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Indian Journal of Advances in Chemical Science

Indian Journal of Advances in Chemical Science 3(2) (2015) 141-146

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

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Received 18th November 2014; Revised 10th March 2015; Accepted 18th March 2015

ABSTRACT

Salen and salophen type of ligands have the ability to form stable complexes with Zn^{2+} and thereby can increase the availability of Zn^{2+} for plants when used as growth promoters in ppm level. Four Schiff base ligands and their Zn^{2+} complexes were synthesized and characterized by different analytical and spectroscopic techniques. These Schiff bases and their Zn^{2+} 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^2-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 $(AcO)_2 \cdot 2H_2O$, 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

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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 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 ν(C=N) (in the region 1640 cm^{-1}-1610 cm^{-1}), ν(O-H) (in the region 1400 cm^{-1}-1000 cm^{-1}) for aromatic ring, ν(C-H) and ν(N-H) (in the region 3100 cm^{-1}-2500 cm^{-1}). The appearance of the ν(N-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 ν(C-H), ν(C=N), ν(C-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 cm^{-1}-3200 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_1 ($C_{16}H_{16}N_2O_2$) *N, N′* - Ethylene bis (salicylidenediamine)	268.31	92.27	71.6 (71.3)	5.9 (5.9)	10.2 (10.5)
L_2 ($C_{20}H_{16}N_2O_2$) *N, N′* - (*o*-Phenylene) bis (salicylidenediamine)	316.35	90.39	75.9 (75.8)	5.1 (5.1)	8.9 (9.0)
L_3 ($C_{18}H_{20}N_2O_4$) *N, N′* - Ethylene bis (3-methoxysalicylidenediamine)	328.36	91.21	65.9 (65.7)	6.1 (6.2)	8.5 (8.4)
L_4 ($C_{22}H_{20}N_2O_4$) *N, N′* (*o*-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^{-1}) of the prepared four ligands.

Ligand	ν (C=N)	δ (O-H)	Aromatic ring vibrations	N (C-H)+ν (N-H)	ν (C-O)
L_1	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_2	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_3	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_4	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_1 ($C_{16}H_{16}N_2O_3Zn$) [*N, N′*ethylenebis (salicylidenediamine)] monohydrate	349.7	82.04	54.9 (54.8)	4.6 (4.7)	8.0 (8.1)
C_2 ($C_{20}H_{16}N_2O_3Zn$) [*N, N′*(*o*-phenylene) bis (salicylidenediamine)] monohydrate	397.74	81.71	60.5 (60.2)	4.0 (4.2)	7.1 (7.1)
C_3 ($C_{18}H_{20}N_2O_5Zn$) [*N, N′*ethylene bis (3-methoxysalicylidenediamine)] monohydrate	409.75	81.28	52.8 (52.5)	4.9 (5.0)	6.9 (6.8)
C_4 ($C_{22}H_{20}N_2O_5Zn$) [*N, N′* (*o*-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^{-1}) of the prepared four zinc (II) complexes.

Complex	(ν (C=N)+δ (O-H) H_2O)	(ν (O-H) H_2O)	Aromatic ring vibrations	ν (C-H)	ν (C-O)
C_1	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_2	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_3	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_4	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^{th} and 30^{th} 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_4 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_4 one. When it was subjected to a number of leaves, again C_4 complex showed the best result. The trend of ligands and complexes imparted identical trajectory at both the 15th day and 30th day interval after the seed germination.

The statistical analysis of the observations on leaf chlorophyll content of papaya seedlings at 15th day and 30th day interval showed both non significant and significant variations which is revealed in Table 7. On 15th day leaf, chlorophyll content for all ligands and complexes ranged from 28.33 mg to 34.54 mg/100 g. On 30th day, maximum leaf chlorophyll content was observed in C_4, which was least in control. A significant variation was also observed in leaf zinc content of papaya seedlings. At 15th day after germination, it was highest for C_4 and lowest for the control. Some sort of reduced range of leaf zinc content was observed in 30th 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.

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_1	12.52	22.51	71.07
L_2	12.01	20.73	73.40
L_3	11.69	21.98	70.52
L_4	12.13	20.23	74.44
C_1	11.05	18.36	77.65
C_2	10.82	17.92	78.32
C_3	10.94	18.41	76.21
C_4	10.09	16.67	80.83
Control	12.47	23.07	72.45
$CD_{0.05}$	0.74	1.03	2.06
SE±m	0.39	0.54	0.97

SE: Standard error

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^{2+} 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 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_1	7.36	13.13	6.21	11.10	3.07	4.32
L_2	7.02	13.22	6.42	11.34	3.11	5.09
L_3	6.87	12.75	6.10	12.00	3.34	4.68
L_4	6.98	13.34	7.34	11.56	3.23	4.92
C_1	8.12	15.31	8.56	13.37	3.67	5.24
C_2	8.23	14.86	9.29	13.25	3.56	5.41
C_3	8.50	15.23	9.13	14.05	3.81	6.10
C_4	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
$CD_{0.05}$	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

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_1	31.51	41.65	12.42	12.00
L_2	28.33	39.27	13.11	12.87
L_3	30.52	39.45	12.52	12.36
L_4	29.31	40.16	12.27	11.89
C_1	32.64	45.40	17.12	16.75
C_2	34.42	46.38	16.33	16.21
C_3	33.33	44.79	16.54	16.30
C_4	34.54	52.10	17.97	17.65
Control	29.87	40.56	12.20	11.99
$CD_{0.05}$	NS	3.43	0.98	0.92
SE±m	NS	1.67	0.41	0.45

SE: Standard error

clearly shows that C_4 has the maximum beneficial effect on germination and seedling growth of papaya. Hence, C_4 may be used as an effective seed soaking chemical for papaya. However, C_4 would be further studied in order to confirm it's 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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