Indian J. Applied & Pure Bio. Vol. 40(3), 2123-2130 (2025). **ISSN: 0970-2091**
A web of Science Journal

Synthesis and spectral characterization of Molybdenum, Iron, complexes and their effect on germination of leguminous plants *Glycine max* (L.) Merr. {Soybean seed} and *Psium sativum* L. {Pea seed}

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Abstract

A new series of Schiff base ligands and their metal complexes were prepared by reacting an amino acid (glycine) with derivatives of benzaldehyde in a 1:2 ratio under appropriate reaction conditions. These ligands were further complexed with metal ions of iron, and molybdenum by reacting them with their respective metal salts. The ligand was characterized by elemental analysis (C, H, N), and the complexes were characterized using UV-Visible, IR, AAS, and conductivity methods. The ligands were synthesized using a known literature method. Seeds of soybean and pea were treated with M-L solution, ligand solution, metal ion solution, and a control solution of 0.1 M, respectively. Their effects on germination in a soil-less medium were studied by measuring various germination parameters.

Key words : Ligands, Complexes, Germination, Metal ion, Soybean, peanuts.

Schiff bases can form complexes with nearly all transition metal ions due to the versatility of their donor sites. Although well-known, their applications across various fields continue to make them a subject of active interest. Transition metal ion complexes with Schiff bases represent a significant and expanding class of compounds, particularly valued for their roles in stereochemistry and magnetochemistry. They can serve as synthetic models for the metal-containing active sites of metalloproteins and metalloenzymes. Unsymmetrical Schiff base ligands offer notable advantages over their symmetrical

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counterparts when elucidating the composition and geometry of metal-binding sites in biological systems, as well as the selectivity of these systems toward synthetic materials. Schiff base transition metal complexes are often preferred over conventional micronutrient supplements. Due to the simplicity of their preparation, these compounds can be produced on a large scale.

Heavy metal Schiff base complexes may be used as supplements to compensate for nutritional deficiencies while simultaneously mitigating the toxicity associated with the application of certain heavy metals. However, to fully assess their effectiveness, these factors must be thoroughly investigated under a range of environmental conditions.

The biological activity of metals is well studied. It has been found that both metals and their complexes exhibit notable activity in biological systems. Greson *et al.*[5] reported that the activity of metal chelates is considerably enhanced compared to that of the free metal ions and ligands. Shel *et al.*[3] observed antifungal and antibacterial activities of metal complexes. Sayid Amir *et al.*[3] studied the effects of some heavy metals on the seed germination of wheat, sunflower, and canola, and evaluated their phytoremediation potential. K. Abraham *et al.*[7] also examined the impact of heavy metals on the seed germination of *Arachis hypogaea*.

It is well known that amino acid complexes exhibit biological and metabolic enzyme activity. Antimicrobial and toxicological activities of metal-ligand complexes have been demonstrated in many studies[6,12]. Sharma *et al.*[9] observed the biological activity of certain rare earth metals. Similarly, the pharmaceutical applications of metal complexes have been explored in several investigationsy. Studies have revealed that metal complexes often show greater antifungal and antimicrobial activity than free ligands or metal ions (Shel *et al.* and Shashindharam[10-11]). The effect of chloro-substituted pyrazole and its complexes on spinach at different pH levels was studied by A.A. Ranteke *et al.*[8].

The present study aims to observe the effect of Schiff base complexes on the germination of soybean and pea seeds in a soil-less medium. Schiff base ligands, which contain nitrogen donor sites, form stable complexes with most transition metals. These ligands interact with metal ions through sigma-donating and pi-accepting molecular orbitals.

Experimental analysis :

Reagents : The chemicals used were of AR (Analytical Reagent) grade. Chloro benzaldehyde, alanine, glycine, and ethanol were purchased and used without any further purification. Double-distilled water was used throughout the experiments.

Synthesis ligands by literature method :

1. (E)-2-((4-chlorobenzylidene) amino) propanoic acid (L1)
2. (E)-2-((4-chlorobenzylidene) amino) acetic acid (L2)

The above two ligands were synthesized using a standard method reported in the literature.

By adding 40 mL of ethanol to 1.5 g

of 4-chlorobenzaldehyde in a 250 mL round-bottom flask, followed by 1.20 g of the respective amino acids (alanine for L1 and glycine for L2) and 1.66 g of NaOH, the reaction mixture was refluxed with constant stirring for 4 hours. After refluxing, the solution was cooled in an ice bath for 3 hours. The resulting crystals were filtered, washed with ethanol, and dried in an oven.

Synthesis of metal complexes with ligands [$L_{1,}$ $L_{2,}$] :

The complexes of Mo and Fe were separately prepared by mixing the ligands (L1 and L2) with metal salts such as Mo, O(acac), and ferric chloride in a 1:2 molar ratio in a 50 mL Schlenk vessel containing 20 mL of methanol. The mixtures were refluxed for 5–6 hours, and the resulting solutions were cooled in an ice bath until crystals of the corresponding complexes formed. The crystals were then collected separately.

Germination of seeds in soil less media :

It includes the Collection of seeds and the Seeds of soybean and peas were collected from well known seed store, viability of seeds were checked before used.

Treatment of seeds :- The seeds were treated with the prepared ligand solutions, metal-ligand complex solutions, and a control solution of distilled water. The soaked seeds were placed for germination on Whatman filter paper No. 1 in well-labeled trays, with each tray containing 20 seeds. Each tray was regularly watered with 250 mL of distilled water as needed. The treated samples were kept under natural sunlight and ambient temperature conditions.

Parameters measured Plant growth was assessed based on several parameters, including seedling height, root length, shoot length, root-to-shoot ratio, percentage of germination, and chlorophyll content of the leaves.

Estimation of chlorophyll content of leaves:

Procedure:

Freshly obtained leaves of the respective plants were chopped and ground into a fine powder, with the addition of 1 mL of water to obtain a homogeneous mixture. From each mixture, 1 mL was taken and mixed with 5.0 mL of 80% acetone. The solutions were shaken and then centrifuged. The supernatant was collected, and the optical density (OD) was measured at 650 nm and 665 nm.

Table-1. The elemental analysis results were in accordance with the theoretical estimated calculations with a percentage error of +-1-2

Ligands	I.U. P.A.C Name	Molecualr Formulla	Formula Wt.	Yield	% Found Calculated C H N
L_1	(E)-2-((4-chlorobenzylidene) amino) propanoic acid	$C_{10}H_{10}Cl\ NO_2$	211.65	92.03	56.75,4.76,6.62
L_2	(E)-2-((4-chlorobenzylidene)	$C_9H_8Cl\ NO_2$	197.02	91.25	54.70,4.08,7.09

Spectral study of complexes :

Table-2. Infra-red spectral study.FTIR spectra were obtained as KBR Pellets. The important bands observed were as follows

Com-pounds	v (C=N) cm^{-1}	V (O-H) cm^{-1}	Aromatic. Ring vibrations	N-H vibration	C-O vibration	M-O
L_1	1619-1660	3238.67	1545.76,768.06,774.08, 735.4,672.6	3004.6	1700,1690	500-550 cm^{-1}
L_2	1623-1670	3457.37	753.6,632.4,546.5,536.9	2936.5	1190.7,1176.3	

The study of IR spectra helps predict the nature of functional groups attached to the central metal atom and the ligand. The important IR bands of the ligands and their respective metal complexes are summarized in Table-2. The ligands show an intense broad band in the range of 1700–1690 cm^{-1}, corresponding to the C=O stretching vibration of the carboxylic acid group. Similarly, an intense band in the range of 3200–3600 cm^{-1} confirms the presence of an –OH group in the ligand. The stretching frequency of the aromatic ring appears in the range of 2800–3000 cm^{-1}.

Strong absorption bands observed at 1615–1670 cm^{-1} are attributed to the C=N (azomethine) stretching vibration. In the spectra of all metal complexes, these bands shift to lower frequencies (1590–1599 cm^{-1}), indicating the involvement of the –C=N nitrogen atom in coordination with the metal ionx . Additionally, the aromatic C=C stretching appears between 1400 and 1550 cm^{-1}. These observations suggest that the ligands function as tetradentate chelating agents.

This is further supported by the appearance of a new band in the range of 452–478 cm^{-1}, which corresponds to M–N bond formation. The complexes [ML1] and [ML2] also exhibit medium intensity bands in the range of 500–550 cm^{-1}, which are likely due to M–O bond vibrations.

Table-3. U.V –Visible sp

compounds	Molar Conductance Ohm'1cm^2 mol'1	μeff (B.M.)	Absorption Maxima cm'1(nm)		
			V1	v2	v3
L_1M_1	54.7	4.89	9623(1036)	19764(634)	23764(521)
L_1M_2	49.7	5.36	9875(1085)	19769(612)	23776(576)
L_2M_1	42.5	5.87	9879(1098)	19323(632)	22456(543)
L_2M_2	58.2	5.78	9345(1053)	19674(587)	23756(576)

Table 4 provides an overview of the electronic spectral characteristics of [ML1] and [ML2] complexes. Three key features are observed in the electronic spectra of all the complexes, which were recorded in DMF medium. The intra ligand charge transfer transition (π→π*) appears as one or two peaks in the 250–295 nm region. Peaks in the 300–370 nm range correspond to the n→π* transitions. Additionally, a strong peak in the 410–460 nm region is attributed to ligand-to-metal (L→M) charge transfer transitions.

For ligand L1, absorption bands were observed at 256, 311, and 425 nm, corresponding to π→π*, n→π*, and L→M transitions, respectively. In the electronic absorption spectra of the [CuL1] complex, weak bands were observed at 261, 305, and 427 nm, which are also assigned to π→π*, n→π*, and L→M transitions.

Table 4. Effect of M-L Complex, ligand, M^{+2}, and water of 0.1 molar solution on germination of soybean in soil less media .(0.1 M) pH.7.0

Complexs	Germin-ation %	Root length	Shoot length	Root-shoot ratio	A.V No. of leaves	Choloro-fillmg\100 gm
	15 days	15 days	15 days	15 days	15 days	
L_1M_1	88	15.22	15.48	0.85	06	34.24
L_1M_2	91	14.11	16.32	0.86	05	32.04
L_2M_1	78	14.31	15.61	0.91	05	32.09
L_2M_2	85	13.12	15.76	0.84	07	34.01
L_1	74	13.65	14.76	0.92	06	30.14
L_2	79	12.33	14.95	0.82	06	29.78
Control	69	13.02	13.89	0.86	05	30.65
M_1^{+2}	67	10.98	13.02	0.84	03	30.89
M_2^{+2}	63	09.55	13.67	0.69	03	31.03

According to Table-5, during the first 15 days of germination, the lowest germination percentage was observed for the metal ion treatment (M1), whereas the highest germination percentage was recorded for the complex L_2M_1. The metal ions exhibited a low germination rate, while the ligands and control solution produced nearly identical results. This suggests that the metal complexes are more effective in enhancing germination compared to the other experimental treatments. Similarly, the L2M1 complex yielded the greatest root length, while the L_2M_2 complex resulted in the highest shoot length. The highest root-to-shoot ratio was found with the L_1 ligand, and the number of leaves per plant was highest in the L2M2 treatment, with the ligands and control solution showing similar outcomes.

Table-5. Effect of M-L Complex, ligand, M^{+2}, and water of 0.1 molar solution on germination of Pea seeds in soil less media .(0.2 M) pH.7.0

Complexs	Germi-nation % 15 days	Root length 15 days	Shoot length 15 days	Root-shoot ratio 15 days	No.of leaves 15 days	Choloro-fillmg\100 gm
L_1M_1	89	14.34	16.43	0.87	08	32.08
L_1M_2	92	14.15	15.23	0.92	07	32.66
L_2M_1	88	14.76	15.77	0.93	07	31.98
L_2M_2	87	14.12	16.76	0.84	08	32.67
L_1	73	13.61	14.36	0.94	06	30.07
L_2	76	12.89	14.71	0.87	06	29.68
Control	56	11.02	14.89	0.74	06	30.73
M_1^{+2}	51	12.98	13.32	0.97	05	26.98
M_2^{+2}	54	12.55	13.77	0.91	04	26.09

Table-6. Effects of Ligands (L1–L2), Fe (M1), Mo (M2), and Their Complexes on Germination Parameters

Compound	SVI (Mean ± SD)	GP (Mean ± SD)	GI (Mean ± SD)	CVG (Mean ± SD)
Control	1137.04 ± 64.24	77.33 ± 1.22	11.74 ± 1.22	0.274 ± 0.06
L1	1253.38 ± 184.40	78.66 ± 2.61	13.78 ± 1.18	0.368 ± 0.09
L2	1377.88 ± 161.45	73.50 ± 2.77	14.98 ± 0.82	0.334 ± 0.07
L1M1	1467.87 ± 126.36	84.35 ± 1.63	15.51 ± 1.91	0.348 ± 0.09
L1M2	1365.96 ± 233.60	86.86 ± 1.84	15.75 ± 2.23	0.383 ± 0.07
L2M1	1356.46 ± 132.82	84.45 ± 2.43	16.65 ± 1.98	0.367 ± 0.05
L2M2	1276.86 ± 156.60	79.47 ± 1.13	11.73 ± 1.68	0.127 ± 0.01
M1 (Fe)	1142.97 ± 166.45	56.32 ± 1.39	11.88 ± 1.86	0.189 ± 0.04
M2 (Mo)	1235.77 ± 175.50	57.38 ± 1.23	12.75 ± 1.68	0.169 ± 0.00

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

From the table-4 it is evident that germination percentage significantly decreased in seeds treated with metal ion solutions, with the lowest germination observed for the metal ion M_2 during the first 15 days. In contrast, the highest germination percentage was recorded for the complex L_1M_1. The ligands and control solutions showed nearly similar results, indicating minimal impact compared to the metal complexes.Similarly, root length was greatest in the L_1M_1 treatment, while shoot length was highest in L_1M_2. The highest root-to-shoot ratio was observed with the L_1 ligand. The number of leaves per plant was highest in the L_2M_2 treatment, while the ligand and control groups produced comparable results. However, there was a reduction in the average number of leaves per plant in the metal ion-treated samples. These results suggest that increasing the concentration of metal ion solutions significantly reduces germination percentage and negatively affects plant growth and development. In contrast, increasing the concentration of metal in its complexed form does not notably alter germination or growth rates.Overall, it is clear that metal complexes, in both media types, exhibit higher efficiency as plant growth regulators. Notably, results in the soil-less medium were more favorable compared to those in soil-based conditions.

1. *Negative Impact of Free metals :*
   - o Both Fe (M1) and Mo (M2) treatments alone reduced germination performance compared to the control.
   - o Significant reductions were seen in Germination Percentage (GP) and Coefficient of Velocity of Germination (CVG), especially in Fe (56.32%) and Mo (57.38%), indicating toxicity at the applied concentration.

2. *Ligands Alone Showed Mild Improvement:*
   - o Both L1 and L2 alone showed moderate improvement in germination parameters (SVI, GI, and CVG) compared to the control.
   - o Particularly, L2 had the highest Germination Index (14.98), suggesting a stimulatory role in early seed metabolism.

3. Metal-Ligand Complexes Significantly Improved Performance:
   - o All metal-ligand complexes (L1M1, L1M2, L2M1, L2M2) outperformed the free metal treatments and even surpassed the control in most parameters.
   - o L1M2 (Mo complex) had the highest Germination Percentage (86.86%) and GI (15.75), while L2M1 (Fe complex) had the highest GI (16.65) and strong overall values.
   - o This indicates that complexation of metals with ligands mitigates metal toxicity, possibly by regulating metal availability and enhancing uptake efficiency.

4. *Lowest CVG Observed in L2M2 :*
   - o Despite decent GP and SVI, L2M2 showed a very low CVG (0.127), suggesting slower germination speed, which may be due to delayed onset or uneven germination.

Complexation of Fe and Mo with ligands (L1 and L2) significantly improves seed germination parameters by reducing metal toxicity and enhancing physiological performance. Among the treatments, **L1M2 and L2M1** emerged as the most effective complexes, suggesting their potential for promoting germination and early seedling vigor in metal-stressed environments.

The authors are indebted to the USIC, University of Kashmir (UT of J&K); the Department of Chemistry, Awadhesh Pratap Singh University, Rewa (M.P.); the Science Instrumentation Center, I.C.S.C. of Science and Commerce, Srinagar, Kashmir (UT of J&K); and the Department of Pharmaceutical Sciences, Jamia Hamdard University, New

Delhi, for providing access to A.A.S. facilities (Perkin Elmer A.A.S-800), UV-Visible spectrophotometer (UV-1601, SHIMADZU), FTIR spectrophotometer (Jasco Model-4100), and CHN analysis equipment (CHNO ELEMENTAR-VARIO-III Elemental Analyzer), respectively.

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