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.[1] Although Vanadium was discovered in early eighteenth century, very little work was done on Vanadium before the 1950s.[2] 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.[3] However, interest intensified when Arnon and Wessel (1953) concluded that Vanadium is essential for some plants.[4] Subsequent studies showed that Vanadium is generally toxic to terrestrial plants when applied in amounts greater than pico-molar levels.[5] But it has been found to be beneficial for plant growth and development when applied in trace amount.[6,7] 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+}).[8] Moreover V^{4+}, predominantly found in the soil, is responsible for the development of the plants.[9] Study revealed that Vanadium acts as constituent of the cofactors in vanadate-dependent haloperoxidases and vanadium nitroginase.[10] Many important electron transfer processes and plasma membrane hydrogen (H^+)-translocation ATPase are largely dependent upon vanadium.[11] 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.[12]

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.[13] 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.[14,15] 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.[16] 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 NH_4VO_3 and the vanadyl complex were thoroughly studied in terms of relative water content, biochemical components, oxidative stress markers and overall tolerance level, *etc*.

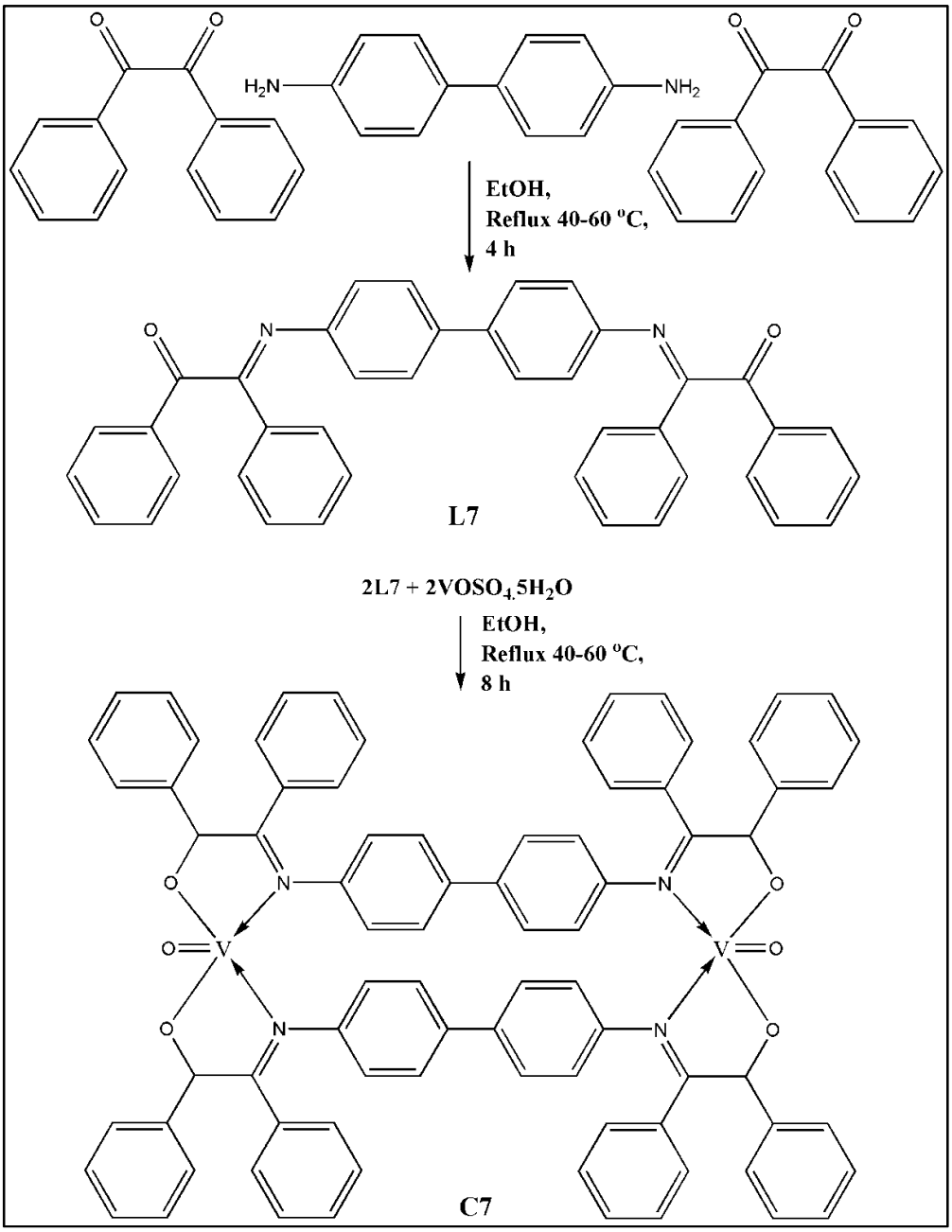
5.2. Materials and methods

5.2.1. Synthesis of the ligands and its vanadyl complex

The synthesis of a polydentate Schiff base ligand (**L7**) and its vanadyl (VO^{2+}) complex (**C7**) are discussed in Chapter II. These compounds were prepared by following literature method[17] (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 ± 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 H_2O and transferred to 20% Steiner nutrient solution (1.8 mM $Ca(NO_3)_2.4H_2O$, 0.8 mM $MgSO_4.7H_2O$, 0.2 mM KH_2PO_4, 0.6 mM KNO_3, 0.6 mM K_2SO_4, 89.31 μM Fe, 42.37 μM Mn, 7.12 μM Zn, 39.98 μM B, 2.93 μM Cu, 1.80 μM 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 μM) of the Schiff base ligand (**L7**), its vanadyl complex (**C7**) and NH_4VO_3 (AV) along with a control (no treatment of nutrient solution the ligand, the complex and NH_4VO_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.[17] 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 cm^{-1} appeared due to $\boldsymbol{\nu_{C=N}}$, $\boldsymbol{\nu_{C=O}}$ and $\boldsymbol{\nu_{O-H}}$ vibrations, respectively for the ligand (L7). After complexation the $\boldsymbol{\nu_{C=N}}$ band shifted to 1624 cm^{-1} due to coordination bond formation. For the complex two new bands appeared at 982 and 491 cm^{-1} due to $\boldsymbol{\nu_{V-O}}$ and $\boldsymbol{\nu_{V-N}}$ vibrations, respectively. The electronic spectra were measured with 5×10^{-4} molar solutions in dimethylformamide for both the compounds. The ligand (C7) has a characteristic $\boldsymbol{\lambda_{max}}$ at 263.1 nm due to π-π^* 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.[17]

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

	Molecular Formula	Colour	Formula Weight	m. p (°C)	% Found (Calculated)		
					C	H	N
L7	$C_{40}H_{28}N_2O_2.2H_2O$	Olive green	605.67	230	78.75 (79.45)	4.96 (5.3)	5.28 (4.6)
C7	$C_{80}H_{56}N_4O_6V_2.5H_2O$	Pale green	1361.28	> 280	70.01 (70.58)	4.59 (4.8)	5.33 (4.12)

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

	$\boldsymbol{\nu_{C=N}}$	$\boldsymbol{\nu_{O-H}}$	$\boldsymbol{\nu_{C=O}}$	$\boldsymbol{\nu_{V-O}}$	$\boldsymbol{\nu_{V-N}}$	$\boldsymbol{\lambda_{max}}$
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 5µM, 10µM and 20µM of L7, C7 and NH_4VO_3 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 20µM concentration of NH_4VO_3 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.

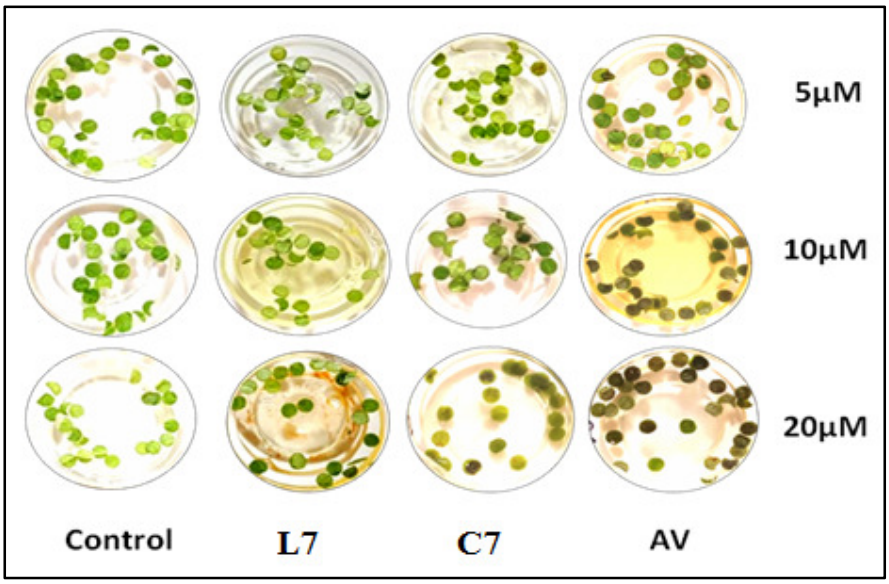


Fig 5.1. Leaf senescence assay: phenotypic changes observed as chlorophyll bleaching in response to 5µM, 10µM and 20µM of L7, C7 and NH_4VO_3 for 7 days.

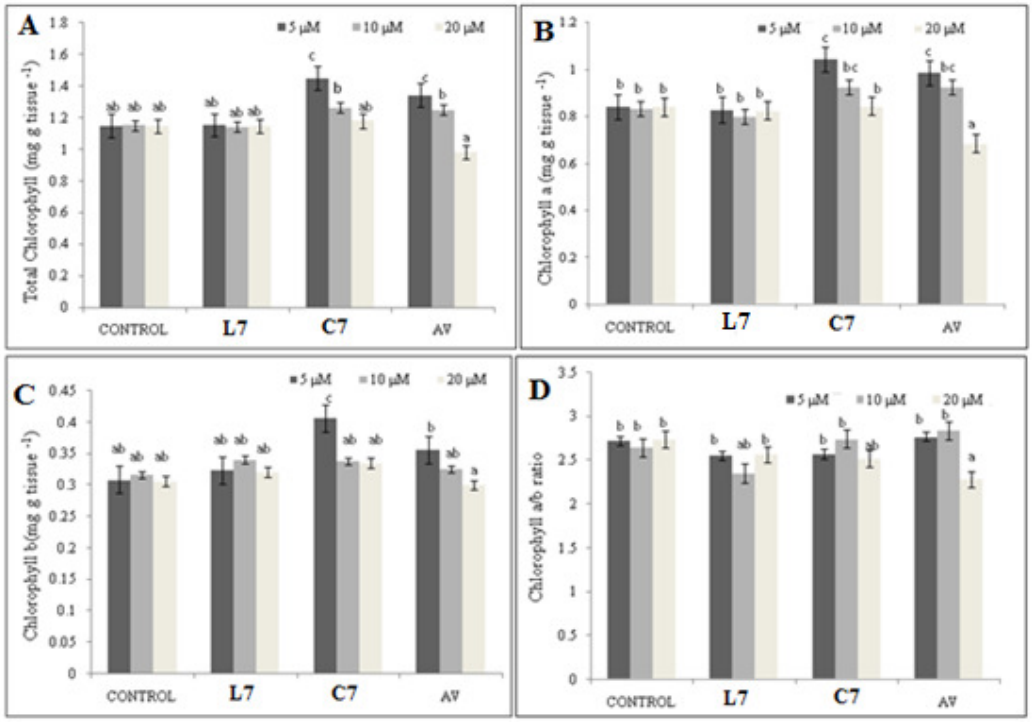


Fig 5.2. Effects of the control, L7, C7 and NH_4VO_3 (AV) on: A, total chlorophyll content; B, chlorophyll a content; C, chlorophyll b content and D, chlorophyll a/b ratio of mung bean at various concentrations. Values are represented as mean ± SD (n=3). Bars with different letters are significantly 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 °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 NH_4VO_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 NH_4VO_3 treated plants signified higher stress in cells (shown in Table 5.3).

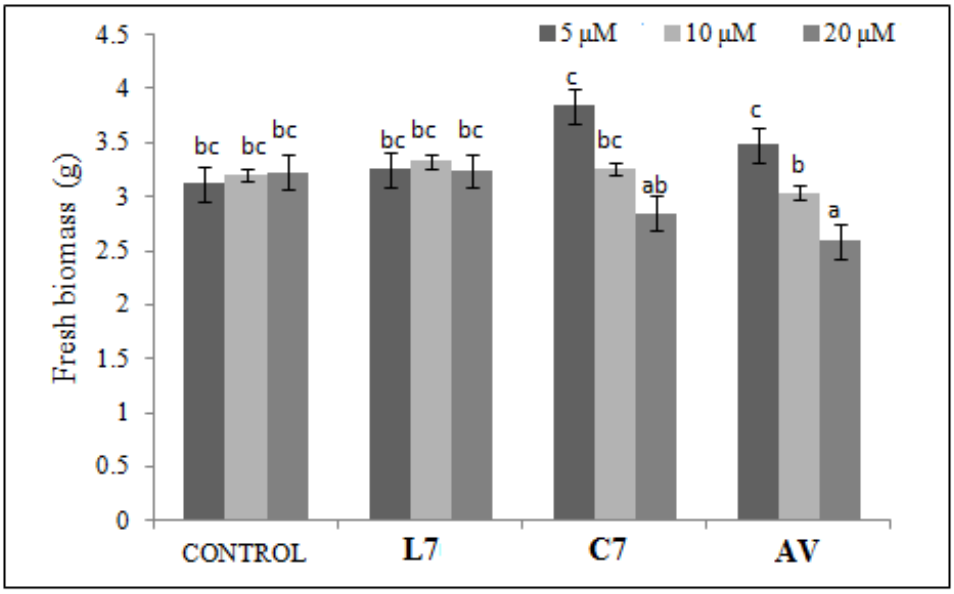


Fig 5.3. Effects of the control, L7, C7 and NH_4VO_3 (AV) on fresh biomass of mung bean at various concentrations. Values are represented as mean ± SD (n=3). Bars with different letters are significantly different at $P \leq 0.05$ according to Fischers LSD set.

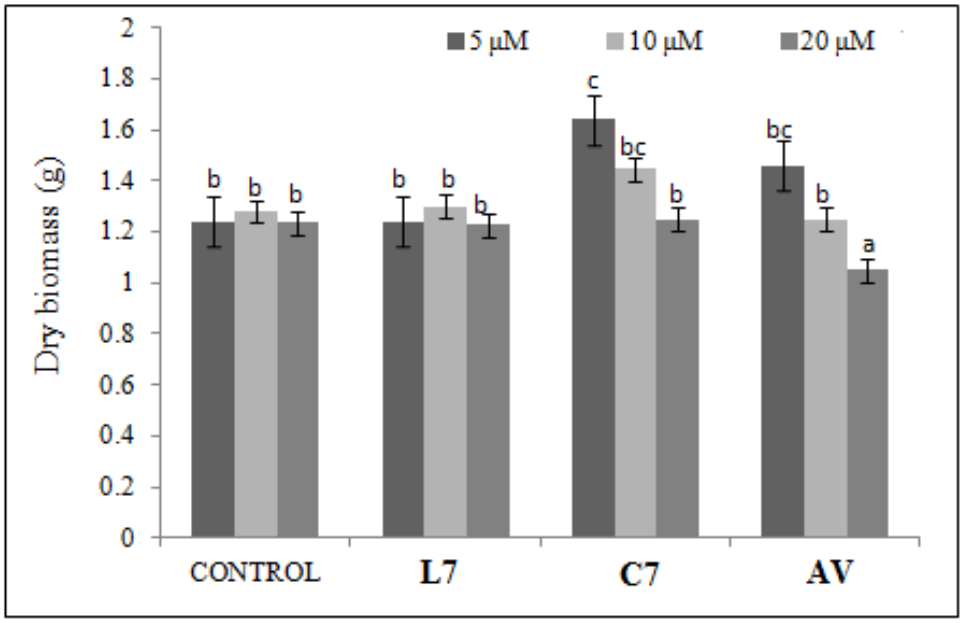


Fig 5.4. Effects of the control, L7, C7 and NH_4VO_3 (AV) on dry biomass of mung bean at various concentrations. Values are represented as mean ± SD (n=3). Bars with different letters are significantly different at $P \leq 0.05$ according to Fischers LSD set.

Table 5.3. Effects of the control, L7, C7 and NH_4VO_3 (AV) on relative water content (RWC) of mung bean at various concentrations. Values are represented as mean ± 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 μM	81.29 ± 2.12c	81.16 ±1.95	81.53 ±1.45c	80.75 ± 2.01c
10 μM	81.60 ±2.45c	81.29 ±1.56	76.63 ±1.58c	68.29 ±1.92b
20 μM	81.53 ±2.74c	79.92 ±1.87c	68.93 ±1.94b	59.75 ±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.[18-20] Amongst a variety of ROS, H_2O_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.[21,22] Lipid peroxidation produces malonaldehyde as the end product in a chain of reactions with membrane phospholipid molecules.[23] 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 NH_4VO_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.[24-26] To have a better understanding of the adverse impact of different concentrations of the Schiff base VO^{2+} complex(C7) and NH_4VO_3 (AV) on plants; stress markers such as electrolyte leakage (EL), Hydrogen peroxide (H_2O_2) and peroxidation of membrane lipids (MDA) and cell viability were assessed (illustrated Fig 5.5).

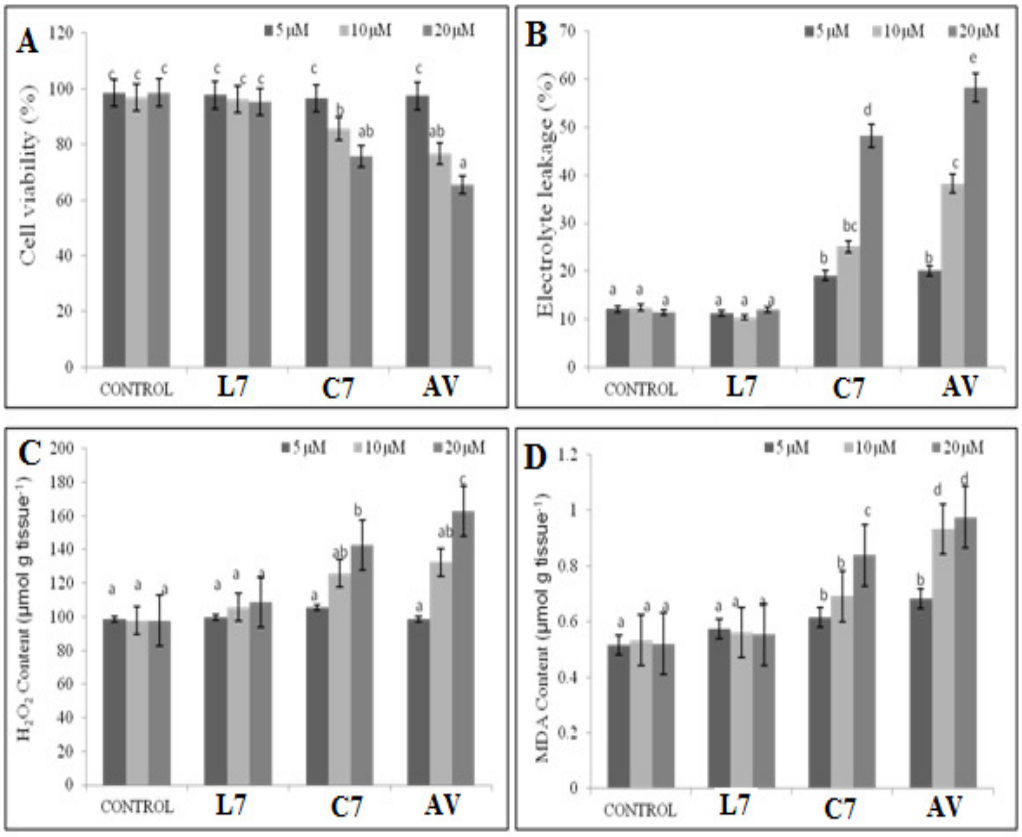


Fig 5.5. Effects of the control, L7, C7 and NH_4VO_3 (AV) on: A, Cell viability; B, Electrolyte leakage; C, H_2O_2 content and D, MDA content respectively of mung bean at various concentrations. Values are represented as mean ± SD (n=3). Bars with different letters are significantly different at $P \leq 0.05$ according to Fischers LSD set.

In all the plants, electrolyte seepage from the membranes increased gradually with the increasing concentration of the Schiff base complex (C7) and NH_4VO_3 (AV) whereas in L7 there were no such changes in these parameters in relation to control. At 5 µM level electrolyte leakage (EL) remained similar for both C7 and AV whereas 10µM and 20µM of AV caused considerable membrane leakage as compared to C7 treated plants. H_2O_2 accumulation was pretty similar in the Schiff base complex (C7) and AV treated plant leaves up to 10µM while at 20µM, there was greater H_2O_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 20µM whereas in AV treated plants significant induction was observed from 20µM. 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 5μM. 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 (5μM) 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.[27] 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 (5μM). But at higher concentration (20μM) 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.

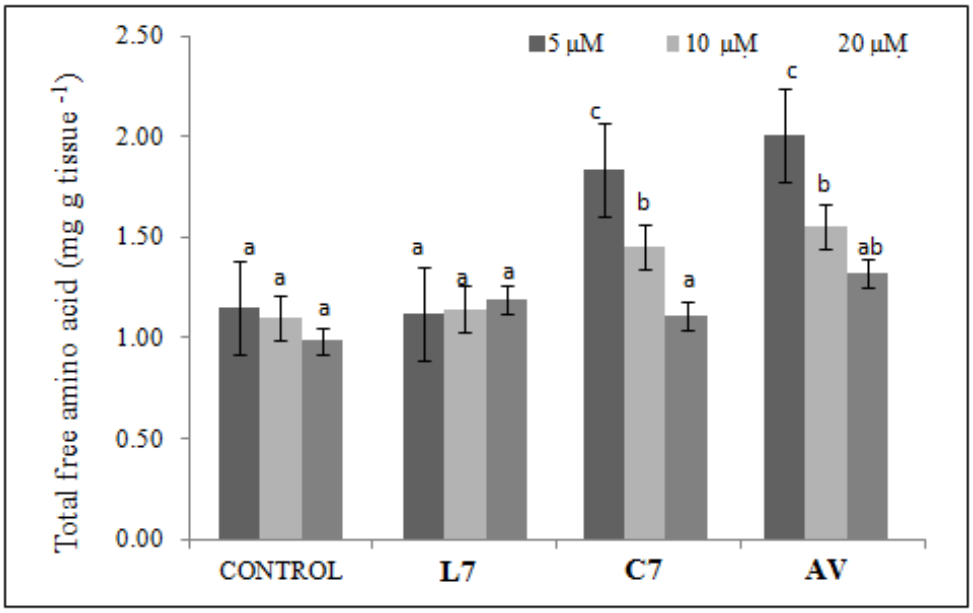


Fig 5.6. Effects of the control, L7, C7 and NH_4VO_3 (AV) on total free amino acid content of mung bean at various concentrations. Values are represented as mean ± SD (n=3). Bars with different letters are significantly different at $P \leq 0.05$ according to Fischers LSD set.

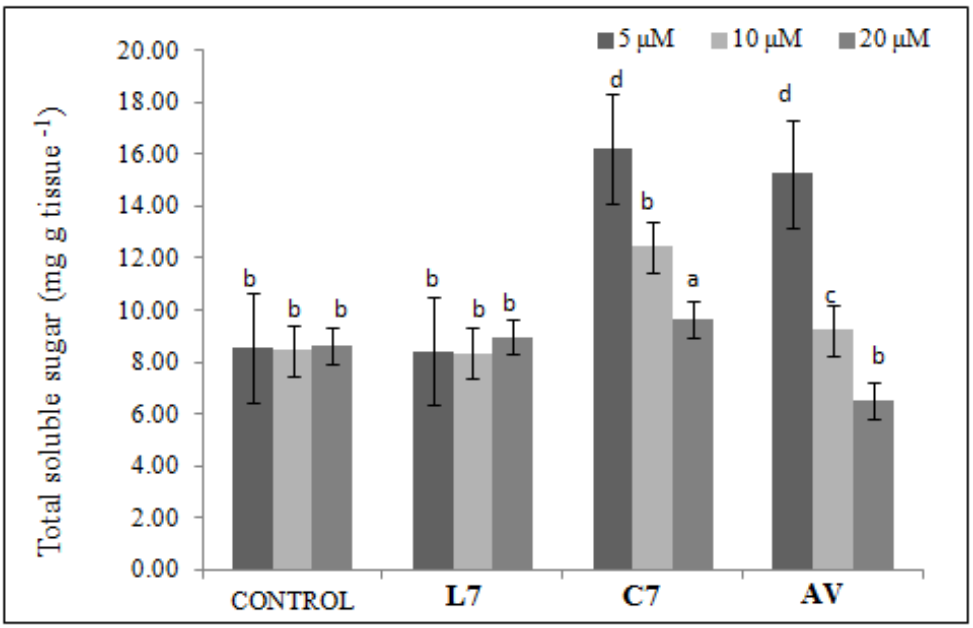


Fig 5.7. Effects of the control, L7, C7 and NH_4VO_3 (AV) on total soluble sugar content of mung bean at various concentrations. Values are represented as mean ± SD (n=3). Bars with different letters are significantly different at $P \leq 0.05$ according to Fischers LSD set.

5.4. Conclusions

Outcomes of the present experiment reveal that the Schiff base complex (C7) has less toxic effects than NH_4VO_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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