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Antifungal Studies of Complexes of Schiff Base Derived from Anthracene Carboxaldehyde-L-Tyrosine

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Tyrosine is a potential chelating agent, because this forms stable and coloured complexes with various metal ions. Schiff bases derived from tyrosine and their metal complexes have wide application in biological science. Mn(II), Co(II), Ni(II) and Cu(II) complexes of the type $ML_2[H_2O]_2$ were synthesized and have been screened for their antifungal behaviour against *Phytophthora capsici*, the casual organism of foot rot of black pepper (*Piper nigrum* L.). The results show that the antifungal behaviour increases with increase in concentration.

Key Words: Anthracene carboxaldehyde-L-tyrosine, Antifungal studies, *Phytophthora capsici*, Schiff base.

INTRODUCTION

The antifungal study of *Phytophthora capsici* is gaining momentum in the field of agricultural research. Chemicals will always be needed for the control of diseases like foot rot of pepper[1]. New systemic compounds are considered as highly active with specific modes of action. Kaars[2], Fehrmann[3], Siegel[4], Byrde and Richmond [5] suggested that antifungal agents possess considerable selectivity. The use of Bordeaux mixture as a contact fungicide against foot rot of black pepper has been reported by many workers[6–13]. Schiff bases derived from tyrosine and their metal complexes have a wide application in biological science. A review by Yufeng[14] on the metal complexes of Schiff bases derived from amino acids points out the current interest of this type of compounds. The present paper reported the synthesis of the Schiff base anthracene carboxaldehyde-L-tyrosine and its four metal complexes and investigated their use as some new antifungal agents.

EXPERIMENTAL

Preparation of ligand

KOH (0.56 g, 0.01 mol) dissolved in methanol and tyrosine (1.81 g, 0.01 mol) in methanol were mixed and stirred vigorously. A hot methanol solution of anthracene-9-carboxaldehyde (2.06 g, 0.01 mol) was added to it with constant

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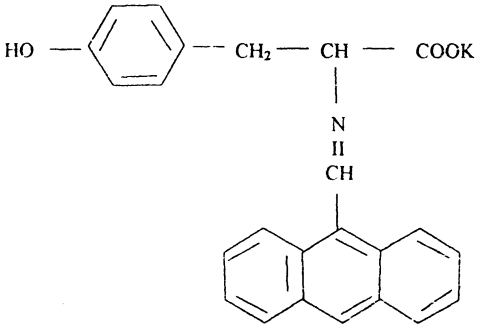
stirring. The orange-yellow precipitate obtained was filtered, recrystallized from methanol and dried in vacuum desiccator. Melting point was found to be 79°C.



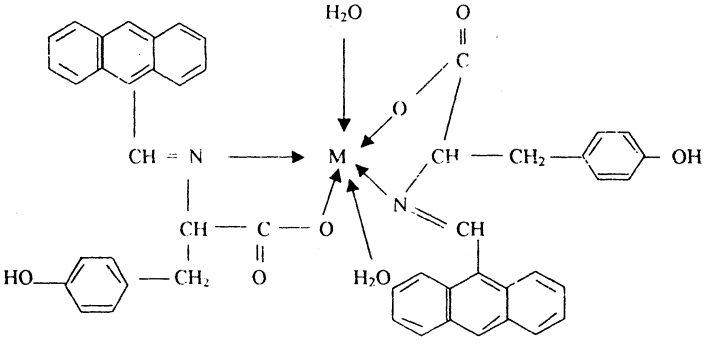
Structure of the ligand

Preparation of complexes

The complexes were generally prepared by adding slowly a hot aqueous methanol solution of the metal acetate or chloride to a refluxing solution of the ligand in methanol medium. The complexes possess 1 : 2 stoichiometry.

Mn(II), Co(II), Ni(II) and Cu(II) were prepared by adding methanol solution of the metal acetate (0.005 mol) to a refluxing solution of the ligand (0.01 mol), in methanol after adjusting its pH (7–7.5) with acetic acid. The precipitated complex was filtered, washed with methanol-water (1 : 1) mixture and dried in a vacuum desiccator.

The UV and IR spectra and elemental analysis confirm the general structure of complex as:



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

Antifungal activity

For the study of the antifungal activity of the transition metal complexes [Co(II), Cu(II), Ni(II) and Mn(II)] of Schiff base anthracene carboxaldehyde-L- tyrosine to the causal organism of foot rot of black pepper, *Phytophthora capsici*, the facilities of Indian Institute of Spices Research, Calicut, Kerala, India, were used.

Methods

Test solutions of complexes have been prepared by dissolving appropriate amounts of complexes in 3 : 1 methanol-water mixture. In this work the pathogen *Phytophthora capsici* isolated from infected pepper plant and the culture already maintained in National Repository of *Phytophthora*, IISR, Calicut, was used for the studies. The effect of these complexes on mycelial growth, sporulation, zoospore release and zoospore germination, the four critical stages in the life cycle of *Phytophthora capsici* were studied. For growth studies carrot agar medium was used (Griffin 1977)[15]. For mycelial growth study the test solution in concentrations 0.5 and 1% were taken and for the other three stages 200 ppm, 400 ppm, 500 ppm and 1000 ppm were used. Test solutions were prepared in 3 : 1 methanol-water solution.

Poisoned food technique[16] was used to study the antifungal property of the complexes and the ligand. The test solution in the desired concentration was taken and appropriate volume mixed with molten carrot agar. This chemical amended medium was transferred into 9 cm petri plates. In control sets appropriate quantities of methanol-water solution was incorporated in place of test solution. Three plates were kept for each concentration. The growth of the colony in methanol control set was compared with that of various treatments and the per cent inhibition was calculated.

Different test concentrations of complexes were prepared in 3 : 1 methanol-water mixture. For testing the effect of complexes on sporulation, *Phytophthora capsici* was grown on carrot agar in dark for 48 h at 20°C and 1 cm diameter discs of mycelium were cut and placed in petri plates (5 discs per plate). 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. Number 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. capsici* cultures 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 in fluorescent light as described above. Such sporulating discs were taken in petri plates, test solution of desired concentration 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. Due to cold shock, zoospores formed inside the sporangia were released. 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 methanol 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 release of zoospores. Zoospores were collected in test tubes and vortexed. The

zoospores which settle at the bottom, 50 μL of zoospore suspension was placed in the cavities of cavity slides and 50 μL test solution was poured and mixed . 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. 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 Schiff base complexes of Mn(II), Co(II), Ni(II) and Cu(II) metal ions and the Schiff base anthracene carboxaldehyde-L-tyrosine have been synthesized. These complexes and the ligand were tested for their antifungal action. The results of mycelial growth studies, sporulation study, zoospore release studies and zoospore germination studies are discussed here. The effect of this ligand and complexes on different growth phases of *P. capsici* is presented in Tables 1–4.

The test solutions, with two concentrations, ranging from 0.5 and 1%, were used for mycelial growth studies. Comparison of potassium salt of anthracene carboxaldehyde-L-tyrosine and its complexes showed that all the metal complexes and the ligand are active. Among these, Ni(II) complex is more active against *P. capsici* having a percentage inhibition of 67.3% at higher concentration. The comparative study of all the results given in Table-1 shows that at lower concentration, the ligand and the complexes were found to be inactive. In the case of Co(II) complex of anthracene carboxaldehyde-L-tyrosine (ACT), the activity increased with increase in concentration. The complexes affected the mycelial growth of *P. capsici* in the order ACT < Co(II) < Ni(II) < Cu(II) < Mn(II) at lower concentration.

TABLE-1
EFFECT OF ACT AND ITS COMPLEXES ON MYCELIAL GROWTH OF *P. CAPSICI*

Complex	Mycelial growth in mm (at 0.5%)	Inhibition percentage	Mycelial growth in mm (at 1%)	Inhibition percentage
$MnL_2(H_2O)_2$	32.44	46.20	16.67	67.06
$CoL_2(H_2O)_2$	39.22	34.95	21.56	57.40
$NiL_2(H_2O)_7$	36.22	39.93	16.56	67.28
$CuL_2(H_2O)_2$	32.56	46.01	20.33	59.82
ACT	43.78	27.40	18.22	63.99
Control	60.30		50.60	

Cu(II) complex of ACT was found to be active at all concentrations. It possesses 100% inhibition on sporulation phase, whereas Mn(II) complex of ACT was not so effective at lower concentration (200 ppm). At higher concentration (1000 ppm) it was very effective. ACT and its complexes exhibited the percentage inhibition of 100% for ACT, $Cu(ACT)_2$ $(H_2O)_2$, $Mn(ACT)_2$ $(H_2O)_2$, $Ni(ACT)_2$

$(H_2O)_2$ and 98.75% for $Co(ACT)_2(H_2O)_2$ at 1000 ppm concentration. Percentage inhibition increases as concentration increases. In the case of ACT, it possesses 100% inhibition at high concentration. The result of sporulation study on *P. capsici* with different concentrations of ligands and complexes is given in Table-2. Co(II) complex of ACT was found to be less active at low concentration (200 ppm).

TABLE-2
EFFECT OF ACT AND ITS COMPLEXES ON SPORULATION OF *P. CAPSICI*

Complex	Concentration in μg/mL	Sporangia per field	Percentage inhibition
$MnL_2(H_2O)_2$	200	6.6	86.19
	400	0	100
	500	0	100
	1000	0	100
$CoL_2(H_2O)_2$	200	25.8	46.02
	400	21	56.07
	500	6.4	86.61
	1000	0.6	98.74
$NiL_2(H_2O)_2$	200	0	100
	400	0	100
	500	0	100
	1000	0	100
$CuL_2(H_2O)_2$	200	0	100
	400	0	100
	500	0	100
	1000	0	100
ACT	200	6	87.45
	400	0	100
	500	0	100
	1000	0	100
Control		47.8	

The effect of this ligand and complexes on zoospore release of *P. capsici* is presented in Table-3. Zoospore release was completely inhibited by Mn(II) complex of ACT.

TABLE-3
EFFECT OF ACT AND ITS COMPLEXES ON ZOOSPORE RELEASE OF *P. CAPSICI*

Complex	Concentration in μg/mL	Total no sporangia	Zoospores released	% of release	% of inhibition
$MnL_2(H_2O)_2$	200	150.33	3.00	2.05	83.52
	400	192.33	7.33	3.96	75.35
	500	464.33	5.33	1.12	94.33
	1000	164.77	0.00	0.00	100.00
$CoL_2(H_2O)_2$	200	190.67	6.00	4.18	67.42
	400	219.67	7.33	2.95	81.66
	500	173.67	4.33	2.49	87.41
	1000	390.67	1.67	0.44	97.68
$NiL_2(H_2O)_2$	200	132.67	8.00	5.95	52.14
	400	130.33	2.33	1.90	88.19
	500	439.00	20.33	4.65	76.50
	1000	318.33	6.67	2.61	86.17
$CuL_2(H_2O)_2$	200	177.33	2.33	1.31	89.44
	400	143.66	3.33	2.45	80.29
	500	247.67	2.00	0.85	94.74
	1000	242.67	1.00	0.71	97.49
ACT	200	164.00	4.33	2.61	79.04
	400	190.33	6.00	3.10	80.68
	500	584.00	9.00	1.54	92.20
	1000	474.00	7.33	1.55	91.79
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. The Mn(II) complex of ACT, $[Mn(ACT)_2(H_2O)_2]$, $[NI(ACT)_2(H_2O)_2]$ and $[Mn(ACH)_2(H_2O)_2]$ shows the maximum percentage inhibition of 100%. The four complexes of ACT had percentage inhibition in the order Ni(II) ≅ Mn(II) > Co(II) > Cu(II). Ligand is active at higher concentration, than at lower concentration. From this it is clear that due to chelation, percentage inhibition of these complexes on zoospore germination of *P. capsici* increases. The Schiff base anthracene carboxaldehyde-L-tyrosine and its complexes were synthesized for the first time. Based on the results obtained above, these complexes and ligands are found to be effective antifungal agents. This study helped to locate new potent antifungal agents.

TABLE-4. EFFECT OF ACT AND ITS COMPLEXES ON ZOOSPORE GERMINATION OF *P. CAPSICI*

Complex	Concentration in µg/mL	Total no. of zoospores	Zoospores germinated	% of germination	% of inhibition
$MnL_2(H_2O)_2$	200	8	0	0.00	100.00
	400	12	0	0.00	100.00
	500	15	0	0.00	100.00
	1000	17	0	0.00	100.00
$CoL_2(H_2O)_2$	200	18	5	27.77	16.67
	400	11	2	22.22	48.14
	500	20	0	0.00	100.00
	1000	14	0	0.00	100.00
$NiL_2(H_2O)_2$	200	28	0	0.00	100.00
	400	11	0	0.00	100.00
	500	18	0	0.00	100.00
	1000	9	0	0.00	100.00
$CuL_2(H_2O)_2$	200	11	3	27.27	18.18
	400	14	4	28.57	14.28
	500	9	1	11.11	66.67
	1000	7	1	14.29	57.14
ACT	200	25	7	28.00	16.00
	400	21	6	28.57	14.28
	500	19	3	15.78	52.63
	1000	15	1	6.66	80.00
Control		27	9	33.33	

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