub> O <sub>2</sub>	Orange	84	70.6 (70.57)	5.2 (5.0)	17.58 (17.64)	
C1	C <sub>28</sub> H <sub>22</sub> N <sub>6</sub> O <sub>2</sub> Cu	Green	73	62.55 (62.5)	4.02 (4.12)	15.53 (15.62)	11.76 (11.81)

Note : Decomposition occurs with conc. nitric acids, and the resultant solution was used after suitable dilution for metal analysis

**Table 2: IR spectroscopic data of the Schiff bases and their complexes**

Compound	$\nu(\text{C}=\text{N})$	$\nu(\text{phenolic C-O})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
L2	1610	1578	1285	
C2	1630	1592	1258	530 430
L1	1640	1588	1290	
C1	1627	1578	1257	514 449

**Table 3: Electronic spectral data of the Schiff bases and their Cu(II) complexes**

$\lambda_{\text{max}}(\epsilon, \text{L/mol cm})$ in DMF			
L2	368.5	318	254
C2	398	318	251
L1	362.5	270.5	
C1	396	266	

### CONCLUSION

Offshoots of the present study reveals that Schiff base Cu(II) complexes have less toxic effects than copper sulphate on rice seedlings and provide better tolerance to copper toxicity than copper sulphate. Maximum positive impact of Schiff base complexes was found mostly at 50 ppm concentration. Though different stress marker and reactive oxygen species accumulation were less and minimum pigment damage was noticed in Schiff base Cu(II) complex treated seedlings but the optimum positive impact of these Schiff base Cu(II) complexes largely depends on dose. Beyond certain concentration these complexes may have inhibitory

effects on rice. Cu(II) schiff base complexes can be used as a supplement to turn down micronutrient deficiency at the same time minimize the toxicity generated by application of different ionic form of Cu(II) may open a new direction research.

### ACKNOWLEDGEMENT

The authors are grateful to the Departmental Special Assistance Scheme under the University Grants Commission, New Delhi (SAP-DRS-III, No. 540/12/DRS/2013) for financial support. Authors (K. Acharjee and J. Sarkar) are thankful to UGC, India and Department of science and technology, India respectively for providing fellowships.

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