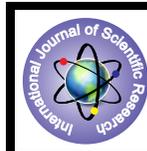


# Designing, Synthesis, Characterization And in Vitro Screening of Metal Complexes Against The Fungus Causing Wilt Disease of Tomato



## Chemistry

**KEYWORDS :** Mixed ligand, bipyridine, *Fusarium oxysporum* f. sp. lycopersici, metal complexes, antifungal screening.

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### ABSTRACT

A series of mixed ligand transition metal complexes of the type  $[M(P)_2(B)_2]$  were synthesized and characterized by modern methods like elemental analysis, molar conductance, magnetic susceptibility measurements, electronic spectra and IR spectroscopy. In these complexes M stands for bivalent metal ions Mn(II), Co(II), Ni(II), Cu(II) and Zn(II), P for picrate ion and B for 2, 2' bipyridine. Spectral studies provide strong evidence in support of ligands in octahedral manner. Antifungal screening indicates that the complexes show enhanced antifungal activities as compared with parent compounds against an important multi drug resistant, soil borne fungal pathogen *Fusarium oxysporum* f. sp. lycopersici, which attacks on tomato plants all over the world. All complexes show enhanced antifungal activities against the fungal strain.

### INTRODUCTION

Fungi are significant destroyers of foodstuffs, rendering them unfit for human consumption by retarding their nutritive value. Fusarium wilt (caused by the soil-borne fungus *Fusarium oxysporum* f. sp. lycopersici, FOL) is one of the most damaging diseases of tomato (*Lycopersicon esculentum* Mill.) causing economic losses in India. The disease is favoured by high temperature and humidity. Antifungal studies of transition metal complexes have been an active field of research<sup>1</sup>. It has been observed that metal complexes with appropriate ligands are chemically more significant and specific than the metal ions and ligands as original<sup>2</sup>. Bi-oactive donor sites of N and O atoms in organic ligand moieties are useful in the development of metal based drugs.

As a part of our ongoing work<sup>3</sup> on mixed ligand complexes, in this paper we are reporting the newly synthesized complexes of bivalent metal ions with picric acid and 2, 2' bipyridine. Their antifungal activities investigated to perform primary selection of these complexes as the therapeutic agents for the mycelial growth of FOL *in vitro*.

### EXPERIMENTAL

#### Materials and methods

All the chemicals used were of analytical grade. Metal salts used were metal di chlorides. Used picric acid was of highest purity, 2, 2' bipyridine was used as received. Methanol, ethanol and benzene were further purified by double distillation. Mixed ligand transition metal complexes were prepared as reported earlier<sup>4</sup>.

Carbon, hydrogen and nitrogen were estimated microanalytically on Elementar Vario EL III elemental analyzer at SAIF, CDRI Lucknow. Conductivity measurements were made on ELICO EQ 660 Conductivity Bridge using DMF as a solvent. Metal contents were estimated by standard methods<sup>5</sup>. Magnetic susceptibility measurement were made at room temperature by Gouy's method using  $Hg[Co(SCN)_4]$  as calibrant. The diamagnetic correction of metal-ligand system was calculated using Pascal's constant. The purity of metal complexes was checked by TLC method along with standard ligands. IR spectra in the range 4000-400  $cm^{-1}$  were recorded at CDRI Lucknow on a shimadzu FTIR 8201 P C spectrometer where as spectra in the range 4000-250  $cm^{-1}$  were recorded on a Perkin Elmer infra red spectrophotometer 521 at the department of chemistry IIT Roorkee. The electronic spectra of the compounds were recorded at CDRI Lucknow on a Shimadzu UV 1601 spectrophotometer. *In vitro* antifungal activities were carried out at IIVR Varanasi and Microbiological Lab IFTM University Moradabad.

#### Fungi and cultures

The fungus FOL was obtained from the fungal collection of the laboratory of IIVR Varanasi. All strains were selected for their

aggressiveness among tomato. The pathogenic fungus were cultured and purified on potato dextrose agar medium (PDA)<sup>6</sup>. *In vitro* fungal proliferation of different complexes at concentrations of 100, 500, 1000, 2000, 3000 ppm respectively were done. Flasks without fungicides used as control. The method used is that described in the literature<sup>7</sup>.

### RESULTS AND DISCUSSION

Elemental analysis data and molar conductivities of different complexes are given in Table 1.

**Table 1- Characterization data of mixed ligand metal complexes.**

Complex	Percentage Calculated (Found)				$\Lambda_m$
	C	H	N	Metal	
Mn(P) <sub>2</sub> (B) <sub>2</sub>	46.67 (49.01)	2.44 (2.52)	17.01 (17.34)	6.67 (7.02)	20.98
Co(P) <sub>2</sub> (B) <sub>2</sub>	46.44 (46.81)	2.44 (2.12)	16.93 (17.28)	7.12 (7.38)	26.04
Ni(P) <sub>2</sub> (B) <sub>2</sub>	46.46 (45.98)	2.44 (2.01)	16.93 (16.52)	7.09 (6.89)	29.45
Cu(P) <sub>2</sub> (B) <sub>2</sub>	46.19 (45.92)	2.42 (3.10)	16.83 (16.99)	7.64 (8.02)	27.56
Zn(P) <sub>2</sub> (B) <sub>2</sub>	46.09 (46.40)	2.42 (2.55)	16.79 (16.98)	7.84 (8.10)	25.35

Where  $\Lambda_m$  is calculated in  $ohm^{-1}cm^2 mol^{-1}$

All the complexes are coloured solid and quite stable at room temperature. These are stable up to 250°C. But above this they dissociates. The complexes are sparingly soluble in water, insoluble in common organic solvents but completely soluble in DMSO and DMF. Conductivity data also confirm this nature. The purity of complexes was checked by TLC. On the basis of elemental analysis the complexes are formulated as  $[M(P)_2(B)_2]$ .

#### Magnetic properties

Room temperature magnetic susceptibility measurements indicate paramagnetic nature for Mn, Co, Ni and Cu complexes. The magnetic moment observed for Mn(II) complex is 5.89 BM which is consistent with the octahedral geometry of the complex<sup>8</sup>. The six co-ordinate Co(II) complex exhibit magnetic moment of 4.96 BM suggesting octahedral geometry for Co(II) complex<sup>9</sup>. Ni(II) complex showed magnetic moment 3.39 BM slightly higher than the spin only value, indicating an octahedral environment around Ni(II) ion<sup>10</sup>. The observed magnetic moments for Cu (II) complex is 1.86 BM suggesting a distorted octahedral geometry around Cu(II)<sup>11</sup>. The Zn(II) complex was found to be diamagnetic, while all other complexes were paramagnetic with magnetic moment values close to the spin only values<sup>12</sup>.

**Electronic spectra**

Electronic spectra of Mn(II) mixed ligand complexes showed  $\nu_1, \nu_2$  and  $\nu_3$  transitions at 15200, 19100 and 25600  $\text{cm}^{-1}$  respectively which may be attributed to  ${}^4A_{1g} \rightarrow {}^4T_{1g}$  (G),  ${}^6A_{1g} \rightarrow {}^4T_{2g}$  (G) and  ${}^6A_{1g} \rightarrow {}^4A_{1g}$  (G) respectively. The  $\nu_2/\nu_1$  value is in accordance with Mn(II) octahedral complexes<sup>13</sup>. In Co(II) complexes the bands observed at 9250, 18500 and 20350  $\text{cm}^{-1}$  may be due to  ${}^4T_{1g}$  (F)  $\rightarrow$   ${}^4T_{2g}$  (F),  ${}^4T_{1g}$  (F)  $\rightarrow$   ${}^6A_{2g}$  (F) and  ${}^4T_{1g}$  (F)  $\rightarrow$   ${}^4T_{1g}$  (P) charge transfer transitions respectively in an octahedral field<sup>14</sup>. Ni(II) complex showed three transitions at 9910, 16090 and 25310  $\text{cm}^{-1}$  as  $\nu_1, \nu_2$  and  $\nu_3$  due to  ${}^3A_{2g} \rightarrow {}^3T_{2g}$ ,  ${}^3A_{2g} \rightarrow {}^3T_{1g}$  (F) and  ${}^3A_{2g} \rightarrow {}^3T_{1g}$  (P) respectively. The  $\nu_2/\nu_1$  value for the present compound is in the usual range (1.60-1.82) reported for majority of octahedral Ni (II) complexes<sup>14,15</sup>. The copper(II) complex showed one broad band at 15665  $\text{cm}^{-1}$ . Which may be due to  ${}^2E_g \rightarrow {}^2T_{2g}$  transition showing distorted octahedral geometry<sup>12,13,16</sup>. Ligand field parameters 10 Dq, B,  $\beta$  and  $\beta^\circ$  have been calculated from the electronic spectra. The values of ligand field parameters are given in Table 2.

**Table 2- Electronic spectra and ligand field parameters.**

Complex	10Dq ( $\text{cm}^{-1}$ )	B ( $\text{cm}^{-1}$ )	$\beta$	$\beta^\circ$
Mn(P) <sub>2</sub> (B) <sub>2</sub>	15833.33	735.55	0.855	14.5
Co(P) <sub>2</sub> (B) <sub>2</sub>	9250	740.00	0.762	23.8
Ni(P) <sub>2</sub> (B) <sub>2</sub>	9910	778.00	0.755	24.5
Cu(P) <sub>2</sub> (B) <sub>2</sub>	15665	--	--	--

These data are in accordance with the octahedral/ distorted octahedral geometry of complexes.

**IR spectra**

The infrared spectra of the mixed ligand complexes of bivalent '3d' transition metal ions with picric acid and 2, 2' bipyridine have been recorded. The proposed assignments of the IR spectral bands of the synthesized metal(II) picrate complexes are given in the Table 3.

**Table 3- Important IR spectral bands ( $\text{cm}^{-1}$ ) of mixed ligand complexes**

Complexes	Mn(P) <sub>2</sub> (B) <sub>2</sub>	Co(P) <sub>2</sub> (B) <sub>2</sub>	Ni(P) <sub>2</sub> (B) <sub>2</sub>	Cu(P) <sub>2</sub> (B) <sub>2</sub>	Zn(P) <sub>2</sub> (B) <sub>2</sub>
$\nu(\text{C}=\text{N})$ or $\nu(\text{C}=\text{C})$	1598s	1600 s	1599s	1601s	1599s
$\nu(\text{NO}_2)$	1493s	1493m	1495m	1495m	1496m
$\nu(\text{C}-\text{O})$	1292m	1266m	1280s	1250m	1260m
$\nu(\text{C}-\text{H})$	772s, 765m	768s	770m	769m	775s
$\nu(\text{M}-\text{O})$	521m	542m	592w, 585m	568m, 548w	542m
$\nu(\text{M}-\text{N})$	430m	440m, 432m	437m	432m	440m, 430m
$\nu(\text{M}-\text{O}-\text{N})$	352w	380m, 369w	350m, 310w	374m, 350m	380m, 350m

the two significant absorption bands at 500 and 3385  $\text{cm}^{-1}$  in the picric acid may be due to  $\nu_{\text{as}}(\text{NO}_2)$  and  $\nu(\text{O}-\text{H})$  (hydrogen bonded) respectively. The absence of  $\nu(\text{O}-\text{H})$  mode<sup>17,18</sup> at 3385  $\text{cm}^{-1}$  and appearance of a medium band at 1260  $\text{cm}^{-1}$  in all the complexes suggest the co-ordination of picrate ion in a monodentate fashion. The appearance of a new medium inten-

sity band in the range 1260-1296  $\text{cm}^{-1}$  is attributed to  $\nu(\text{C}-\text{O})$  because of co-ordination of phenolic oxygen after deprotonation<sup>19</sup>. The mode of co-ordination through the deprotonated phenolic oxygen is further manifested by appearance of new bands in the region 529-585  $\text{cm}^{-1}$  due to  $\nu(\text{M}-\text{O})$  vibrations<sup>20,21</sup>. In the IR spectra of the complexes, the band due to ring vibrations of the uncoordinated 2, 2' bipyridine observed at 1631  $\text{cm}^{-1}$  was shifted to 1598  $\text{cm}^{-1}$ . This shift by about 33  $\text{cm}^{-1}$  to a lower frequency shows that 2, 2' bipyridine is coordinated to the metal centres<sup>22</sup>. The strong bands at about 1598 and 770  $\text{cm}^{-1}$  may be assigned to  $\nu(\text{C}=\text{N})$  and  $\nu(\text{C}-\text{H})$  respectively<sup>23</sup> the shifting of these bands from their position in the free ligand indicates the coordination through N atom. The intensity of the bands present in the range 765-775  $\text{cm}^{-1}$  clearly indicates the involvement of both the N atoms in coordination confirming the bidentate nature of the ligand 2, 2' bipyridine<sup>24</sup>.

**In vitro antifungal activity**

*In vitro* antifungal screening of the complexes against FOL were done at the concentrations 100, 500, 1000, 2000 and 3000 ppm. The results were compared with control as shown in Table 4.

**Table 4- In vitro antifungal activity of metal complex against FOL (radial growth in mm).**

Doses (ppm)	complexes	Mn(P) <sub>2</sub> (B) <sub>2</sub>	Co(P) <sub>2</sub> (B) <sub>2</sub>	Ni(P) <sub>2</sub> (B) <sub>2</sub>	Cu(P) <sub>2</sub> (B) <sub>2</sub>	Zn(P) <sub>2</sub> (B) <sub>2</sub>
	100	42.0	39.5	36.7	30.5	39.5
500	30.0	33.5	28.9	25.6	30.5	
1000	29.5	30.0	20.2	20.5	23.5	
2000	20.5	18.5	15.2	18.2	20.5	
3000	19.2	10.5	10.2	10.1	18.5	
Control	62.6	62.6	62.6	62.6	62.6	
Mean	33.97	32.43	28.96	27.92	32.60	
CD 5%	1.53	1.50	2.0	2.0	1.47	
CV	4.50	5.30	4.30	5.34	6.35	

Comparison of activities of different metal chelates showed that all the complexes are very effective against *F. oxysporum* f sp. *lycopersici*.

**CONCLUSION**

The work described in this paper involved the synthesis and spectroscopic characterization of manganese, cobalt, nickel, copper and zinc complexes with picric acid and 2, 2' bipyridine ligands. The IR spectra revealed that picrate ions behaves as monodentate ligand co-ordinated to the metal ions through the deprotonated phenolic oxygen and 2, 2' bipyridine co-ordinate through both the nitrogen atoms to the metal ion. The magnetic moment and electronic spectra confirm the octahedral geometry of the complexes. The *in vitro* antifungal activity on the radial growth of the fungus refers that the complexes have significant inhibition efficiency against FOL Transition metal complexes with bioligands, represents a novel group of antimicrobial agents with potential application for the control of fungal infections and are used to treat the drug resistant fungal pathogens.

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