Asian Journal of Chemistry Vol. 16, No. 1 (2004), 493–500

Antifungal Studies of Transition Metal Complexes of Schiff Base Derived from Anthracene Carboxaldehyde-L-Histidine

G. INDIRA DEVI, GEETHA PARAMESWARAN* and V. VEENA †
Department of Chemistry, University of Calicut, Calicut-673 635, India

Transition metal complexes with Schiff bases as ligands have been amongst the most widely studied coordination compounds. Schiff bases and their metal complexes have found a multitude of uses. Amino acid complexes have been attracted by the biochemists because of its vast application. The antifungal behaviour of the Schiff base anthracene carboxaldehyde L-histidine and its complexes with Cu(II), Co(II), Mn(II) and Ni(II) has been studied individually. The effects of the above mentioned complexes were tried on different growth phases of *Phytophthora capsici*, the casual organism of foot rot of black pepper (*Piper nigrum.* L.). The results showed that the antifungal activity of the above complexes depends on metal coordination and the concentration of the test solution used. Due to chelation, the percentage inhibition of these complexes increases.

Key Words: Anthracene carboxaldehyde-L-histidine, Antifungal study, *Phytophthora capsici*, Schiff base.

INTRODUCTION

Antifungal agents are widely used to prevent or control diseases of plants and animals. According to Somers[1] earlier protective fungicides used in agriculture were non-specific poisons, which acted by selective accumulation. The use of fungicide started with Millardet's discovery of Bordeaux mixture[2, 3]. In this work the antifungal activity of transition metal complexes of Schiff bases are carried out. Emphasis is given to *Phytophthora capsici* causing foot rot of black pepper.

Black pepper (*Piper nigrum* L.) the "king of spices" is an important commercial crop. Black pepper is a perennial woody climber with adventitious roots. India is the leading producer and exporter of black pepper. But now this status is diminishing gradually because of low productivity. Foot rot caused by *P. capsici* is a major production constraint for black pepper[4]. The antifungal study of *P. capsici* is gaining momentum in the field of agricultural research. Fry[5] predicts that chemicals will probably always be needed for the control of disease like foot rot of pepper. In this study emphasis is given to the development of modern effective fungicides derived from Schiff base complexes of transition metals. Though the work in this area is limited, Schiff base complexes have long been used for the antifungal activities. Complexes derived from transition metal complexes are effective in antifungal treatment. Some of the complexes have been studied for their mechanism of action. The increases in the inhibition of Cu^{2+} by the addition of KEDTA to the medium have already been reported. Phenyl tin(IV) complexes of Schiff bases derived from the condensation of 2-hydroxy-1-naphthaldehyde and benzaldehyde

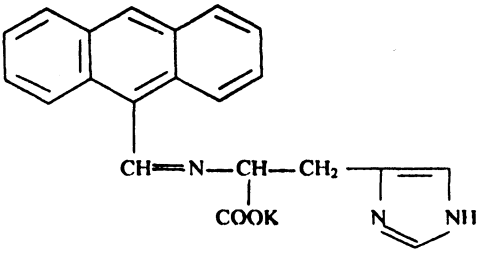
†Indian Institute of Spices Research, PB 1701, Marykunnu P.O., Calicut, India.

with glycine, DL-alanine, L-methionine, D-phenylalanine, 2-amino butyric acid and N-leucine have been synthesized for the above work. The fungicidal activity of the ligand and the complexes were detected using the standard procedure.

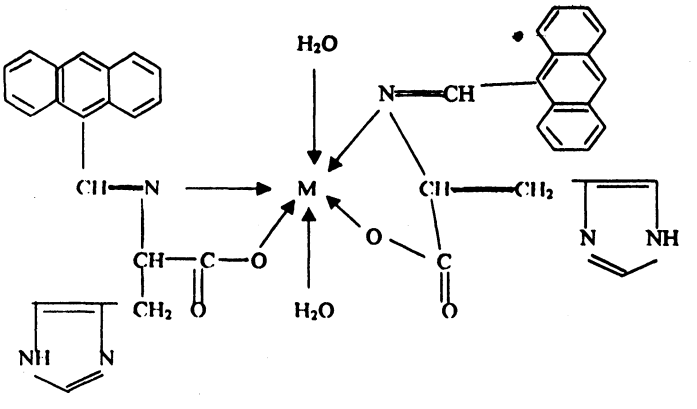
There are only few reports of antifungal activity of Schiff base complexes and no notable work has been reported on the antifungal studies of transition metal complexes of Schiff bases derived from anthracene carboxaldehyde-amino acids. Hence this work seems to be worthwhile in this field. Therefore, in the present study, antifungal activity of metal complexes synthesized from transition metals and anthracene carboxaldehyde L-histidine were tried against four critical stages of life-cycle of *Phytophthora capsici, viz.*, mycelial growth, sporangial production, zoospore production and zoospore germination.

EXPERIMENTAL

The Schiff bases were prepared by refluxing ethanolic/methanolic solutions of anthracene carboxaldehyde with L-histidine. The structure of the ligand is as follows.



The metal complexes of the Schiff base anthracene carboxaldehyde L-histidine were prepared by adding slowly a hot aqueous methanolic/ethanolic solution of metal acetate to a refluxing solution of the ligand in methanl/ethanol medium after adjusting its pH to 7–7.5. The complexes possess 1 : 2 metal to ligand stoichiometry. The precipitated complex was filtered and washed with methanol water mixture and dried in a vacuum desiccator. The general structure of the complexes is given below.



M - Mn (II), Co (II), Ni (II), Cu (II)

For the study of the antifungal activity of the transition metal complexes [Co(II), Cu(II), Ni(II) and Mn(II)] of the Schiff base, anthracene carboxaldehyde L-histidine to the causal organism of foot rot in black pepper, *P. capsici*, the facilities of Indian Institute of Spices Research, Calicut, Kerala, India, were used. Test solution of complexes have been prepared by dissolving appropriate amounts of complexes in 3 : 1 methanol-water mixture. In this work the pathogen *P. capsici* isolated from infected pepper plant and the culture already maintained at National Repository of *Pytophthora*, IISR, Calicut, was used. The antifungal activity was tested at the four critical stages in the life-cycle of the pathogen, *viz.*, mycelial growth, sporulation, zoospore release and zoospore germination. For mycelial growth study the test solution in concentrations ranging from 0.5% to 1% were taken and for the other three stages dilutions of 200 ppm, 400 ppm, 500 ppm and 1000 ppm were used.

For the studies on growth of mycelium poisoned food technique[6] was used. The test solution in the definite concentration form was taken and appropriate volume mixed with molten carrot agare medium to obtain final concentrations ranging from 0.5–1%. This chemical amended medium was poured into 9 cm petri plates. *P. capsici* from black pepper was cultured on carrot agar; 1 cm diameter mycelial discs of actively growing culture were cut with cork borer and placed in the center of petri plate containing carrot agar and test solution mixture. In control sets appropriate quantities of methanol-water solution were incorporated in place of the test solution. Three plates were kept for each concentration. The plates were incubated at 25 ± 1°C, growth of colony (fungal growth) was measured in every 25 h, at three points along the diameter of the plate and the mean of these three readings taken as the diameter of the colony. The growth of the colony in methanol control sets was compared with that of various treatments and the per cent inhibition was calculated.

For testing the effect of complexes on sporangial production, *P. capsici* was grown on carrot agar in dark for 48 h at 25 ± 1°C and 1 cm diameter discs of mycelium were cut and placed in petri plates. Methanolic solutions of different concentrations of complexes were placed on these discs and incubated under continuous light for 48 h. In control, the discs were covered with aqueous methanol. Numbers of sporangia produced per microscopic field were counted under 10x magnification. The average of 5 fields for each replication was counted and compared with that of methanol control.

To study the effect of complexes on zoospore release (indirect germination of sporangium) *P. capisici* culture grown on carrot agar medium for 48 h were taken. 1 cm diameter discs were cut and put in sterile distilled water and allowed to sporulate by incubating under fluorescent light as described above. Such sporulating disc were taken in petri plates, test solution of desired concentrations ranging from 200–1000 ppm placed over them and incubated at 4°C for 10 min (kept in the freezer of a refrigerator for 10 min). These plates were taken out and kept at laboratory temperature for 30 min before observation. For control set 3 : 1 methanol-water mixture was used in place of complex solution. The number of sporangia, which released zoospores, was counted. Six microscopic fields were

observed for each replication and per cent inhibition was calculated by comparing with control plates.

To evaluate the effect of complexes on germination of zoospore, sporulating discs were subjected to cold shock at 4°C for 10 min as described above to get the release of zoospores. Zoospores were collected in test tubes and vortexed. The zoospores settled at the bottom. 50 µL of the zoospore suspension was placed in the cavity of the cavity slides and 50 µL test solution was poured and mixed to form the final concentration of 200 to 1000 ppm. In control 3 : 1 methanol water mixture was used. All slides were incubated at room temperature inside petri plates lined with moist filter paper for 12 h. The treatment was replicated three times. In each slide, the microscopic fields were observed for number of zoospores present and the number germinated were counted. Inhibition percentage was calculated in comparison with methanol control.

RESULTS AND DISCUSSION

The results of mycelial growth studies, sporulation study, zoospore release studies and zoospore germination studies are discussed here. Four metal complexes and the ligand anthracene carboxaldehyde L-histidine were tested for their antifungal action. The effect of this ligand and complexes on different growth phases of *P. capsici* is presented in Tables 1 to 4. Based on the inhibition action on the mycelial growth, these compounds were selected for further studies.

TABLE-1
EFFECT OF ACH AND ITS COMPLEXES ON MYCELIAL GROWTH OF *PHYTOPHTHORA CAPSICI*

Complex	Mycelial growth in mm (at 0.5%)	Inhibition percentage	Mycelial growth in mm (at 1%)	Inhibition percentage
$MnL_2(H_2O)_2$	24.1	60.0	17.5	65.40
$CoL_2(H_2O)_2$	24.3	59.7	13.5	73.30
$NiL_2(H_2O)_2$	33.4	44.6	23.5	53.50
$CuL_2(H_2O)_2$	31.0	48.5	7.0	86.16
ACH	49.6	17.7	27.5	45.70
Control	60.3		50.6	

The test solutions, at two concentrations 0.5 and 1% were used for mycelial growth inhibition study. Among the ligand, potassium salt of anthracene carboxaldehyde L-histidine and its complexes with Cu(II), Co(II), Mn(II) and Ni(II), the maximum percentage of inhibition was shown by Cu(II) at 1% concentration. At 500 ppm the maximum effect was shown by $MnL_2^1(H_2O)_2$ (60%) and $CoL_2^1(H_2O)_2$ (59.7%), whereas at 1000 ppm the maximum effect was expressed by $CuL_2^1(H_2O)_2$ (86.2%) followed by $CoL_2^1(H_2O)_2$ (73.3%). In both concentrations the ligand possess lower percentage inhibition when compared with the complex. At lower concentration, the percentage inhibition of the ligand ACH and its complexes are in the order Co(II) ≅ Mn(II) > Cu(II) > Ni(II) > ligand ACH. At

the higher concentration, they are in the order Cu(II) > Mn(II) > Co(II) > Ni(II) > ligand ACH. The ligand is least resistant to this fungus when compared to the complexes. Among the complexes Ni(II) complex inhibited mycelial growth by 53.5% and found not to have a significant action at both lower and higher concentration.

TABLE-2
EFFECT OF ACH AND ITS COMPLEXES ON SPORULATION OF PHYTOPHTHORA CAPSICI

Complexes	Concentration in μg/mL	Sporangia per field	Percentage inhibition
$MnL_2(H_2O)_2$	200	30.8	63.59
	400	11.8	86.05
	500	0.0	100.00
	1000	0.0	100.00
$CoL_2(H_2O)_2$	200	37.8	55.23
	400	11.4	86.61
	500	0.0	100.00
	1000	0.0	100.00
$NiL_2(H_2O)_2$	200	30.08	64.43
	400	15.6	81.59
	500	2.0	95.81
	1000	0.0	100.00
$CuL_2(H_2O)_2$	200	0.0	100.00
	400	0.0	100.00
	500	0.0	100.00
	1000	0.0	100.00
ACH	200	44.0	47.99
	400	32.2	61.93
	500	22.4	73.52
	1000	5.8	93.14
Control		84.6	

Cu(II) complexes of ACH were found to be active at all concentrations. It possessed 100% inhibition on sporulation phase, whereas Mn(II) complex of ACH was not so effective at lower concentration (200 ppm). At higher concentration (1000 ppm) it was very effective. The sporulation inhibitory activities of ACH and its complexes were found to be in the order Cu(II) > Mn(II) ≅ Co(II) > Ni(II) > ligand ACH. The results of sporulation study on *P. capsici* with different concentrations of ligand and complexes are given in Table-2. The ligand

ACH was found to be less active at low concentration (200 ppm), whereas at 500 ppm and 1000 ppm, the ligand and its complexes were very active (73.5% and 93.14% respectively).

The effect of this ligand and complexes on zoospore release of *P. capsici* is presented in Table-3. Zoospore release was completely inhibited by Co(II) complex of ACH at higher concentration (1000 ppm). Cu(II) and Mn(II) complexes inhibited zoospore germination by nearly 100% at 500 ppm and 1000 ppm. The ligand (ACH) had inhibitory effect on zoospore release at all concentrations.

TABLE-3
EFFECT OF ACH AND ITS COMPLEXES ON ZOOSPORE RELEASE OF *PHYTOPHTHORA CAPSICI*

Complex	Concentration in μg/mL	Total no. of sporangia	Zoospores released	% of release	% of inhibition
$MnL_2(H_2O)_2$	200	144.67	5.67	4.14	66.71
	400	84.33	0.33	0.35	97.79
	500	102.33	0.67	0.53	97.30
	1000	403.33	15.00	4.25	77.53
$CoL_2(H_2O)_2$	200	207.00	1.67	0.86	93.06
	400	136.33	0.00	0.00	100.00
	500	372.00	0.00	0.00	100.00
	1000	526.00	0.00	0.00	100.00
$NiL_2(H_2O)_2$	200	148.67	2.00	1.34	89.24
	400	123.00	3.33	2.68	83.30
	500	467.33	1.33	0.27	98.63
	1000	124.67	1.00	0.75	96.02
$CuL_2(H_2O)_2$	200	185.00	5.33	2.74	78.01
	400	169.67	0.33	0.18	98.90
	500	285.33	1.33	0.34	98.25
	1000	341.33	1.33	0.24	98.71
ACH	200	175.67	3.33	1.88	84.87
	400	151.00	3.33	2.32	85.57
	500	398.67	6.00	1.41	92.85
	1000	177.00	3.00	1.68	81.13
Control	200	174.66	21.33	12.44	
	400	157.00	22.00	16.07	
	500	249.33	49.00	19.76	
	1000	425.67	72.67	18.90	

The percentage inhibition in zoospore germination of *P. capsici* is given in Table-4. Mn $(ACH)_2(H_2O)_2$ and $Co(ACH)_2(H_2O)_2$ shows the maximum percent-

age inhibition of 100%. The four complexes of ACH exhibited percentage inhibition in the order Mn(II) = Co(II) > Ni(II) > Cu(II). Ligand is more active at high concentration than at lower concentration. Complexes possess more percentage inhibition, when compared to the ligand. From this it is clear that due to chelation, percentage inhibition of these complexes on zoospore germination of *P. capsici* increases.

TABLE-4
EFFECT OF (ACH) AND THEIR COMPLEXES ON ZOOSPORE GERMINATION OF *PHYTOPHTHORA CAPSICI*

Sample	Concentration in µg/mL	Total no. of zoospores	Zoospores germinated	% of germination	% of inhibition
$MnL_2(H_2O)_2$	200	9	0	0.00	100.00
	400	14	0	0.00	100.00
	500	27	0	0.00	100.00
	1000	12	0	0.00	100.00
$CoL_2(H_2O)_2$	200	4	0	0.00	100.00
	400	7	0	0.00	100.00
	500	8	1	12.50	62.50
	1000	6	0	0.00	100.00
$NiL_2(H_2O)_2$	200	15	1	5.18	84.44
	400	18	2	8.64	74.07
	500	18	2	8.64	74.07
	1000	11	0	0.00	100.00
$CuL_2(H_2O)_2$	200	32	9	21.80	34.38
	400	22	4	14.14	57.58
	500	27	2	5.75	82.72
	1000	24	0	0.00	100.00
ACH	200	16	5	31.25	6.25
	400	19	4	21.05	36.84
	500	11	2	18.18	45.45
	1000	18	2	11.11	66.67
Control		27	9	33.33	

The Schiff base anthracene carboxaldehyde L-histidine and its complexes were synthesized for the first time. *In vitro* anti-microbial activity of new organo tin (IV) complexes of Schiff bases derived from amino acids has been reported by Nathmala and Yadav [7]. The antifungal study of these complexes was a part of the search of new antifungal agents. This detailed study shows that these compounds are 100% effective at 1000 ppm concentration and can be used as effective antifungal agents.

REFERENCES

1. E. Somers, *Science Progress*, **50**, 218 (1962).
2. A. Millardet, Phytopathological Classics, No. 3, American Phytopathological Society, St. Paul, Minnesota, 25 pp. (1885).
3. E.C. Large, The Advance of the Fungi, Jonathan Cape, London, 488 pp (1940).
4. P. Holliday and W.P. Mowat, Phytopathological Paper No. 5, Commonwealth Mycol. Inst., Kew, Surrey, p. 62 (1963).
5. W.E. Fry, Management with Chemicals, Plant Diseases, Vol. I, Academic Press, New York, pp. 213–238 (1977b).
6. G.A. Zentmyer, *Phytopathology*, **45**, 398 (1955).
7. Nathmala and R. Yadav, *Bull. Chem. Soc. (Japan)*, **70**, 1331 (1997).

(*Received*: 29 July 2003; *Accepted*: 7 November 2003) AJC-3214