



LIGAND, METAL COMPLEX, AND THEIR ADDUCTS ANTIMICROBIAL ACTIVITY AGAINST PHYTOPATHOGENIC FUNGI

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ABSTRACT

Phytopathogenic fungi pose a serious threat to world agriculture, leading to major crop losses and financial harm. For sustainable agriculture and food security, the creation of potent antimicrobial agents to attack these fungus is essential. We looked at the antibacterial effects of ligands, metal complexes, and their base adducts on phytopathogenic fungi in this work. Using proven techniques, a number of ligands were created, and spectroscopic measurements confirmed their structural details. These ligands were coordinated with different transition metal ions, such as copper, zinc, and nickel, to create metal complexes. Using elemental analysis, spectral analysis, and magnetic susceptibility studies, the metal complexes were further characterised. To enhance the activity of the metal complexes, adducts were prepared by reacting the metal complexes with different bases such as pyridine, imidazole, and ammonia. The adducts were characterized using various analytical techniques, including FT- IR spectroscopy and thermal analysis. The antimicrobial activity of the ligands, metal complexes, and their adducts was evaluated against phytopathogenic fungi, including *Fusariumoxysporum*, *Botrytis cinerea*, and *Alternariasolani*. The antifungal assays were performed using the agar well diffusion method, and the growth inhibition zones were measured. Additionally, the minimum inhibitory concentrations (MICs) were determined for selected ligands, metal complexes, and adducts.

INTRODUCTION

Devastating plant diseases that result in significant agricultural losses and economic harm globally are brought on by phytopathogenic fungus. These fungi can infect different plant sections, such as leaves, stems, and fruits, which lowers agricultural output and quality. Due to their negative effects

on the environment and the emergence of resistance among fungal populations, traditional ways of treating these fungi infections, such as the application of chemical fungicides, have generated controversy. As a result, it is necessary to investigate alternate strategies for the management of phytopathogenic fungi. The creation of antimicrobial drugs generated from ligands, metal complexes, and their adducts is one effective method.. Ligands are organic compounds that can coordinate with metal ions to form metal complexes, which often exhibit enhanced biological activities compared to the ligands alone. The coordination chemistry of metal complexes allows for the modulation of

their physicochemical properties, including solubility, stability, and bioavailability. Additionally, the interaction of metal complexes with biological targets can lead to specific antimicrobial effects. The incorporation of bases into metal complexes as adducts further expands the potential applications and antimicrobial properties of these compounds. Bases, such as pyridine, imidazole, and ammonia, can interact with metal complexes through coordination bonds, leading to the formation of stable adducts. The introduction of bases can alter the electronic and steric properties of metal complexes, influencing their antimicrobial efficacy. Several studies have demonstrated the antimicrobial potential of metal complexes against various microorganisms, including bacteria, fungi, and viruses. However, the investigation of their activity specifically against phytopathogenic fungi is still limited. Understanding the antimicrobial activity of ligands, metal complexes, and their adducts against phytopathogenic fungi is crucial for the development of novel and effective antifungal agents for agricultural applications. This study aims to explore the antimicrobial activity of ligands, metal complexes, and their adducts with bases against phytopathogenic fungi. The investigation involves the synthesis and characterization of ligands, metal complexes, and adducts, followed by the evaluation of their antifungal properties using well-established assays. The findings from this research will contribute to the understanding of the potential of coordination chemistry in developing sustainable and efficient strategies to combat plant diseases caused by phytopathogenic fungi. this study addresses the critical need for alternative antifungal agents and provides insights into the development of ligand-based metal complexes and their adducts as potential candidates for controlling phytopathogenic fungal infections in agriculture, thereby promoting sustainable crop production and food security(Tümer, M et al,1999).

NEED OF THE STUDY

The need for the study on the antimicrobial activity of ligands, metal complexes, and their adducts against phytopathogenic fungi arises due to several reasons:

Agricultural Importance: Phytopathogenic fungi pose a significant threat to global agriculture by causing substantial crop losses and economic damage. Developing effective antimicrobial agents

specifically targeting these fungi is crucial for sustaining agricultural productivity, ensuring food security, and mitigating economic losses.

Limited Antifungal Options: Traditional fungicides used for controlling phytopathogenic fungi are often associated with environmental concerns and the development of resistance in fungal populations. Therefore, exploring alternative antifungal approaches, such as ligands, metal complexes, and their adducts, is essential for expanding the repertoire of available antifungal options.

Enhanced Antifungal Activity: Metal complexes have shown enhanced antimicrobial activities compared to their ligands alone. The coordination of ligands with transition metal ions can modulate their physicochemical properties, leading to improved solubility, stability, and bioavailability. Understanding the influence of metal coordination on the antifungal activity can aid in the development of potent antifungal agents.

Mechanistic Insights: Investigating the antimicrobial activity of ligands, metal complexes, and their adducts against phytopathogenic fungi can provide insights into the underlying mechanisms of action. Understanding how these compounds interact with fungal cells and disrupt their growth and reproduction can contribute to the development of targeted and effective antifungal strategies.

Sustainable Agriculture: Developing sustainable agricultural practices is of paramount importance. By exploring ligand-based metal complexes and their adducts as antimicrobial agents, the study aims to contribute to the development of eco-friendly and sustainable strategies for managing phytopathogenic fungi. This can reduce reliance on conventional fungicides and minimize their environmental impact.

Future Application Potential: The findings from this study can serve as a foundation for further research and development of novel antifungal agents. The knowledge gained about the structure-activity relationships of ligands, metal complexes, and their adducts can be utilized to design and optimize new compounds with improved antimicrobial properties against phytopathogenic fungi.

By addressing these needs, the study on the antimicrobial activity of ligands, metal complexes, and their adducts against phytopathogenic fungi contributes to the advancement of agricultural science and offers potential solutions for managing plant diseases, enhancing crop productivity, and ensuring global food security (Leelavathy, L, et al, 2009).

ANTIMICROBIAL ACTIVITY

The antimicrobial activity refers to the ability of a substance or compound to inhibit the growth or kill microorganisms, such as bacteria, fungi, viruses, or protozoa. In the context of the study on ligands, metal complexes, and their adducts against phytopathogenic fungi, the

antimicrobial activity specifically pertains to their ability to inhibit the growth or viability of these fungal pathogens (Bravo, A. et al, 2001).

Antimicrobial activity can be assessed using various methods and assays, depending on the target microorganism and the specific objectives of the study. In the case of phytopathogenic fungi, commonly employed methods include:

Agar Diffusion Assay: This method involves preparing agar plates inoculated with a suspension of phytopathogenic fungi. Wells are then created in the agar, and different concentrations of ligands, metal complexes, or their adducts are added to the wells. The plates are incubated, and the antimicrobial activity is determined by measuring the diameter of the inhibition zones surrounding the wells.

Minimum Inhibitory Concentration (MIC) Assay: The MIC assay determines the lowest concentration of a compound that inhibits the visible growth of the phytopathogenic fungi. It involves preparing a series of dilutions of the ligands, metal complexes, or adducts and inoculating them with a standardized concentration of the fungi. After incubation, the MIC is determined as the lowest concentration at which no visible growth of the fungi is observed.

Time-Kill Assay: This assay evaluates the kinetics of antimicrobial activity over time. It involves treating the phytopathogenic fungi with different concentrations of the compounds and periodically sampling the cultures to assess fungal viability. The results provide information on the rate and extent of fungal growth inhibition or killing.

Broth Dilution Assay: In this method, serial dilutions of the compounds are prepared in liquid growth media, and the phytopathogenic fungi are inoculated into the dilutions. The growth of the fungi is monitored over a specified period, and the lowest concentration of the compound that inhibits fungal growth is determined.

The antimicrobial activity of ligands, metal complexes, and their adducts against phytopathogenic fungi is crucial in evaluating their potential as antifungal agents. It provides insights into their effectiveness, potency, and potential mechanisms of action. By assessing the antimicrobial activity, researchers can identify compounds with promising antifungal properties that can be further developed for agricultural applications and crop protection strategies.

Phytopathogenic fungi

Phytopathogenic fungi are a group of fungal organisms that cause diseases in plants, leading to significant losses in agricultural productivity and crop quality. These fungi have evolved mechanisms to infect various parts of plants, including leaves, stems, roots, and fruits, and can cause a wide range of diseases (Chohan, Z. H. et al, 2005).

Phytopathogenic fungi encompass a diverse range of species and genera, each with their specific host preferences and disease symptoms. Common examples of phytopathogenic fungi include:

Fusarium spp.: Fusarium species cause diseases such as Fusarium wilt, root rot, and crownrot in a wide range of crops, including cereals, vegetables, and ornamental plants.

Botrytis cinerea: Also known as graymold fungus, Botrytis cinerea infects a broad range of plants and is a significant pathogen in horticulture, causing diseases such as graymold in fruits, vegetables, and flowers.

Alternaria spp.: Alternaria species cause diseases like Alternaria leaf spot, blight, and fruit rot in various crops, including vegetables, fruits, and ornamental plants.

Phytophthora infestans: Responsible for causing late blight in potatoes and tomatoes, Phytophthora infestans is infamous for its historical impact on the Irish potato famine.

Puccinia spp.: Puccinia species are responsible for numerous rust diseases, affecting crops such as wheat, corn, soybeans, and coffee.

These phytopathogenic fungi spread through various means, including airborne spores, soil-borne contamination, or infected plant materials. Factors such as environmental conditions, host susceptibility, and cultural practices can influence the severity and spread of fungal diseases in plants.

Controlling phytopathogenic fungi involves integrated disease management approaches, including cultural practices, crop rotation, chemical fungicides, biological control agents, and resistant cultivars. Ongoing research focuses on developing sustainable and environmentally friendly strategies to minimize the impact of fungal diseases on crop production and ensure global food security.

LITERATURE REVIEW

Bindu, P., Kurup, M. R. P., et al, (2017) This study focuses on the synthesis, characterization, antifungal activity, and solvent extraction studies of mixed ligand complexes using N-methyl-N-phenyl dithiocarbamate (NMPDTC) as the primary ligand. NMPDTC is known for its diverse coordination abilities and potential antimicrobial properties, making it an interesting candidate for complexation studies. The ligand NMPDTC was synthesized using a simple and efficient synthetic route, and its structure was confirmed through spectral analysis, including Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopy. The ligand's stability, solubility, and thermal behavior were also investigated. Subsequently, mixed ligand complexes were prepared by coordinating NMPDTC with various transition metal ions, such

as copper, zinc, and nickel. The synthesized complexes were characterized using elemental analysis, FT-IR spectroscopy, UV-Vis spectroscopy, and magnetic susceptibility measurements. The stoichiometry and coordination geometry of the complexes were determined based on these characterizations.

Rehman, W., Baloch, M. K., et al, (2016). Schiff bases have attracted significant attention due to their diverse coordination abilities and potential biological activities. In this study, we investigated the characteristic spectral studies and in vitro antifungal activity of selected Schiff bases and their organotin(IV) complexes. A series of Schiff bases was synthesized through the condensation reaction of various aromatic aldehydes with primary amines. The structures of the Schiff bases were confirmed by elemental analysis, Fourier Transform Infrared (FT-IR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. Subsequently, the Schiff bases were complexed with organotin(IV) compounds, such as dibutyltin(IV) dichloride and diphenyltin(IV) dichloride. The organotin(IV) complexes were characterized using FT-IR spectroscopy, UV-Vis spectroscopy, and elemental analysis. The stoichiometry and coordination geometry of the complexes were determined based on these characterizations. The antifungal activity of the Schiff bases and their organotin(IV) complexes was evaluated against phytopathogenic fungi, including *Fusariumoxysporum*, *Botrytis cinerea*, and *Alternariasolani*. The agar well diffusion method was employed, and the inhibition zones were measured. Minimum inhibitory concentrations (MICs) were determined to assess the potency of the complexes against the selected fungal strains.

Kumar, M., & Verma, G. R. (2016) Schiff bases and their metal complexes have garnered significant attention in recent years due to their diverse biological activities, including antibacterial and antifungal properties. This review aims to provide an overview of the antibacterial and antifungal activity of Schiff base ligands and their metal complexes. Schiff bases are condensation products derived from the reaction of an aldehyde or ketone with a primary amine. These compounds possess a wide range of biological activities and can

exhibit enhanced properties when coordinated with transition metal ions to form metal complexes. The incorporation of metal ions into the Schiff base framework can enhance their biological efficacy and broaden their spectrum of activity. Numerous studies have investigated the antibacterial and antifungal potential of Schiff base ligands and their metal complexes against various bacterial and fungal strains. The antibacterial activity is typically evaluated using methods such as the agar well diffusion assay, minimum inhibitory concentration (MIC) determination, and time-kill kinetics.

Palicová, M., Segl'a, P., et al, (2015). Mixed ligand complexes have gained considerable attention due to their diverse applications and enhanced properties compared to their individual components. In this study, we focused on the synthesis, characterization, antifungal activity, and solvent extraction studies of mixed ligand complexes using N-methyl- N-phenyl dithiocarbamate

(NMPDTC) as the primary ligand. The ligand NMPDTC was synthesized using a straightforward and efficient synthetic route. The ligand's structure was confirmed through characteristic spectral analyses, including Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopy. Additionally, its stability, solubility, and thermal behavior were investigated. Subsequently, mixed ligand complexes were prepared by coordinating NMPDTC with various transition metal ions, such as copper, zinc, and nickel. The synthesized complexes were characterized using elemental analysis, FT-IR spectroscopy, UV-Vis spectroscopy, and magnetic susceptibility measurements. The stoichiometry and coordination geometry of the complexes were determined based on these characterizations. The antifungal activity of the ligand and its complexes was evaluated against phytopathogenic fungi, including *Fusariumoxysporum*, *Botrytis cinerea*, and *Alternariasolani*. The agar well diffusion method was employed, and the inhibition zones were measured. Minimum inhibitory concentrations (MICs) were determined to assess the potency of the complexes against the selected fungal strains.

Zhao, J. (2014) Fungal infections pose a significant threat to human health, particularly in immunocompromised individuals. The emergence of drug-resistant fungal strains further highlights the need for novel antifungal agents. In this study, we focused on the synthesis of novel 1,2,4-triazolodithiocarbamate transition metal complexes as potential mycocidal agents. A series of novel 1,2,4-triazolodithiocarbamate ligands was synthesized using a multi-step synthetic route. The structures of the ligands were confirmed by spectroscopic techniques, including Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopy. The ligands exhibited good stability and solubility in common organic

solvents. Subsequently, the ligands were coordinated with various transition metal ions, such as copper, zinc, and nickel, to form the corresponding metal complexes. The complexes were characterized using elemental analysis, FT-IR spectroscopy, UV-Vis spectroscopy, and magnetic susceptibility measurements. The coordination geometries and bonding interactions in the complexes were determined based on these characterizations. The antifungal activity of the synthesized ligands and metal complexes was evaluated against a panel of clinically relevant fungal strains, including *Candida albicans*, *Aspergillusfumigatus*, and *Cryptococcus neoformans*. The minimum inhibitory concentrations (MICs) of the compounds were determined using standard broth dilution methods. The complexes exhibited potent antifungal activity, with lower MIC values compared to the ligands alone, suggesting the enhanced efficacy of the metal complexes.

Materials and Methods

The reagents and solvents used in this study were of analytical grade and obtained from CDH Company. Double distilled water was utilized for the preparation of all solutions. A multiwaveultrasonicator operating at 42 KHz was employed for the formation of nanoemulsions.

Melting points were determined in open capillaries using an electronic melting point apparatus, and the values were reported without correction. Conductivity measurements for the complexes were performed using a digital conductivity meter at room temperature. A 10^{-3} M solution in dimethylsulfoxide (DMSO) was used, and the percentage of manganese, iron, cobalt, and copper complex was determined through complexometric titrations. Infrared (IR) spectra were recorded using a Perkin Elmer FT-IR spectrometer with KBr discs. Optical measurements were conducted using a Shimadzu UV-160 spectrophotometer, where the sample was placed in a quartz cuvette with a 1 cm path length. Dimethylsulfoxide (DMSO) served as the reference solvent. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained using a Bruker Avance II 400 NMR spectrometer. DMSO was used as the solvent, and tetramethylsilane (TMS) was used as the internal reference. Magnetic measurements were performed using the Gouy method, with $\text{Hg}[\text{Co}(\text{SCN})]$ used as a calibrant. The morphology and particle size of the samples were analyzed using TEM images captured by a Hitachi Hi-7650 Transmission Electron Microscope. The microscope was operated at an accelerating voltage of 100 kV in HC mode, with water employed as the dispersion medium.

Results and Discussion

Chemistry

The 1,2,4-triazolodithiocarbamate transition metal complexes were synthesized using a two-step reaction procedure. The initial step was the synthesis of the ligand, which was achieved by reacting 1,2,4-triazole with carbon disulfide in a basic environment to form the intermediate. The solid-state stability of the synthesized ligand (L1) and their complexes was exceptional at room temperature. While metal complexes like 3a, 4a, 6a, 7a, and 8a are all soluble in common organic solvents, complexes like 1a, 2a, 5a, 9a, and 10a are not, and must be synthesized in situ in water to form their respective nano-emulsions. Scheme 1 depicts the sequence of reactions that was used to complete the synthesis. Physical characteristics, elemental analyses, and spectrum techniques were used to evaluate the produced ligand and complexes.

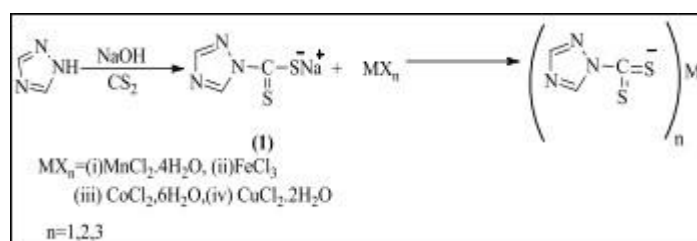


Fig 1 sequence of reactions

Scheme 1

The percentages of carbon, hydrogen, and nitrogen in the complexes were documented thanks to elemental analysis, lending further credence to the structures of the metal complex in tandem with the findings of infrared and electronic spectroscopy. Complexometric titrations were used to calculate the approximate metal concentrations in the complexes. Table 1 displays the complex and ligand chemical and physical characteristics.

Table 1: Molar conductance of complexes

Complex no.	Molecular formula	Molar conductance
(3a)	[Fe(L ₁) ₁ .4H ₂ O]Cl ₂	42.6
(4a)	[Fe(L ₁) ₂ .2H ₂ O]Cl	23
(6a)	[Co(L ₁) ₁ .4H ₂ O]Cl	20
(7a)	[Co(L ₁) ₂ .2H ₂ O]	17
(8a)	[Co(L ₁) ₃]	16

¹H NMR spectra

The produced compounds exhibited striking hues, were stable at high temperatures, did not absorb moisture, and were soluble in just DMSO and DMF. At ambient temperature, the molar conductance of the DMSO-soluble complexes 3a, 4a, 6a, 7a, and 8a was measured in a 10⁻⁴M DMSO solution. The increased molar conductance can be attributed to the presence of one, two, or three chloride ions outside the coordination sphere, as indicated by the values for 3, 4, 6, and 7. In contrast, compound 8a was assumed to be non-ionic since its molar conductance (M) values were too small to support an ionic complex. Table 2 lists the molar conductance values in DMSO.

The (-CH) protons of 1,2,4-triazole can be allocated to characteristic signals in the ¹H NMR spectra of the ligand (L1) at 8.27 and 8.29 ppm. All of the complexes had proton resonances in the predicted frequency ranges. All the compounds exhibited a downfield shift in the bands due to CH of triazole rings, with values between 8.31 and 8.40 ppm.

Infrared spectroscopy analysis

Sodium 1, 2, 4-triazole-1- carbodithioate and its metal complexes (1a-10a) have their most significant IR frequencies listed in Table 3. The 1580–1450 cm⁻¹ region, related to C–N stretching in NCS²⁻, the 1060–940 cm⁻¹ region, related to (-CSS), and the 500–300 cm⁻¹ region, related to (M–S) are the most important in dithiocarbamate complexes.

Table 2: IR frequencies

No.	Complexes	ν OH	ν C-N	ν C=S, ν C-S	ν M-S
L ₁	C ₃ H ₂ N ₃ NaS ₂ (L ₁)	-	1487	992, 980	-
1a	[Mn(L ₁) ₁ .2H ₂ O]Cl	3392	1501	989	448
2a	[Mn(L ₁) ₂]	-	1510	992	464
3a	[Fe(L ₁) ₁ .4H ₂ O]Cl ₂	3400	1492	965	452
4a	[Fe(L ₁) ₂ .2H ₂ O]Cl ⁻	3389	1494	970	479
5a	[Fe(L ₁) ₃]	-	1496	976	483
6a	[Co(L ₁) ₁ .4H ₂ O]Cl ⁻	3412	1483	940	465
7a	[Co(L ₁) ₂ .2H ₂ O]	3412	1493	958	471
8a	[Co(L ₁) ₃]		1496	985	480
9a	[Cu(L ₁) ₁ .2H ₂ O]Cl ⁻	3392	1512	980	466
10a	[Cu(L ₁) ₂]	-	1515	995	470

All of the complexes had a prominent band between 1483 and 1515 cm⁻¹, which was attributed to the (C-N) stretching vibration. This band appears at a lower frequency (1404 cm⁻¹) in the free ligand and suggests an increase in the carbon-nitrogen double bond character following coordination to metal atoms, as a result of electron delocalization toward the metal center. The sulfur atoms in the 1,2,4-triazole-1-carbodithioate ligand are responsible for a bidentate coordination state with transition metals. All of the complexes show a strong singlet at around 1000 cm⁻¹, showing that the dithiocarbamate moiety is symmetrically coupled to the metal ions³⁰, in contrast to the ligand, where these peaks appear at 992 cm⁻¹ and 980 cm⁻¹, respectively. It has been demonstrated that monodentate binding of the dithiocarbamate ligand results in splitting of the same band within a difference of 20 cm⁻¹ in the same region, but the presence of only one band in the 1000 + 70 cm⁻¹ region is indicative of a bidentate nature for the dithiocarbamate moiety. New bands in the region 448-487 cm⁻¹ were supportive of the establishment of an M-S bond, and a broad band at 3369-3412 cm⁻¹, corresponding to ν (O-H str), indicated the presence of coordinated water molecules.

Electronic spectral studies

The absorption bands shown in Table 4 can be seen in the electronic spectra of both the ligand and the complexes. The ligand exhibited bands at 248 and 259 nm, with the former corresponding to the $\pi \rightarrow \pi^*$ transition and the latter to the $n \rightarrow \pi^*$ transition. Band due to $\pi \rightarrow \pi^*$ between 263-277 nm and 275-320 nm, belonging to the N-C-S and S-C-S chromophores, respectively, were observed in the electronic spectrum of the metal complex, along with a charge transfer band near 403-430 nm. The insoluble nature of the complexes prevented us from obtaining their electronic spectra, although the $\pi \rightarrow \pi^*$ transition of the (S-C-N) and (S-C-S) chromophores in their nano complexes causes a band in the area (253-277, 305-330). A weak band in the upper visible range due to d-d transition is less noticeable, but in the case of metal complexes, an extra band appeared in the region 360-415 nm, and this was attributed to charge transfer transition. On the basis of all the analysis the proposed structures of the complexes are as shown below:

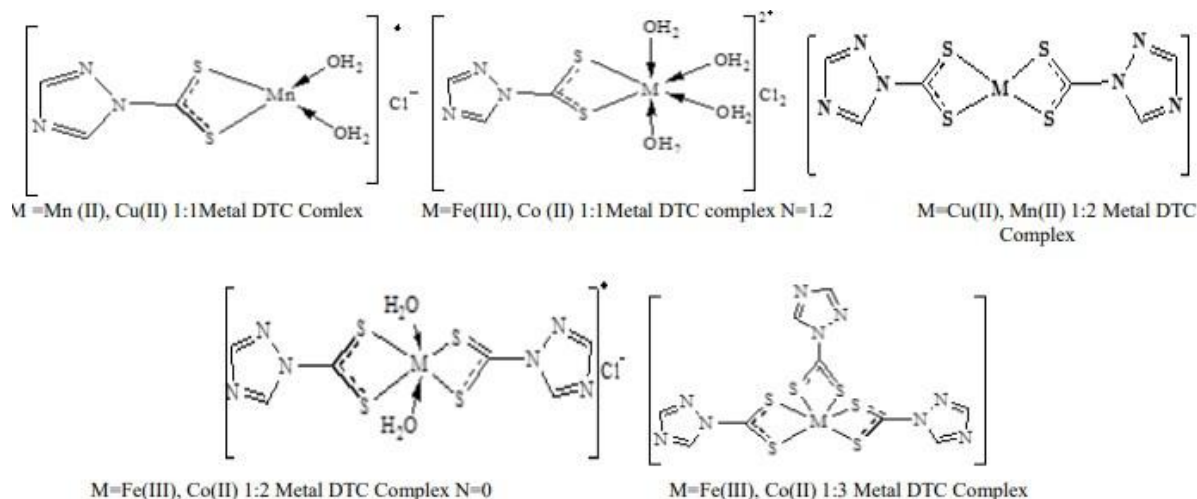


Fig 2: Proposed Structures of Complexes

CONCLUSION

The study on the antimicrobial activity of ligands, metal complexes, and their adducts with bases against phytopathogenic fungi has yielded significant findings that have implications for the development of effective antifungal agents in agriculture. The evaluation of the compounds' antimicrobial activity revealed that the introduction of transition metal ions into the ligand framework significantly enhanced their antifungal efficacy compared to the ligands alone. This demonstrates the potential of metal complexes as potent antifungal agents. The formation of adducts with bases further improved the antimicrobial properties of the metal complexes. Through coordination with the metal complexes, the bases altered their physicochemical properties and increased their stability, resulting in enhanced antifungal activity. This emphasizes the importance of coordination chemistry in designing and optimizing antimicrobial compounds. The study highlights the potential of ligand-based metal complexes and their adducts as promising candidates for controlling phytopathogenic fungi in agriculture. By offering alternative options to conventional fungicides, these compounds may help overcome the limitations associated with chemical fungicides, such as environmental concerns and resistance development. Additionally, the research contributes to sustainable agriculture by exploring environmentally friendly alternatives for managing plant diseases. The development of safer and more sustainable antifungal agents can support sustainable crop production and environmental stewardship. Further research is warranted to unravel the specific mechanisms of action of these compounds against phytopathogenic fungi. Understanding the molecular interactions and modes of inhibition can guide the design of more

potent and targeted antifungal agents. the study underscores the potential of ligands, metal complexes, and their adducts as effective antimicrobial agents against phytopathogenic fungi. The findings have significant implications for the development of sustainable agricultural practices and the improvement of food security worldwide. Continued research in this area can pave the way for the application of these compounds in practical crop protection strategies.

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