four fungi [*A. niger, A. flavus, Alternaria alternata, Rhizopus stolonifer*].

# Experimental

## Chemicals and physical measurements

All chemicals and reagents used in this study were of Analar grade (BDH/Aldrich). They include; 2-hydroxyacetophenone, L-tyrosine, 4-dimethylaminobenzaldehyde, 2,4-dinitrophenylhydrazine, $CoCl_2.6H_2O$, NiCl2.6H2O, $CuCl_2.2H_2O$, ZnCl2, $FeCl_3.6H_2O$, absolute ethanol and Dimethylformamide (DMF). The (CHN) analyses were done by using 2400 elemental analyzer. The molar conductance measurements were performed on a BC 3020 Professional Benchtop Conductivity Meter, Benghazi University. Magnetic susceptibility was determined using a Johnson Matthey instrument at room temperature
(25°C) with $Hg[Co(SCN)_4]$ as blank. The infrared spectra were recorded as KBr disc on a Perkin-Elmer 1430 IR Spectrophotometer. The nuclear magnetic resonance spectra of the Schiff bases (HL1 and HL2) and their Zn(II) mixed ligand complex were recorded on Varian Gemini 200-200MHz spectrometer using TMS as internal standard and d6-DMSO as a solvent The electronic spectra were recorded on a Unicam Model UV-2 spectrophotometer. The mass spectra were carried out by using Shimadzu QP-2010 Plus. The EPR spectra were recorded by using EMX ESR spectrometer (Bruker) 1998 Y. All the mentioned analyses except molar conductivity were done at Micro analytical center, Cairo University, Giza, Egypt [2].

## Synthesis of Schiff bases and their mixed ligand complexes

The Schiff bases of (HL1) Scheme 1, and (L2) Scheme 2 and their mi

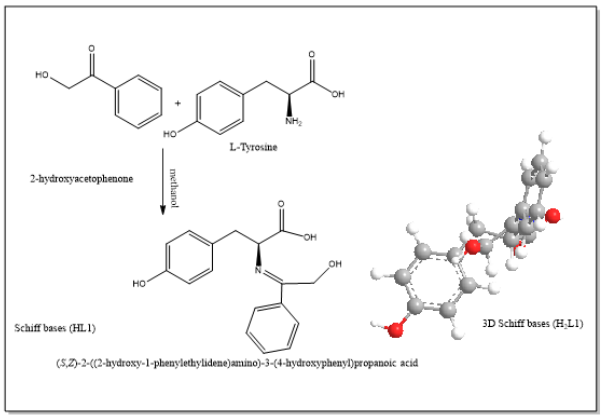


**Figure1:** Structural of schiff base (HL1).

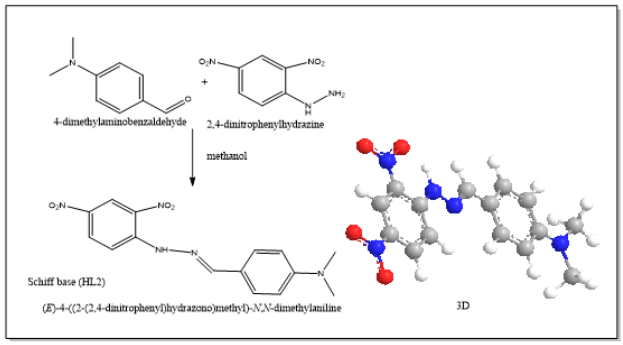


**Figure2:** Schiff base (HL2).

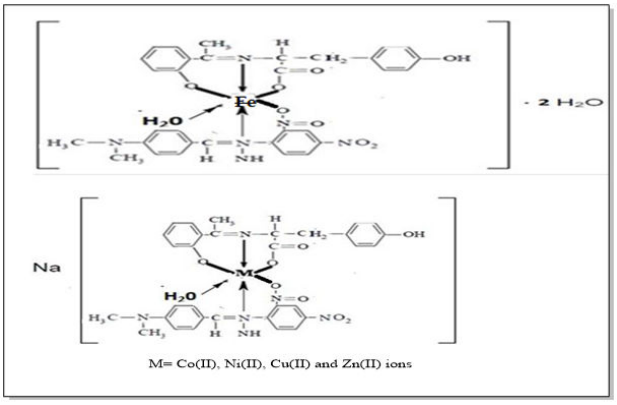


**Figure3:** Structure of mixed ligand complexes.

**Fungal species:** Four test organisms, *Aspergillus niger, Aspergillus flavus, Alternaria alternate* and *Rhizopus stolonifer*. Were collected from the Laboratory of Applied Microbiology, University of Omar AL-Mukhtar, Libya. They were cultured in Petri plates containing Potato dextrose agar (PDA) media and incubated at 27°C for three days with periodic sub-culturing at 4°C.

## Screening for antifungal assay

**Antifungal activity test:** The antifungal activity of the all compounds (ligands, metal salts and their complexes (were evaluated by the agar well diffusion method. All fungi were sub-cultured and prepared for the assessment of ligands and their complexes activity. The compounds were dissolved in DMF solution. The (PDA )medium was poured in to the sterile petri plates and allowed to solidify. The inoculum used was prepared using the fungal species from a 72-hour culture on (PDA).The fungal suspension of each test fungi was evenly spread over the media by sterile cotton swabs [3]. The plates have been kept to dry and a sterile cork borer (7 mm in diameter) were then used to punch wells in the agar medium. Subsequently, wells were filled with 20 µl of each compounds at various concentration of (25, 50 and 100) mg/mL and allowed to diffuse at room temperature for 15 min. The plates were incubated at 27°C for 48-72 hrs. After the incubation the plates were observed for formation of clear inhibition zone around the well indicated the presence of antifungal activity evaluated by measuring the diameter of the inhibition zone around the whole (mm) as below:

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | DMF(Control) | - | - | - | - | - | - | - | - | - | - | - | - |

**Table1:** Antifungal activity for the metal salts, Schiff bases and mixed ligand complexesagainst some pathogenic fungi.

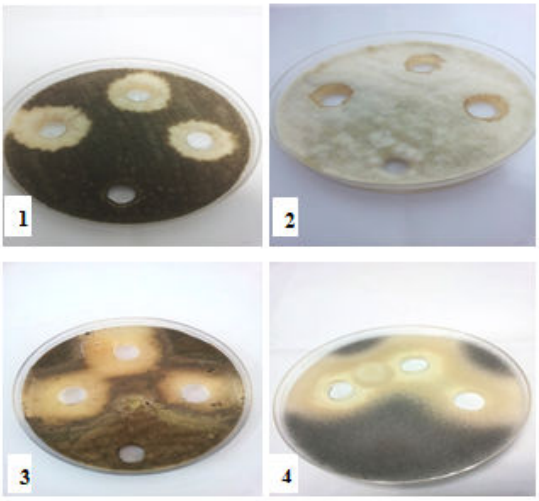


**Figure4:** Effect of $CoCl_2.6H_2O$ on 1- *Aspergillus niger* , 2- *Aspergillus flavus*, 3- *Alternaria alternata* and 4- *Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentrations, respectively (from right to left) compared with control.

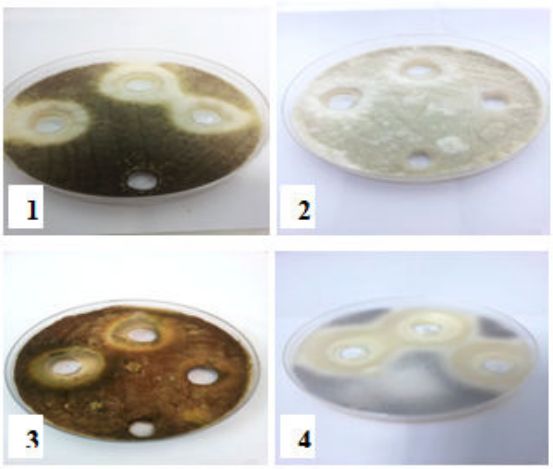


**Figure5:** Effect of $NiCl_2.6H_2O$ on 1- *Aspergillus niger*, 2- *Aspergillus flavus*, 3- *Alternaria alternate* and 4- *Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentrations, respectively (from right to left) compared with control.

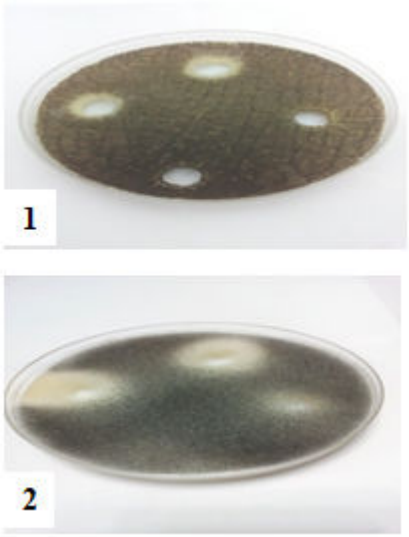


**Figure6:** Effect of $CuCl_2.2H_2O$ on 1- *Aspergillus niger* and 2- *Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentrations, respectively (from right to left) compared with control.

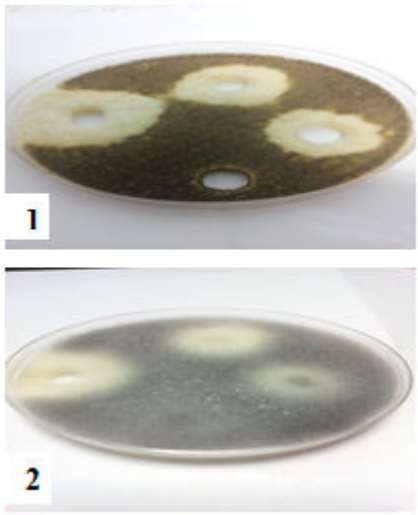


**Figure7:** Effect of $ZnCl_2$ on 1- *Aspergillus niger* and 2- *Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentrations, respectively (from right to left) compared with control.

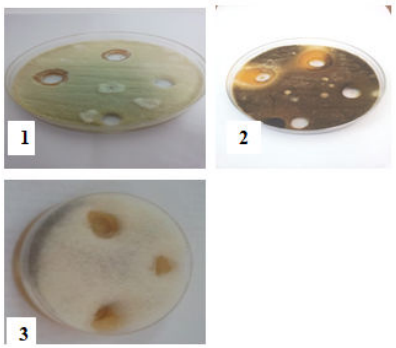


**Figure8:** Effect of $FeCl_3.6H_2O$ on 1- *Aspergillus flavus*, 2- *Alternaria alternate* and 3-*Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentrations, respectively (from right to left) compared with control.

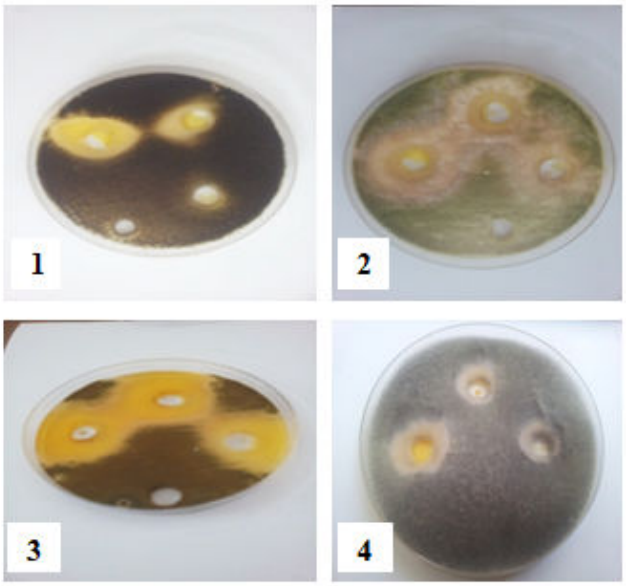


**Figure9:** Effect of $C_{18}H_{21}NO_5$ (HL1) on 1- *Aspergillus niger*, 2- *Aspergillus flavus*, 3- *Alternaria alternata* and 4- *Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentrations, respectively (from right to left) compared with control.

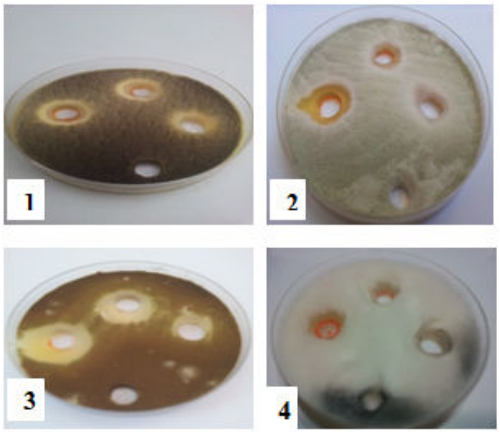


**Figure10:** Effect of $(Fe(L1)(L4)H_2O).2H_2O$ on 1- *Aspergillus niger*, 2- *Aspergillus flavus* , 3- *Alternaria alternata* and 4- *Rhizopus stolonifer* at 25, 50 and 100 mg/ml concentration, respectively (from right to left) compared with control.

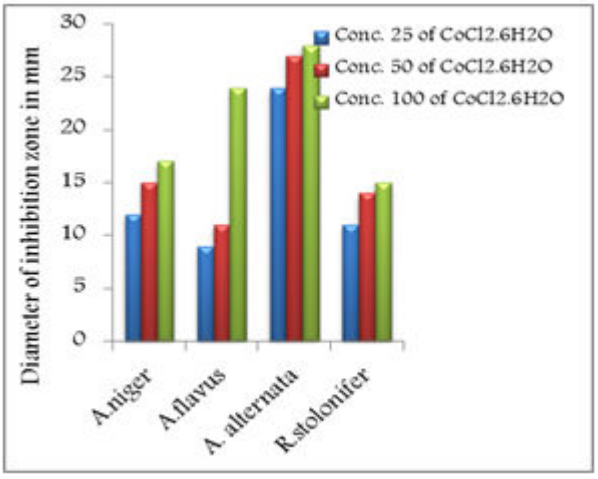


**Figure11:** Effect of $CoCl_2.6H_2O$ on different types of fungi.

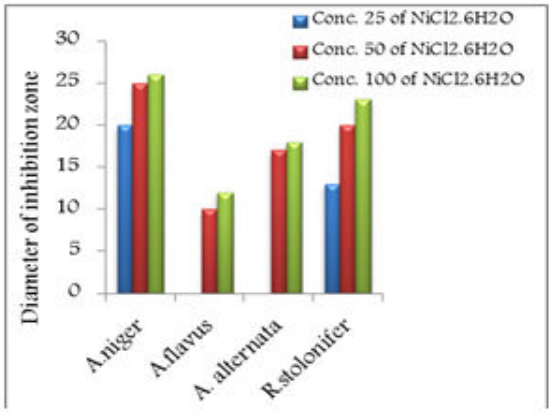


**Figure12:** Effect of $NiCl_2.6H_2O$ on different types of fungi.

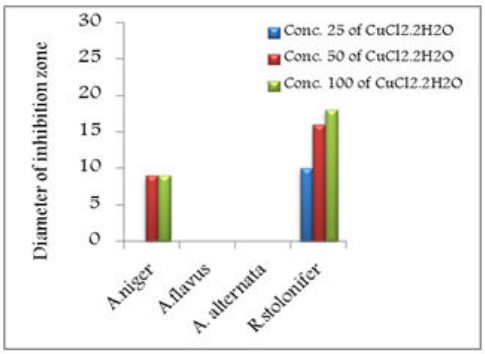


**Figure 13:** Effect of $CuCl_2.6H_2O$ on two types of fungi.

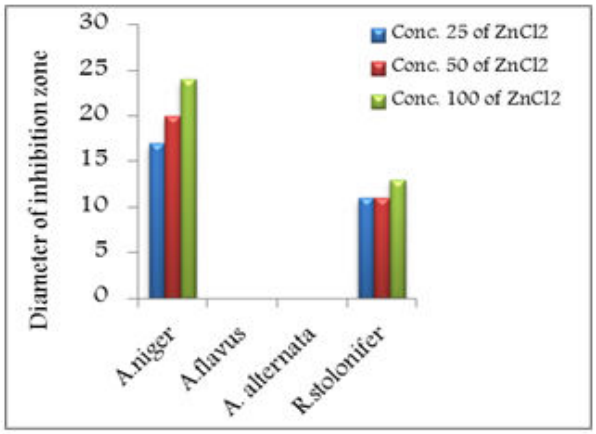


**Figure 14:** Effect of $ZnCl_2$ on two types of fungi.

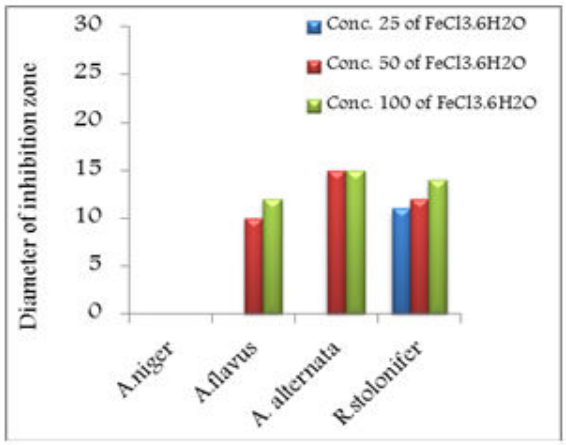


**Figure 15:** Effect of $FeCl_3.6H_2O$ on three types of fungi.

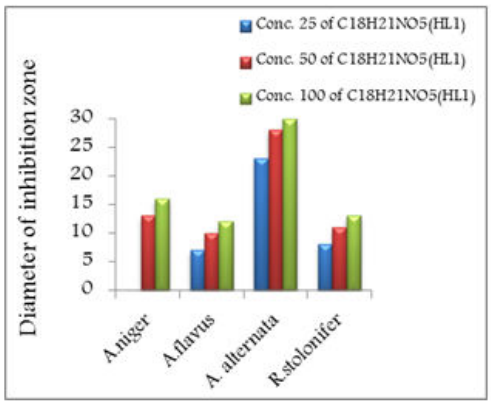


**Figure 16:** Effect of $C_{18}H_{21}NO_5$ (HL1) on different types of fungi.

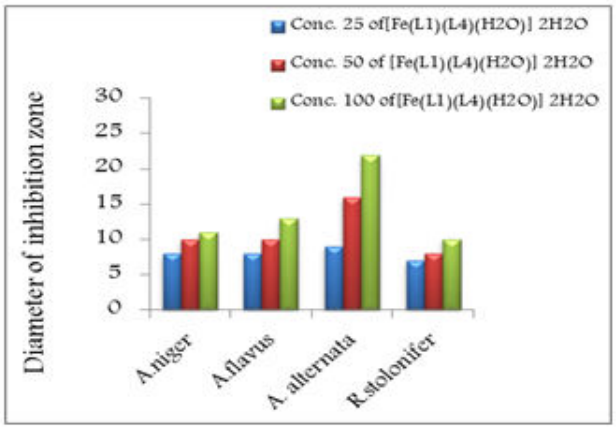


**Figure 17:** Effect of $[Fe(L1)(L4)(H_2O)])2H_2O$ on different types of fungi.

## Conclusion

The mixed ligand complexes of Co(II), Ni(II), Cu(II) Zn(II) and Fe(III) ions with Schiff bases formed the condensation of [2-hydroxyacetophenone and L-Tyrosine] (HL1) as primary ligand and [4-dimethylaminobenzaldehyde with 2,4-dinitrophenylhydrazine] as secondary ligand (HL2) were electrolytes types in nature, whereas, Fe(III) mixed ligand complex with the same Schiff bases revealed to be nonelectrolyte in nature.

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