International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **2(6),** 16-21, June **(2013)** *Int. Res. J. Biological Sci.*

Synthesis and Antifungal studies of Glycine and Glycine-metal complexes on Phytophthora Capsici

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Available online at: www.isca.in
Received 6th March 2013, revised 25th April 2013, accepted 19th May 2013

Abstract

Phytophthora Capsici is an important pathogenic species attack on plants. Several metal complexes are known to act as antifungal agents. Amino acid complexes of transition metals are long been used for antifungal studies. The antifungal behavior of the glycine - copper acetate complex and glycine - cobalt acetate complex has been studied individually. The effect of the above mentioned glycine complexes were tried on mycelial growth phase and sporulation phase of Phytophthora Capsici, which is a typical fungus. Foot rot caused by Phytophthora Capsici is a major production constraint in black pepper, the king of spices. The results showed that glycine copper (II) complex is a very good antifungal agent. Even in lower concentration also these amino acid metal complexes are good inhibitors against two growth phases of Phytophthora Capsici.

Keywords: Glycine, Antifungal study, *Phytophthora Capsici*, mycelial growth, sporulation, metal complex.

Introduction

Among various diseases affecting black pepper in India, foot rot known as Quick wilt is the most important one[1-3]. Quick wilt is caused by a fungus called *Phytophthora Capsici.* It is the fungus which attack hundreds of species of plants and cause higher economic loss and environmental damage[4]. The thallus of this fungus is called mycelium. It consists of large number of tubular structures. The most common and characteristic asexual spore is called sporangium. Antifungal agents are used to reduce damage of materials caused by fungal attack. According to Somer's[5] earlier protective fungicide used in agriculture were non specific poison which acted by selective accumulation. Antimicrobial and cytotoxic activities of four newly synthesized iron complexes derived from succinimide and amino acids were carried out [6].

Antifungal study of phosphorus compounds reveals that they are powerful and selective in their antifungal activities[7-10]. Bordeaux mixture is used as a contact fungicide against foot rot of black pepper and metalaxyl is used as a systematic fungicide to control *Phytophthora Capsici* infections in various crops[11]. Metalaxyl is reported to inhibit protein and nucleic acid synthesis[12]. Spray with metalaxyl give good control on the attack of Phytophthora *Capsici* on black pepper[13]. Isolation and identification of antifungal agent N – butyl benzene sulphonamide, glycolipids and oligomycin was also reported[14-16]. Antifungal activity of some amino acids complexes have been determined against *candida albicans, a* single celled fungus[17].

Aminoacid complexes have wide variety of applications[18]. Copper binding amino acid complexes used to generare super oxide ion. Invitro antimicrobial activity of new organo stanous complexes of Schiff bases derived from amino acids, and anti fungal mechanism of zoxamide and its effects on cellular micro tubules were studied[19,20]. Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi was also determined[21]. The antifungal activity of amino acid metal complexes derived from anthracine carboxaldehyde L histidine was studied[22]. Coordination complexes of transition metals have been widely studied[23] for their antimicrobial, antifungal activities and as potent cytotoxic chemotherapeutic agents. Synthesis, characterization and biological studies of Cu(II) and Ni(II) complexes[24] and Antimicrobial Activity of Phospholipid Compound Produced by Acidophilic *Bacillus subtilis* were also carried out[25].

The antifungal activity of the transition metal chelates viz. Cu^{2+}, Ni^{2+}, Co^{+2}, Mn^{2+} and Zn^{2+} of quinolinols were investigated against various fungi. The metal complexes exhibit good activity against fungal strains compared with parental compound[26]. It is shown that certain root extracts are very effective against microbial infection[27]. Recently 1, 3, 5-Thiadiazin compounds have been synthesized and assayed for their antibiological activity[28]. In this study emphasis is given to the development of modern effective fungicides derived from amino acid complex of copper and cobalt. Amino acids are very small biomolecules and the simplest member is glycine. Amino acid like glycine and histidine easily forms complexes with almost all metal ions. In ceruloplasmin, histidine residues are involved in bonding to copper ions. Here the synthesis of two amino acid metal complexes, and their antifungal activities against the fungal pathogen *Phytophthora Capsici* were carried out.

Material and Methods

The pathogen Phytophthora Capsici was isolated from infected plant parts i.e. from infected leaf of pepper. The infected plant part was collected from the experimental plot of IISR, Calicut.

For multiplication of pathogen carrot agar medium was used. Materials used for the preparation of complexes and for antifungal study are of analar quality. Solvents used were purified by standard methods. Test solution of amino acid glycine and glycine copper complex has been prepared by dissolving in distilled water. Test solution of glycine cobalt complex has been prepared by dissolving in HCL distilled water mixture. In this the pathogen, *Phytophthora Capsici* isolated from the infected pepper plant and the culture already maintained in National Repository of *Phytophthora* IISR, Calicut was used. The effects of complexes on the growth of capsici and capsici sporulation are studied.

Glycine copper complex is prepared using glycine and copper acetate in the ratio 2:1.This mixture refluxed on a water bath for about 2 hour. The hot solution cooled in ice bath. Complex separated is filtered off.

Glycine - cobalt acetate complex is prepared by using 0.01 molar substances in 2:1 proportion of glycine and cobalt acetate. Using accurately weighed amount of metal acetate solution and glycine solution the complex is prepared.

Accurately weighed glycine copper acetate complex and glycine cobalt acetate complex is taken and then incinerated. From the mass of complex after incineration, the metal percentage is determined. The result is shown in the table. From the calculated value of metal percentage, formulae of complex are assigned.

For stock test solution 100% glycine copper and glycine cobalt complex solutions are made. For antifungal study of glycine-copper acetate complex following three stock solutions has to be prepared: i. By dissolving appropriate amount of glycine – copper acetate complex in hot water. ii. By dissolving appropriate amount of glycine in hot water. iii. Hot water.

For antifungal study of glycine-cobalt acetate complex following three stock solutions have to be prepared: i. By dissolving appropriate amount of glycine – cobalt acetate complex in aqueous HCl, ii. By dissolving appropriate amount of glycine in aqueous HCl, iii. HCl and distilled water.

The concentrations of complexes tested were 10-20%. For this, 1ml, 1.5 ml and 2 ml of each stock test solution were added to 9 ml, 8.5 ml and 8 ml of water. Add a definite volume of this test solution and 2mg chloramphenicol anti bacterial agent in to 49.5 ml of warm sterile carrot agar medium. 20 ml of this amended medium was poured in Petri dishes and allowed to solidify. After solidification mycelial discs were cut and placed in the centre of the medium. The radial growth of the colony was measured. The Percentage inhibition is calculated using the formula I = (C - T) / C x 100.

The effects of complexes and amino acid on sporulation of *Phytophthora* were studied by incubating the culture disc under light in different test concentration of the complex and amino acid. The concentrations of amino acid and complexes were 1, 2, 10, 15, and 20%. For this 1,2,10, 15 and 20 ml of stock test solutions were used. 48 hour old culture of *Phytophthora Capsici* was used. Six discs from the above culture placed in the Petri dish containing test solution. A compound microscope at 10 X magnification was used for the study.

Results and Discussion

The complex prepared was characterized on the basis of determining the percentage of metal and melting point..Melting point of blue coloured copper complex was 297^0 C and that of pale brown cobalt complex was 345^0C. From the metal percentage determination following formulae is assigned to the complexes. Cu $(COOCH_2\ NH_2)_2\ (H_2O)$ for copper complex and Co $(COOCH_2\ NH_2)_2$ for cobalt complex.

Antifungal activity of aminoacid glycine and its two metal complexes namely glycine copper complex and glycine cobalt complex were carried out. The results of mycelial growth studies, sporulation studies are discussed here. The effect of the amino acid and complexes on different growth phases of *Phytophthora Capsisi* is presented in tables.

The test solution with three concentrations ten percentage, fifteen percentage and twenty percentage were used for mycelial growth studies. Amino acid glycine and its complexes with copper (II) and Cobalt (II) solutions were tried on *Phytophthora Capsici* for its mycelial growth phase.

Glycine Cu (II) complex has high inhibitory power as the concentration of the solution increases. At lower concentration inhibitory power is less. At 10% concentration, the percentage of inhibition is 22.95%, for 15% it is 35.45% and at 20% it is 41.67%. That is as the concentration increases the percentage of inhibition increases, the percentage of inhibition is represented graphically. Hence on coordination with metals, antifungal activity of amino acids increases.

Glycine cobalt complex has 44.76 % inhibition at 10% concentration, at 15% it is 47.13% and at 20%, it is 50.72%. From this study, glycine cobalt (II) has higher inhibitory power when compared to glycine copper (II) complex in the mycellial growth phase of *Phytophthora*. Thus glycine Cobalt (II) complex can use as a better antifungal agent for mycelial growth of *Phytophthora Capsici.*

Glycine Cu (II) complex inhibit 100% in higher concentrations. The percentage of inhibition for 1% is 82.88% and that of 2% is 90%. The number of spores present in control for Glycine Cu (II) complex is almost same. In this case water control is used. In 10%, 15% and 20% there is 100% inhibition. AS concentration increases, percentage inhibition also increases. On coordination with metals, amino acid acts as antifungal agent.

Table-1
Analytical data of amino acid metal complexes

Metal complexes	Colour	Melting point	Metal percentage Experimental	Metal percentage Theoritical
Copper complex	Blue	297	25.56	25.68
Cobalt complex	Pale brown	345	27.61	28.47

Table-2
Effect of control on Mycelial growth of Phytophthora Capsici

Control Concentration	Mycelial growth in mm		
10%	19	20	22
15%	20	21	22
20%	20	23	21

Table-3
Effect of glycine and Copper II complex on Mycelial growth of Phytophthora Capsici

	Mycelial growth in mm									% of inhibition		
Concentration	10%			15%			20%			10%	15%	20%
Glycine	17	14	17	20	21	20	20	21	22	16.94	5.82	2.6
	15	15	19	19	18	18	21	21	21			
	16	18	21	21	20	21	21	20	20			
Complex	15	17	14	14	13	15	10	12	13	22.95	35.45	41.67
	17	18	16	14	12	14	12	11	16			
	16	13	15	14	14	12	13	10	15			

Table-4
Effect of control on Mycelial growth of Phytophthora Capsici

Control Concentration	Mycelial growth in mm		
10%	10	12	13
15%	9	10	10
20%	8	9	6

Table-5
Effect of glycine and cobalt complex on Mycelial growth of Phytophthora Capsici

Concentration	Mycelial growth in mm									% of inhibition		
	10%			15%			20%			10%	15%	20%
Glycine	8	9	10	9	9	7	7	6	6	15.24	16.09	17.39
	9	12	11	10	8	8	6	4	7			
	11	10	9	8	7	7	8	7	6			
Complex	9	6	5	4	3	4	5	6	3	44.76	47.13	50.72
	3	9	6	6	5	4	4	4	5			
	9	7	4	5	6	6	3	2	2			

Table-6
Effect of Glycine – Copper complex on sporulation of *Phytophthora Capsici*

Control Concentration	Number of spores formed in each concentration			
1%	39	45	54	60
2%	45	36	21	30
10%	36	38	42	49
15%	52	47	37	58
20%	35	50	36	65

Table-7
Effect of Glycine Cu(II) Complex on sporulation of Phythophthora capsici

Concentration		Number of spores		percentage of inhibition	
		Glycine Cu(II) complex	Glycine	Glycine Cu(II) complex	Glycine
1%	Field 1	7	55	82.88	5.73
	Field 2	8	38		
	Field 3	9	46		
	Field 4	10	42		
2%	Field 1	2	70	90	< 0
	Field 2	3	65		
	Field 3	4	46		
	Field 4	3	43		
10%	Field 1	3	145	100	< 0
	Field 2	0	235		
	Field 3	0	430		
	Field 4	0	300		
15 %	Field 1	0	185	100	< 0
	Field 2	0	282		
	Field 3	0	264		
	Field 4	0	320		
20%	Field 1	0	300	100	< 0
	Field 2	0	325		
	Field 3	0	225		
	Field 4	0	360		

Table-8
Effect of Glycine – Cobalt complex on sporulation of *Phytophthora Capsici*

Control Concentration	Number of spores formed in each concentration			
1%	10	12	14	11
2%	6	7	8	5
10%	1	1	0	1
15%	0	1	0	0
20%	1	0	0	0

Glycine Co(II) complex is also inhibit 100% in higher concentration. In this also percentage of inhibition increases with increase in concentration .The percentage of inhibition for 1% is 51.71% and that of 2% is 74.25%. It is less than the inhibition produced by Cu(II) complex.

The number of spores formed in control for Co (II) complex is very less. Water containing HCl is used as solvent in the case of Co (II) complex. Increasing the concentration the number spores produced is zero. In this case HCl itself acts as an inhibitor.

From the study upon sporulation it is found that in Glycine Cu(II) complex percentage of inhibition is higher than that of Glycine Co(II) complex. Thus Glycine Cu(II) complex is more effective in sporulation phase of *Phytophthora Capsici*. That is we can use Glycine Cu(II) complex as a good antifungal agent for sporulation of *Phytophthora Capsici* among these two complexes.

Conclusion

In this study two metal complexes glycine Cu(II) complex and glycine Co(II) complex and the amino acid glycine were tried on two major stages of life cycle of *Phytophthora Capsici*, the mycelium growth and sporulation. From this study it is found that glycine Co (II) complex is a good inhibitor for mycelium growth of *Phytophthora Capsici*. glycine Cu (II) complex is also a good inhibitor, but less inhibitory action compared to glycine Co (II) complex.

Glycine Cu (II) complex is a good inhibitor for the spore formation of Phytophthora *Capsici*. The percentage inhibition is very high even in low concentration. Thus it is a good antifungal agent than glycine Co (II) complex. Through this study of Antifungal action of amino acid - glycine and its copper and cobalt complex it is found that glycine support and its metal complexes inhibit the growth of *Phytophthora Capsici*, the casual organism which causes a serious disease, foot rot in black pepper.

Table-9
Effect of Glycine Co(II) Complex on sporulation of Phythophthora capsici

Concentration		Number of spores		percentage of inhibition	
		Glycine Cu(II) complex	Glycine	Glycine Cu(II) complex	Glycine
1%	Field 1	6	39	51.71	< 0
	Field 2	8	38		
	Field 3	5	27		
	Field 4	4	48		
2%	Field 1	2	33	74.25	< 0
	Field 2	1	42		
	Field 3	4	25		
	Field 4	0	40		
10%	Field 1	0	170	100	< 0
	Field 2	0	129		
	Field 3	0	140		
	Field 4	0	126		
15 %	Field 1	0	192	100	< 0
	Field 2	0	223		
	Field 3	0	264		
	Field 4	0	207		
20%	Field 1	0	229	100	< 0
	Field 2	0	280		
	Field 3	0	320		
	Field 4	0	225		

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