

## **CHAPTER III**

### **Morphological and biochemical effects of some 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.\***

#### **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<sub>2</sub>O<sub>2</sub> coordination sites and their Zn<sup>2+</sup> 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*<sup>2</sup> 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 $\cdots$ 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<br>(71.3)       | 5.9<br>(5.9) | 10.2<br>(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<br>(75.8)       | 5.1<br>(5.1) | 8.9<br>(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<br>(65.7)       | 6.1<br>(6.2) | 8.5<br>(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<br>(70.1)       | 5.3<br>(5.2) | 7.5<br>(8.0)   |

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

| Ligand | $\nu_{\text{C=N}}$ | $\delta_{\text{O-H}}$ | Aromatic ring vibrations | $\nu_{\text{C-H}} + \nu_{\text{N-H}}$ | $\nu_{\text{C-O}}$ |
|--------|--------------------|-----------------------|--------------------------|---------------------------------------|--------------------|
| 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)]<br>Monohydrate                        | 349.7          | 82.0      | 54.9<br>(54.8)       | 4.6<br>(4.7) | 8.0<br>(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)]<br>Monohydrate          | 397.74         | 81.7      | 60.5<br>(60.2)       | 4.0<br>(4.2) | 7.1<br>(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)]<br>Monohydrate                | 409.75         | 81.3      | 52.8<br>(52.5)       | 4.9<br>(5.0) | 6.9<br>(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)]<br>Monohydrate | 457.79         | 79.6      | 57.8<br>(57.5)       | 4.4<br>(4.6) | 6.1<br>(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%<br/>seed germination</b> | <b>Period of<br/>germination (days)</b> | <b>Germination<br/>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<br>(cm) |         | Root length<br>(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            |

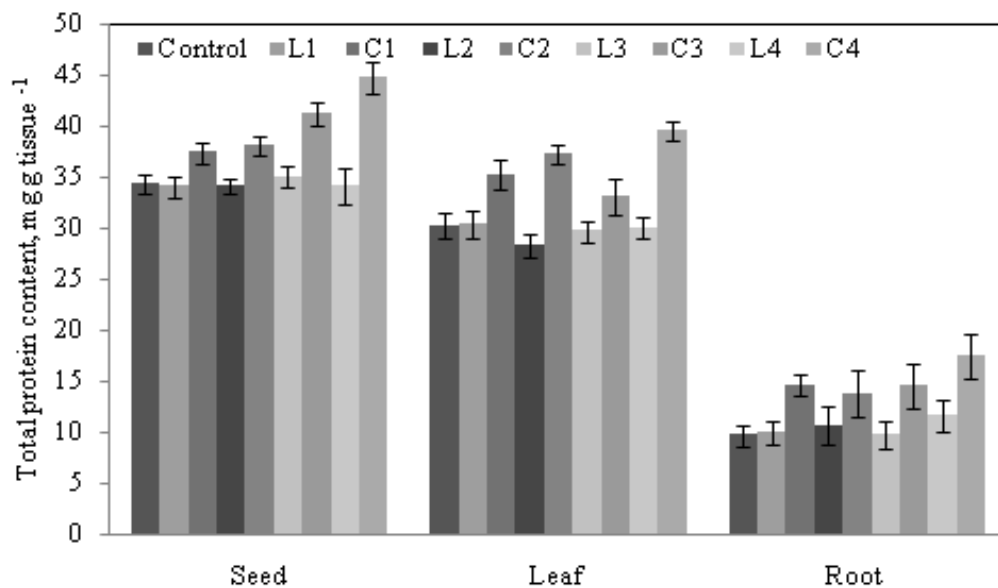
SVI = Seedling vigour index, GP = Germination percentage, GI = Germination index, CVG = Coefficient of velocity of germination. LSD values are computed (at  $P \leq 0.05$ ) to evaluate mean performances amongst different compounds.

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.

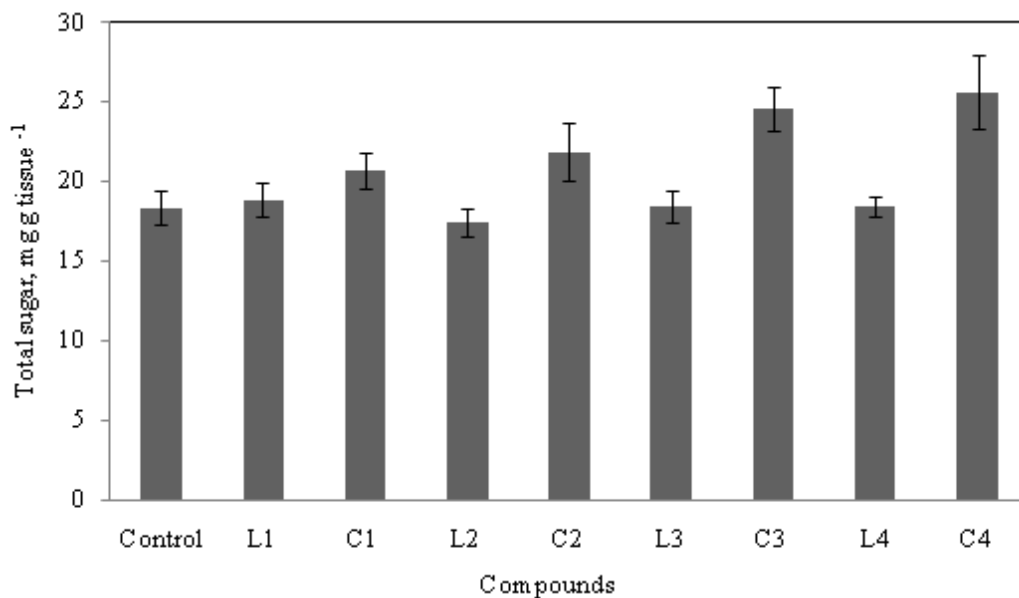
**Table 3.9.** Effects of the ligands, their Zn(II) complexes on various growth parameters (Mean  $\pm$  S.D). LSD values are computed (at  $P \leq 0.05$ ) to evaluate mean performances amongst different compounds.

| <b>Compounds</b>         | <b>Seedling length<br/>(cm)</b> | <b>Hypocotyl length<br/>(cm)</b> | <b>Radicle length<br/>(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<br>( $P \leq 0.05$ ) | 1.09                            | 2.06                             | 1.88                           |

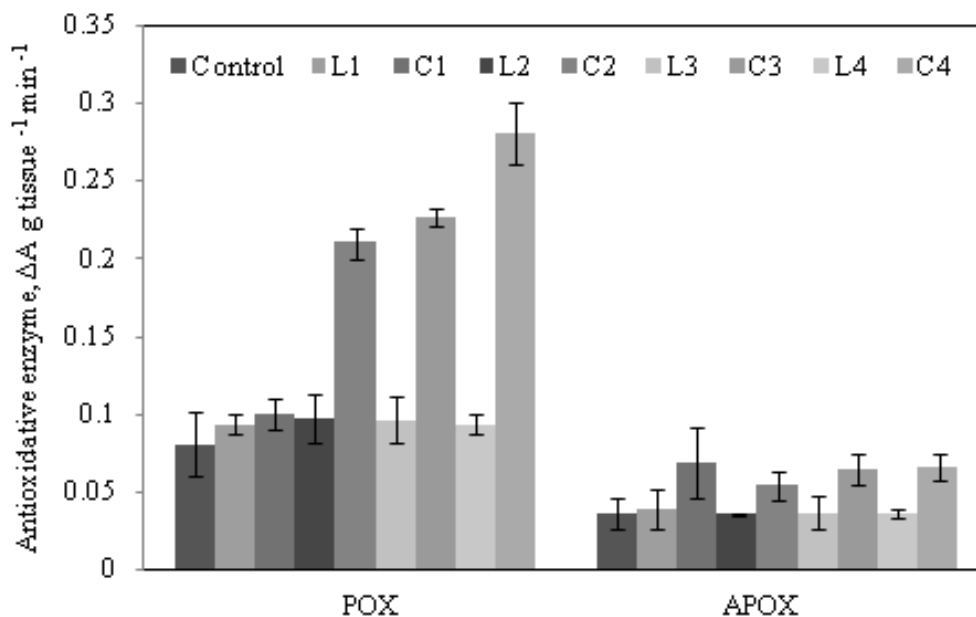
In plant system, Zn is present as cofactor of more than 300 enzymes.<sup>23</sup> based on the above biochemical parameters it is found that total soluble protein content gradually increases in seed, root and shoot after the treatment with Zn(II) complexes and these increments range from 9.07-30.50%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

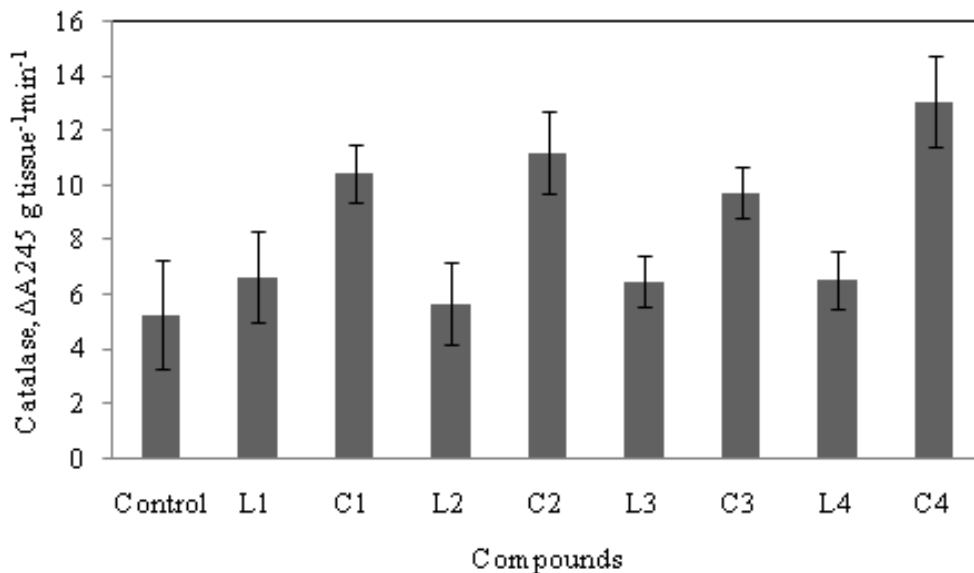


**Fig 3.2.** Total sugar estimation after the treatment with the ligands, their Zn(II) complexes and the control. Results are represented as mean  $\pm$  SD (n=3).



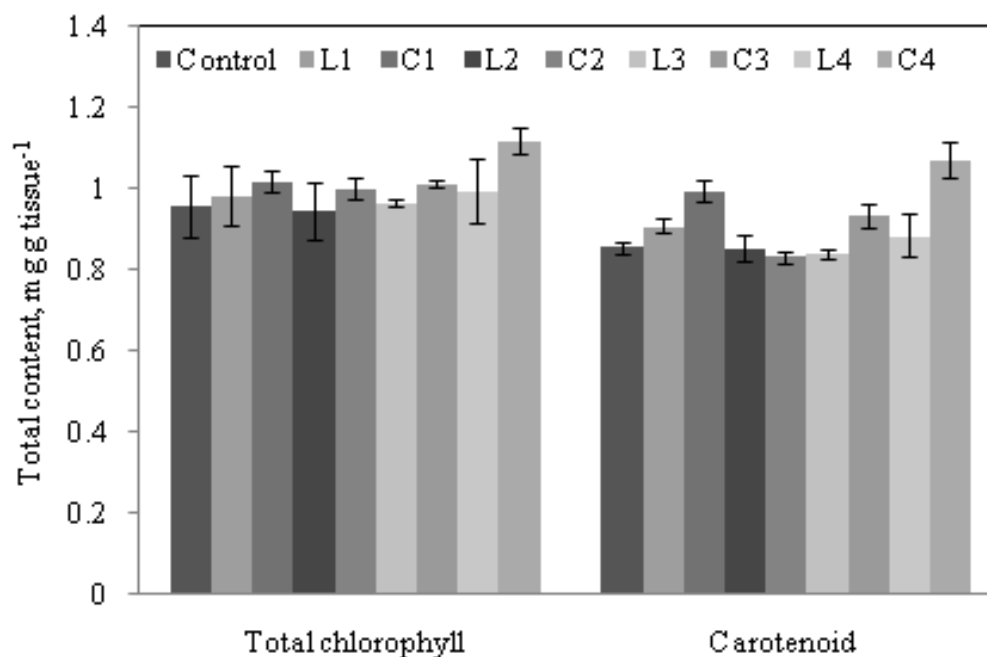
**Fig 3.3.** Effects of the ligands, their Zn(II) complexes and the control on antioxidative enzymes, Peroxidase ( POX) and Ascorbate Peroxidase (APOX) in leaves of desi chickpea. Results are represented as mean  $\pm$  SD (n=3).

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  $H_2O_2$  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<br>( $\mu\text{g g}^{-1}$ ) |
|---------------------|--|
| 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.

### **References**

- 1) D. Bryce-Smith, *Chem. Brit*, **25** (1989) 783.
- 2) A. Camp, B. Fudge, *Soil Science* **60** (1945) 157.
- 3) G. Rout, S. Samantaray, P. Das, *Plant Sci.* **137** (1978) 89.
- 4) P. Aravind, M. Narasimha , V. Prasad, *Braz. J. Plant Physiol.* **17** (2005) 3.
- 5) B. Vallee, D. Auld, *Biochemistry* **29** (1990) 5647.
- 6) S. Thind, D. Rowell, *Biol Fertil Soils.* **28** (1999) 162.
- 7) R. Brennan, D. Bolland, *Aust J Exp Agric.* **46** (2006) 1341.
- 8) H. Khoshgoftarmanesh, R. Schulin, R. Chaney, B. Daneshbakhsh, M. Afyuni, *Agron Sustain Dev.* **30** (2010) 83.
- 9) A. Wallace, A. Wallace, *Iron nutrition and interactions in plants* (1982) 975.
- 10) A. Butler, C. Carrano, *Coord Chem Rev.* **109** (1991) 61.
- 11) T. Katsuki, *Coord. Chem. Rev.* **140** (1995) 189.
- 12) D. Boghaei, S. Mohebbi, *Tetrahedron* **58** (2002) 5357.
- 13) S. Mohebbi, D. Boghaei, A. Sarvestani, A. Salimi, *Appl. Catal. A: Gen.* **278** (2005) 263.
- 14) O. V. Kotova, S. V. Eliseeva, A. S. Averbushkin, L. S. Lepnev, A. A. Vaschenko, A. Yu. Rogachev, A. G. Vitukhnovskii, N. P. Kuzimina, *Russian Chemical Bulletin.* **57** (2008) 1880.

- 15) K. Gomez, A. Gomez, *Statistical Procedures for Agricultural Research*, 2nd ed, New York: John Wiley and Sons (1983).
- 16) D. Chapman, P. Pratt, (1961) *Methods of Analysis for Soils, Plants, and Waters*, Riverside, USA: Division of Agricultural Sciences, University of California, (1961) 4034.
- 17) D. Sauer, R. Burroughs, *Phytopathology* **76** (1986) 745.
- 18) M. Broadley, P. White, J. Hammond, I. Zelko, A. Lux, *New Phytol.* **173** (2007) 677.
- 19) A. Yilmaz, H. Ekiz, I. Gultekin, B. Torun, H. Barut, S. Karanlik, I. Cakmak, *Journal of Plant Nutrition* **21** (1998) 2257.
- 20) A. Ajouri , H. Asgedom , M. Becker, *Journal of Plant Nutrition and Soil Science* **167** (2004) 630.
- 21) N. Slaton, C. Wilson, S. Ntamatungiro, R. Norman, D. Boothe, *Agronomy Journal.* **93** (2001) 152.
- 22) M. Ebrahim, M. Aly, *Journal of Plant Nutrition* **27** (2005) 1859.
- 23) A. Henriques, J. Chalfun, M. Aarts, *Brazilian Journal of Plant Physiology* **24** (2012) 3.
- 24) I. Cakmak, *New Phytol.* **146** (2000) 185.
- 25) Z. Miszalski, I. Ślesak , E. Niewiadomska , R. Bałczekoxide Kwinta , U. Lüttge, R. Ratajczak, *Plant Cell Environ.* **21** (1998) 169.
- 26) N. Smirnov, P. Conklin, F. Loewus, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52** (2001) 437.
- 27) M. Bonnet, O. Camares, P. Veisseire, *J. Expt. Bio.* **51** (2000) 945.
- 28) M. Chvapil, *Life Sci.* **13** (1973) 1041.
- 29) T. Kösesakal, M. Ünal, *Fresenius Environmental Bulletin* **21** (2012) 315.
- 30) W. Aslam, M. Arfan, S. Sahid, F. Anwar, Z. Mahmood, U. Rashid, *International Journal of Chemical and Biochemical Science* **5** (2014) 11.
- 31) T. Khudsar, M. Mahmooduzzafar, M. Iqbal, R. Sairam, *Biologica Planterum* **48** (2004) 255.