SYNTHESIS, CHARACTERIZATION, AND ANTIFUNGAL STUDIES OF FOUR NOVEL BENZILIC ACID AMINO ACID METAL COMPLEXES

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ABSTRACT

*Thermally stable modern effective fungicides can be synthesized from benzilic acid – amino acid and amino acid complexes of transition metals. Cu, Ni, Mn and Co complexes of benzilic acid Phenyl alanine have been synthesized. These complexes were characterized by various methods such as molar conductance, elemental analysis, and spectral measurements and by using magnetic and conductance data. Antifungal activity of the amino acid metal complexes was also carried out. Mn (II), Ni (II), Co (II), and Cu (II) complexes of phenyl alanine and benzilic acid phenyl alanine complexes were used. The molecular formula of the complexes obtained were $\text{CoL}(\text{H}_2\text{O})_3$, $\text{CuL}(\text{H}_2\text{O})_4$, $\text{NiL}(\text{H}_2\text{O})_3$ and $\text{MnL}(\text{H}_2\text{O})_3$, and metal to ligand ratio is found as 1 : 1. A comparative study of antifungal activity of the amino acid metal complexes was tried against mycelia growth phase of *Colletotrichum*, *Rhizoctonia*, and *Phytophthora capsici*. Among these complexes, Copper benzilic acid - Phenyl alanine complexes possess 100% inhibition at higher concentration. The Cobalt benzilic acid Phenyl alanine complexes completely inhibit the growth in almost all concentrations.*

KEYWORDS: Antifungal Activity, Coordination Complex, Benzilic Acid & Phenyl Alanine

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INTRODUCTION

Amino acid transition metal complexes play a vital role in our agricultural and medicinal field. Nowadays their use as antimicrobial agents gains momentum. In the last few years, so many studies have been done on the structure and chemical behavior of several metal complexes to find out an alternative against antimicrobial drugs. The physicochemical investigation on the bivalent metal complexes was carried out by conductometric, magnetic and spectral techniques with the object to compare their properties. Stanila and Braicu from Romania studied the antibacterial activity of copper and cobalt amino acid complexes. They synthesized amino acid complexes of copper and cobalt using ligand methionine, phenylalanine, valine, leucine, lysine and tested their antimicrobial efficiency on E. coli like micro organism. A S K Kuwar et al studied the synthesis, characterization and microbial activities of transition metal complexes of Ni, Cu and Zn. Electronic spectra and Magnetic moment values of these complexes indicate octahedral symmetry. Nathmala et al. have reported Cu (II) complexes of salicylidene amino acid Schiff bases of the type $[\text{Cu}(\text{sal.aa})\text{H}_2\text{O}]\text{nH}_2\text{O}$. Subhasish Saha, et al studied the synthesis, characterization and antimicrobial activity of cobalt metal complex against multi drug resistant bacterial and fungal pathogens. Antifungal activity of organotin complexes of Schiff bases derived from amino acids was studied by Nathmala and Yadav Rakesh. For this, tin (IV) complexes of Schiff bases derived from the condensation of 2 hydroxyl – 1

naphthaldehyde and benzaldehyde amino acid complexes were used.

EXPERIMENTAL

Benzilic acid dissolved in aqueous methanol treated with phenyl alanine refluxed and cooled. Pale yellow precipitate of benzilic acid phenyl alanine is separated. It is washed and dried, and its melting point is noted.

Synthesis of four benzilic acid phenyl alanine metal complexes, Mn (II), Ni (II), Co (II) and Cu (II) were carried out. Benzilic acid and phenyl alanine is dissolved in aqueous methanol, refluxed and then the metal acetate solution is added. Precipitate of complex formed is separated. Phenyl alanine metal complexes were also prepared.

Agar plate technique against viz. Rhizoctonia solani, collectotrichum and phytophthora capsici was used for the anti fungal studies of Benzilic acid Phenyl alanine complexes of copper, cobalt, nickel and manganese. Plates incubated at room temperature and observations were recorded after 24, 48, 72 hours.

RESULTS AND DISCUSSIONS

The metal percentage of the complexes has been obtained from the analytical data summarized in the Table 1. In agreement with the non-electrolytic nature of the complexes, the molar conductance is found to be below $10\Omega^{-1}\text{cm}^2\text{mol}^{-1}$. Determination of coordination of ligands is obtained from the infrared spectra discussed below.

Table 1: Characterization of Complexes

Metal Complex Molecular Formula	Color	Melting Point	M%	Ω^{-1}
$\text{CuL}(\text{H}_2\text{O})_4$	Pale blue	205	11.93 (11.37)	3.4
$\text{Co L}(\text{H}_2\text{O})_3$	pink	324	13.06 (13.18)	7.22
$\text{Mn L}(\text{H}_2\text{O})_3$	white	228	10.85 (10.41)	9
$\text{Ni L}(\text{H}_2\text{O})_3$	Bluish Green	268	11.51 (11.37)	6.2

(L = Benzilic acid Phenyl Alanine)

The Melting point of the ligand benzilic acid phenyl alanine was measured as 152°C . Benzilic acid phenyl alanine complexes prepared were stable and coloured. They are soluble in aqueous methanol and aqueous HCl, but not in water. Metal percentage, colour, molar conductance and melting point are given in the table 1. The ligand is pale yellow whereas the complexes were coloured. Also, the melting point of ligand and metal complexes were different. This shows that there is a coordination of ligand and metal. The molar conductance value of the complex measured in 10^{-3}M aqueous methanol is below $10\text{Scm}^2\text{mol}^{-1}$, indicating the nonelectrolytic nature. Pyrolysis method is used for determining the Metal percentage. The metal to ligand ratio in phenyl alanine metal complexes was 1:1 and in benzilic acid phenyl alanine metal complexes also it was found to be 1:1.

New peaks observed for coordinated water. $\gamma(\text{OH})$, $\gamma(\text{C}=\text{O})$, $\gamma_{\text{asym}}(\text{COO})$ and $\gamma_{\text{sym}}(\text{COO})$ stretching vibrations are observed at 3355–3400 (broad band), 1631, 1594 and 1337cm^{-1} respectively. There is a shift in position of bands from 3248 to 3332, 1621 to 1747, 1493 to 1619 and 1395 to 1455cm^{-1} in the metal complexes, which clearly shows the participation of the carboxylate - oxygen atom in the complexes formation

New bands of $\gamma(\text{M}-\text{O})$ stretching vibrations appeared in the spectra of the complexes in the regions $444\text{--}537\text{cm}^{-1}$. Therefore, from the IR spectra, it is concluded that ligand behaves as a bidentate ligand coordinating to the metal ions via the deprotonated carboxylate O and alcoholic OH in complexes and a band at $420\text{--}488\text{cm}^{-1}$ have been

assigned to $\gamma(\text{M}-\text{N})$ mode of vibration. In all the benzilic acid phenyl alanine complexes, the symmetric stretching vibrations of the carboxylate groups occur at $\approx 1429\text{--}1496\text{ cm}^{-1}$ and asymmetric at $1600\text{--}1650\text{ cm}^{-1}$. Hence the monodentate behaviour of the carboxylate group can be clearly assigned to the complexes. $\gamma(\text{M}-\text{O})$ and $\gamma(\text{M}-\text{N})$ frequencies of copper chelates appeared in the range of $417\text{--}444$ and $444\text{--}518\text{ cm}^{-1}$ respectively.

Similarly, in phenyl alanine – copper and phenyl alanine cobalt complexes new bands in the range of $410\text{--}435$ and $435\text{--}510\text{ cm}^{-1}$ were assigned to $\gamma(\text{M}-\text{O})$ and $\gamma(\text{M}-\text{N})$ respectively. In this case the asymmetric and symmetric stretching vibrations of the carboxylate groups occur at ≈ 1630 and 1470 cm^{-1} which indicates the monodentate behavior of the carboxylate group.

Electronic spectra of Co (II) is having two bands at $\approx 11340\text{ cm}^{-1}$ and 38320 cm^{-1} is assignable to octahedral geometry.⁻¹. The Cu(II) complex electronic spectrum show a broad band in the region $478\text{--}700\text{ nm}$ and is assignable to d-d transition. Two bands have been found at 270 nm and 320 nm in case of free ligand. Pattern of the electronic spectra of metal complexes is different from that of the free ligand, indicating the change in the structure of the complex. In manganese complexes three absorption bands observed at 220 nm , 275 nm , and 306 nm are observed. On the basis of reflectance spectra and magnetic data, an octahedral coordination may be suggested to the Co (II) complex. In phenyl alanine metal complexes two bands present in electronic spectra of Co (II) at $\approx 11340\text{ cm}^{-1}$ and 38320 cm^{-1} . It is assignable to octahedral geometry. The bands at $470\text{--}502\text{ cm}^{-1}$ have been assigned to $(\text{M}-\text{N})$ mode. Three bands present in electronic spectra of Co (II) at $\approx 15625\text{ cm}^{-1}$, 19801 and 38461 cm^{-1} and is assignable to octahedral geometry.⁻¹ to cobalt complex. The aromatic band at 255 nm (39216 cm^{-1}) is attributed to $n\pi\rightarrow\pi^*$ transition, the band around (38461 cm^{-1}) is due to the $n\rightarrow\pi^*$ transition of the $\text{C}=\text{O}$, which is consistent with the proposed structure.

Phenyl alanine complexes of copper and cobalt were prepared for the comparative antifungal studies with Benzilic acid Phenyl alanine complexes of copper, manganese, cobalt and nickel.

Figure 1: Phytophthora Capsici – 72 Hrs

After 72 hours of incubation it is observed that cobalt- benzilic acid phenylalanine complexes have 81.4% inhibition. It possesses moderate inhibition in all concentration. Increased concentrations of complexes show a higher inhibitory power. Copper complexes show average inhibition in all concentrations, where as manganese complexes were poor inhibitors of phytophthora capsici and Rizoctonia. Studies show that cobalt complexes of phenylalanine-benzilic acid are moderate inhibitors of phytophthora capsici and Rizoctonia. The reading of Ni complexes is not up to the standard, may be due to contamination.

Figure 2: Phytophthora Capsici – 48 Hrs

Figure 3: Rhyzotonia Solani – 72 Hrs

Figure 4: Photos of Colletotrichum

Figure 5: Rhyizotonia Solani – 48 Hrs

Figure 6: Phenyl Alanine Cu Complex on Colletotrichum – 48 Hrs

After 48 hours of incubation we found that cobalt – benzylic acid phenylalanine complexes have 100% inhibition. This means that it completely inhibits the growth in all concentration. Cobalt - phenylalanine complex shows an inhibition of 48.68% in 50ppm solutions where as at higher concentrations 100% inhibition

Copper - benzilic acid phenylalanine complexes shows an inhibition of 40.27% in 50 ppm solution and in 100 ppm solution percentage of inhibition increases to 72.57%. But at high concentration solutions of 150, 200 ppm it has 100% inhibition. The antifungal activity of copper phenylalanine complexes has slight difference when compared to copper - benzilic acid phenylalanine complex. It completely inhibits in 200 ppm solution and as the concentration increases, percentage inhibition also increases.

Figure 7: Benzilic Acid Phenyl Alanine Cu Complex on Colletotrichum – 48 Hrs

After 72 hours of incubation with colletotrichum, cobalt- benzilic acid phenylalanine complex completely inhibit the growth in higher concentrations. Cobalt phenyl alanine complex has 47.77% of inhibition in 50ppm. But in 100ppm, 150ppm, 200ppm concentrations there is 100% of inhibition.

In the application of 50ppm copper phenylalanine complexes on colletotrichum, shows a decrease in the percentage of inhibition than the 72 hours of incubation. In all concentrations, copper- benzilic acid phenylalanine-complexes posses anti fungal activity. Cobalt phenylalanine complexes have higher antifungal activity than those with copper.

Figure 8: Benzilic Acid Phenyl Alanine Co Complex on Colletotrichum – 48 Hrs

Figure 9: Phenyl Alanine Cu Complex on Colletotrichum – 72 Hrs

Figure 10: Benzilic acid Phenyl Alanine Cu Complex on Colletotrichum – 72 Hrs

Figure 11: Benzilic Acid Phenyl Alanine Co complex on Colletotrichum – 72 Hrs

CONCLUSIONS

Now a days, diseases due to fungus and bacteria is one of the major problems of agriculture. The use of transition metal complexes as anti fungal agents gains momentum recently. In the present study, new benzilic acid phenyl alanine metal complexes were synthesized and their impact on antifungal activity was studied. Here the benzilic acid –phenyl alanine metal complexes of copper, nickel, manganese and cobalt complexes of phenyl alanine were synthesized and characterized. The ligand and complexes are having different colour and melting point. Metal to ligand ratio obtained as 1:1 and in all these metal is coordinated to water molecule also. From elemental analysis, spectral and thermal studies, the molecular formula of various complexes were assigned as $\text{CoL}(\text{H}_2\text{O})_3$, $\text{CuL}(\text{H}_2\text{O})_4$, $\text{NiL}(\text{H}_2\text{O})_3$ and $\text{MnL}(\text{H}_2\text{O})_3$, where L is the ligand benzilic acid –phenyl alanine. The molar conductance of complexes is found to be below $10 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ and is in agreement with their non electrolytic nature. From electronic spectra, octahedral geometry is assigned to all complexes.

The cobalt complexes are found to be moderate inhibitors at all concentrations on colletotrichum, rhyzoctoni Solani and phytophthora capsici. At low concentration, manganese and copper complexes does not have any noticeable antifungal activity. All these complexes including copper and cobalt phenyl alanine complexes show antifungal activity at very high concentration on the vegetative and reproductive phase of colletotrichum, rhyzoctoni Solani and phytophthora capsici. It is found that, on increasing the concentration the anti fungal activity also increases. Among the four complexes of copper and cobalt, it is found that cobalt benzilic acid phenyl alanine has 100% inhibition and it inhibit in all concentration. Hence cobalt benzilic acid phenyl alanine complexes were found to be very good antifungal agent against colletotrichum, rhyzoctoni Solani and phytophthora capsici at higher concentration.

For rhyzoctoni Solani, 72 hour inhibition found that cobalt phenyl alanine benzilic acid have 81.4% inhibition it moderately inhibit growth in all concentration compared to copper and manganese complexes. In all the cases, as concentration increases, inhibitory power also increases. Copper complexes show average inhibition on both fungi. Results show that manganese complex was a poor inhibitor of phytophthora capsici and rhizotonia. Overall result shows that cobalt complexes can used as antifungal agents for this fungi. This study emphasis the special interest to the discovery of thermally stable, new, amino acid benzilic acid metal complexes which possess high antifungal activity.

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