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Synthesis of Transition Metal Diethyldithiocarbamates and Their Effect on Nodulation and Other Growth Characters in Mungbean, *Vigna radiata*

ANUPAMA ARORA* and C.L. ARORA
Department of Chemistry
Punjab Agricultural University, Ludhiana-141 004, India

The present investigation involves the synthesis of some metal diethyldithiocarbamates and their effect on symbiotic activity of mungbean. Metal diethyldithiocarbamates, $[(C_2H_5)_2NCS_2)]_nM$ (M = Zn, Mn, Cd), were synthesized by the reaction of metal chloride with sodium diethyldithiocarbamate in dichloromethane/water mixture. The metal-complexes were soluble in chloroform, carbon tetrachloride, tetrahydrofuran and benzene. The dithiocarbamate ligand acted as bidentate in all the metal-complexes. The metal-complexes were screened for their effect on growth characters and symbiotic activity of mungbean, *Vigna radiata* ML 267 at various concentrations. The complexes of zinc and manganese significantly increased the dry weight of root and shoot, number of nodules, dry weight of nodules and chlorophyll content of leaves.

Key Words: Transition metal diethyl dithiocarbamates, Effect, Nodulation, Mungbean, *Vigna radiata*.

INTRODUCTION

Metal complexes play an important role in plant and animal life due to their physico-chemical properties. Organosulphur compounds, especially in the form of their metal complexes, exhibit a wide range of biological properties. The complexation has a profound effect on the uptake of micronutrient metal ions. Among sulphur containing compounds, dithiocarbamates constitute a chemically as well as biologically important class. Metal dithiocarbamates have been reported to show fungicidal[1], insecticidal[2] and herbicidal[3] properties. They have been found to act as anticancer agents[4]. They release metal ions in biological system and hence have significant effect on respiration, nitrification and isolated enzyme systems[5]. Hence, the present investigation was undertaken to synthesize some metal diethyldithiocarbamates and to explore them as plant growth regulators. The complexes were checked for their ability to affect nodulation, root and shoot weight and chlorophyll content of leaves in the leguminous crop, mungbean.

EXPERIMENTAL

Preparation of metal diethyldithiocarbamates

Metal diethyldithiocarbamates (M = Zn, Mn, Cd) were synthesized by the reaction of sodium diethyldithiocarbamate with metal chloride in molar ratio in water/dichloromethane (50 : 50) mixture. The organic layer was separated from the aqueous layer and dried over anhydrous potassium sulphate overnight. The supernatant was filtered and distilled out to get the crystals of corresponding metal complex. The crystals were then vacuum dried[6].

$$n[(C_2H_5)_2NCS_2Na] + MCl_n \xrightarrow{CH_2Cl_2/H_2O} [(C_2H_5)_2NCS_2]_nM + nNaCl$$

$$(M = Zn, Mn, Cd)$$

The structure of complexes was confirmed from elemental analysis, solubility, conductance studies and infrared spectra. Yield 80–90%.

Zinc and manganese were estimated in the respective complexes on atomic absorption spectrophotometer and cadmium on ICAP (inductively coupled atomic plasma) emission spectrophotometer[7]. Analysis of carbon, nitrogen and hydrogen was done on elemental analysensteme GmbH Vario EL from Niper, Mohali.

Elico conductivity bridge of type CM 82T fitted with platinized platinum electrodes was used for conductivity measurements. The solutions of the complexes were made in dichloromethane at 5×10^{-3} M concentration. Cell constant of the cell used was 10 m^{-1}. Specific conductance and molar conductance were calculated. Specific conductance has been expressed in terms of Sm^{-1} and molar conductance in terms of $Sm^2\ mol^{-1}$.

Specific conductance (K) = cell constant × observed conductance.

Molar conductance (Λ_m) = 1000 × K/C

where C = concentration of solution in mol/m^3.

The spectra of the complexes were recorded in 4000–600 cm^{-1} on Perkin-Elmer infrared spectrophotometer in nujol mull using sodium chloride optics.

Effect of metal diethyldithiocarbamates on growth and symbiotic activity of *Vigna radiata*

Mungbean seeds, *Vigna radiata* (ML 267), were surface sterilized with 0.1% mercuric chloride, washed and dipped in solutions of various metal (Zn, Mn, Cd) diethyldithiocarbamates at five different concentrations (1.5 mL THF + 8.5 mL distilled water) for 20 min. The concentrations taken were 500 ppm, 200 ppm, 100 ppm, 70 ppm and 40 ppm. The seeds were air dried and sown in soil taken in pots. Each treatment was replicated thrice. The surface sterilized seeds were inoculated with 2 g of *Rhizobium* culture after giving the treatment of complex. Out of ten seeds sown, a single plant per pot was kept after ten days. The surface sterilized seeds with and without *Rhizobium* treatment and without the complex solution treatment were taken as control. The control with *Rhizobium* treatment and without complex solution treatment was denoted as untreated inoculated control while the other without *Rhizobium* treatment and without complex solution treatment was denoted as untreated uninoculated control. The plants were uprooted at pre-flowering stage. The number of nodules, dry weight of nodules, dry weight of root and shoot, and chlorophyll content of leaf were determined.

Number of nodules: The plants were carefully removed from the pots with intact root system and washed with tap water. The nodules were carefully detached from the roots and their number was counted.

Dry weight of nodules: The nodules were dried in oven at 60°C for 24 h and the dry weight per plant was recorded.

Dry weight of root and shoot: Roots were separated from the shoots and both were dried in oven at 60°C for 48 h and dry weight per plant was recorded.

Chlorophyll content of leaves: Chlorophyll content was determined by extracting healthy and fresh leaves in 80% acetone. The optical density (D) of chlorophyll extract of each sample was read at 645 nm and 663 nm using visible spectrophotometer Spectronics-20. 80% acetone was taken as solvent blank[8]. The chlorophyll content was expressed as mg chlorophyll per gram of leaf tissue extract using the following equations:

$$\text{mg chlorophyll 'a'/g tissue} = [12.7\,(D_{663}) - 2.69\,(D_{645})]V/(1000 \times W)$$

$$\text{mg chlorophyll 'b'/g tissue} = [22.9\,(D_{645}) - 4.86\,(D_{663})]V/(1000 \times W)$$

$$\text{mg chlorophyll 'total'/g tissue} = [20.2\,(D_{645}) + 8.02\,(D_{663})]V/(1000 \times W)$$

where D = optical density of chlorophyll extract at the specific indicated wavelength

V = final volume of the 80% acetone chlorophyll extract solution in millilitres

W = fresh weight of the tissue extracted in grams

RESULTS AND DISCUSSION

The elemental analyses were consistent with 1 : 2 metal to ligand stoichiometry for all the metal-complexes (Table-1).

TABLE-1
ANALYTICAL DATA FOR METAL DIETHYLDITHIOCARBAMATES

Complex	% Analysis: Found (Calcd.)			
	C	H	N	Metal
$Zn(DEDTC)_2$	33.73 (33.24)	5.10 (5.54)	7.20 (7.76)	18.99 (18.01)
$Mn(DEDTC)_2$	35.03 (34.19)	6.20 (5.70)	8.28 (7.98)	14.90 (15.67)
$Cd(DEDTC)_2$	28.94 (29.41)	5.40 (4.90)	6.27 (6.86)	27.20 (27.45)

All the complexes were completely soluble in chloroform, carbon tetrachloride and tetrahydrofuran whereas these were insoluble in water. All the complexes gave low specific as well as molar conductance (Table-2). The IR spectra of the complexes gave sharp bands around 1465 cm^{-1} due to C—N stretch[9]. The two bands in 1000–950 cm^{-1} region had been assigned to C—S asymmetric stretch and the absorption around 648 cm^{-1} had been assigned to C—S symmetric stretch[10]. The bands in 1290–1130 cm^{-1} region had been associated with NC grouping[11] (Table-3).

TABLE-2
CONDUCTANCE OF METAL DIETHYLDITHIOCARBAMATES

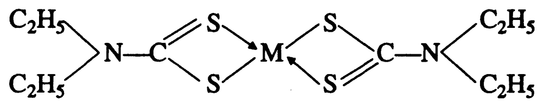
Complex	Observed conductance (S)	Specific conductance (Sm^{-1})	Molar conductance ($Sm^2 mol^{-1}$)
$Zn(DEDTC)_2$	3.22×10^{-4}	3.22×10^{-3}	628×10^{-6}
$Mn(DEDTC)_2$	3.14×10^{-4}	3.14×10^{-3}	644×10^{-6}
$Cd(DEDTC)_2$	3.94×10^{-4}	3.94×10^{-3}	788×10^{-6}

DEDTC = Diethyldithiocarbamate.

TABLE-3
INFRARED SPECTRA (cm^{-1}) OF METAL DIETHYLDITHIOCARBAMATES

Complex	ν(C—N)	ν_{asym}(C—S)	ν_{sym}(C—S)	ν(N—C<S S)
$Zn(DEDTC)_2$	1466	998, 978	684	1300, 1274, 1205, 1150
$Mn(DEDTC)_2$	1462	999, 968	681	1294, 1270, 1196, 1149
$Cd(DEDTC)_2$	1465	988, 977	683	1299, 1270, 1205, 1145

From elemental analysis and spectral studies, the following structure was proposed for these metal-complexes:



(M = Zn, Mn, Cd)

Biological studies

The plants raised from treated seeds and control seeds show differences in various observed characters.

The maximum number of nodules was observed with zinc diethyldithiocarbamate at 100 ppm as compared to untreated inoculated control. Manganese increased the number of nodules again up to 100 ppm. Higher concentrations led to a gradual decrease in the number of nodules (Table-4). An almost similar trend was observed in case of dry weight of nodules. Manganese and zinc complexes had increased the dry weight of nodules up to 100 ppm (Table-5). The dry weight of root and shoot initially increased with increase in concentration of various complexes up to a certain level and after that it started decreasing. Manganese and zinc diethyldithiocarbamates increased the dry weight of root as well as shoot up to 100 ppm. Cadmium had shown negligible effect on the observed characters at lower concentrations (Tables 6 and 7).

TABLE-4

EFFECT OF METAL DIETHYLDITHIOCARBAMATES ON NUMBER OF NODULES/PLANT AFTER 40 DAYS OF SOWING (MEAN OF THREE REPLICATES)

Complex	Concentration (ppm)				
	40	70	100	200	500
$Zn(DEDTC)_2$	16.0	18.0	20.0	16.0	12.0
$Mn(DEDTC)_2$	14.0	16.0	19.0	17.0	11.0
$Cd(DEDTC)_2$	13.0	12.0	10.0	10.0	9.0

Untreated uninoculated control = 12.0; Untreated inoculated control = 14.0; DEDTC = De thyl-dithiocarbamate.

TABLE-5

EFFECT OF METAL DIETHYLDITHIOCARBAMATES ON DRY WEIGHT OF NODULES (mg)/PLANT AFTER 40 DAYS OF SOWING (MEAN OF THREE REPLICATES)

Complex	Concentration (ppm)				
	40	70	100	200	500
$Zn(DEDTC)_2$	12.23	12.84	13.24	11.31	10.02
$Mn(DEDTC)_2$	11.98	12.60	13.10	11.75	10.76
$Cd(DEDTC)_2$	11.97	10.80	10.44	10.00	8.41

Untreated uninoculated control = 10.45; Untreated inoculated control = 11.85

TABLE-6

EFFECT OF METAL DIETHYLDITHIOCARBAMATES ON DRY WEIGHT OF ROOT (g)/PLANT AFTER 40 DAYS OF SOWING (MEAN OF THREE REPLICATES)

Complex	Concentration (ppm)				
	40	70	100	200	500
$Zn(DEDTC)_2$	0.3190	0.3207	0.3225	0.3122	0.3104
$Mn(DEDTC)_2$	0.3141	0.3202	0.3219	0.3115	0.3085
$Cd(DEDTC)_2$	0.3135	0.3145	0.3108	0.3103	0.3044

Untreated uninoculated control = 0.3090; Untreated inoculated control = 0.3125

DEDTC = Diethyldithiocarbamate

TABLE-7

EFFECT OF METAL DIETHYLDITHIOCARBAMATES ON DRY WEIGHT OF SHOOT (g)/PLANT AFTER 40 DAYS OF SOWING (MEAN OF THREE REPLICATES)

Complex	Concentration (ppm)				
	40	70	100	200	500
$Zn(DEDTC)_2$	0.5557	0.5755	0.5893	0.5248	0.5137
$Mn(DEDTC)_2$	0.5520	0.5871	0.5900	0.5500	0.5180
$Cd(DEDTC)_2$	0.5510	0.5475	0.5009	0.4747	0.4340

Untreated uninoculated control = 0.5282; Untreated inoculated control = 0.5498

TABLE-8

EFFECT OF METAL DIETHYLDITHIOCARBAMATES ON CHLOROPHYLL CONTENT (mg g^{-1} FRESH WEIGHT) OF LEAVES AFTER 40 DAYS OF SOWING (MEAN OF THREE REPLICATES)

Complex	Concentration (ppm)														
	40			70			100			200			500		
	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.
$Zn(DEDTC)_2$	0.970	0.514	1.499	1.083	0.591	1.691	1.503	0.884	2.410	0.828	0.452	1.290	0.571	0.375	0.956
$Mn(DEDTC)_2$	0.987	0.473	1.477	1.160	0.649	1.828	1.070	0.527	1.614	0.825	0.425	1.264	0.598	0.320	0.927
$Cd(DEDTC)_2$	0.622	0.376	1.008	0.562	0.397	0.968	0.475	0.347	0.830	0.449	0.360	0.672	0.409	0.143	0.558

	Chl. a	Chl. b	Total Chl.
Untreated uninoculated control	0.608	0.292	0.899
Untreated inoculated control	0.613	0.363	0.986

DEDTC = Diethyldithiocarbamate

The various complexes had increased the chlorophyll contents of leaves to varying levels. Zinc diethyldithiocarbamate increased the chlorophyll content up to 100 ppm whereas manganese diethyldithiocarbamate increased the chlorophyll content up to 70 ppm. Higher concentrations, however, led to a decrease in total chlorophyll content of leaves (Table-8).

Considering the effect of the three complexes on all the observed characters, zinc and manganese diethyldithiocarbamates have stimulatory effect whereas cadmium diethyldithiocarbamate has adverse effect. The reason being, both zinc and manganese are important micronutrients for plants. Zinc is associated with the functioning of carbonic anhydrase and some other enzyme systems (such as carboxylases, dehydrogenases, proteinases, peptidases etc.), auxins, RNA and protein synthesis. Similarly manganese is necessary for the functioning of certain oxidative enzyme systems, araginases and carboxylase groups. It has an important role in nitrogen metabolism and carbon dioxide assimilation. It is necessary for the splitting of water molecule during photosynthesis. Therefore, both these metal ions are indispensible for plant metabolism and the complexes of these important micronutrient metal ions increase the growth rate of plants up to an optimum concentration.

To conclude, zinc diethyldithiocarbamate increased nodulation, dry weight of root and shoot, and the chlorophyll content of leaves at 100 ppm whereas manganese diethyldithiocarbamate increased all these parameters to the maximum at 100 ppm except the chlorophyll content of leaves which was found to be maximum at 70 ppm. Cadmium diethyldithiocarbamate has adverse effect on plant growth.

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