CHAPTER – 5

Synthesis, characterization and antifungal activities of 3d-transition metal complexes of 1-acetylpiperazinyldithiocarbamate, M(acpdtc)2
I. INTRODUCTION

The coordination chemistry of sulfur-containing systems has been growing at a rapid pace due to their close resemblance with biomolecules like amino acids (e.g. methionine, cysteine), peptides such as glutathione, proteins, enzymes and vitamins. Among the various organosulfur systems, dithiocarbamates owe special significance due to their wide biological, industrial, agricultural and chemical applications. The versatility of dithiocarbamato ligands may be attributed to their small bite angle, leading to the stabilization of a wide range of oxidation states of transition metal and group(IV) elements. The complexing ability of dithiocarbamato ligands stems from the presence of the potential sulfur donors which can delocalize positive charge from the metal towards the periphery of the complex. Dithiocarbamato ligands are found to complex almost all the transition metal as well as the main group elements. These dithiocarbamato complexes have been employed as fungicides, pesticides, herbicides, antifouling agents, lubricants, vulcanizers, NO-trapping agents, as froth flotation and transition metal extraction agents. Recently, dithiocarbamates have also been used as structural motifs in the supramolecular chemistry due to their robust complexing ability. A remarkable feature of dithiocarbamato ligands is the ease of their preparation; by the reaction of carbon disulfide with primary or secondary amine in presence of a strong base. However, it has been found that the dithiocarbamates obtained from primary amine are less stable than their secondary amine analogs since the former are susceptible to elimination reactions yielding isothiocyanates.

Piperazine, a cyclic diamine, offers itself as useful motif for the generation of sensor based polyazamacrocyclic complexes because of its electron releasing and molecular recognition properties.
Structurally, piperazine has the ability to exist in its two well studied chair as well as boat form. However, the former is more stable as the later conformer gives rise to a strained five-membered chelating ring\(^{15}\). Another notable feature of piperazine is its broad biological relevance for instance piperazine derivatives are used as dopamine reuptake inhibitors for treating the depression and Parkinson disease\(^{16}\), as antimalarial\(^{17}\), antimicrobial, analgesic and antipyretic agent\(^{18}\). One of the early medicinal uses of piperazine was to irradiate the worms both in human as well as animal subjects. The major advantage of using this diamine is that the excess will be excreted through the urine\(^{19}\).

Hence due to the wide spectrum of biological properties of both dithiocarbamates and piperazine derivatives we thought it worth interesting to study the dithiocarbamato complexes of 1-acetylpiperazine. As piperazine can exist both in chair as well as boat form so this study also aims to explore the conformation of piperazine together with the bonding mode of the dithiocarbamato group. Keeping in mind with the vast biological activity of piperazine and its derivatives, this paper also reports the antimicrobial activity of potassium 1-acetyldithiopiperazine and its 3d-transition metal complexes.

II. EXPERIMENTAL

Hydrated metal chlorides (Merck), 1-acetylpiperazine (Sigma-Aldrich), carbon disulfide, potassium hydroxide (Merck, Mumbai) were used as received. Methanol was distilled prior to use. Elemental analyses (C, H, N and S) were carried out with a Elementar Vario EL III Carlo Erba 1108 analyzer. The metal contents were estimated by complexometric titration\(^20\). IR spectra (4000-400 cm\(^{-1}\)) were recorded on a Perkin Elmer RXI FT-IR spectrometer as KBr disc. The electronic spectra were recorded on a Cintra 5GBC spectrophotometer in DMSO.
The $^1$H-NMR spectra were recorded on a Bruker DRX-300 spectrometer in DMSO-$d_6$ at room temperature. The conductivity measurements were carried out on a CM-82T Elico conductivity bridge in DMSO. Magnetic susceptibility measurements were done with a 155 Allied Research vibrating sample magnetometer at room temperature. TGA was performed with a Perkin-Elmer (Pyris Diamond) thermal analyzer under nitrogen atmosphere using alumina powder as reference. The weight of the sample was between 8 to 12 mg and the heating rate was maintained at 10 °C/min. The melting point of the ligand and its complexes were recorded using Reichert thermovar, Austria using an open capillary method.

A. **Synthesis of Potassium 1-acetylpiperazinyldithiocarbamate, K(acpdte)**

To a 25 ml methanolic solution of 1-acetylpiperazine (20.0 mmol, 2.3 g) was added neat carbon disulfide (20.0 mmol, 1.2 ml) dropwise with continuous stirring maintained at 0°C. To this reaction mixture, KOH (20.0 mmol, 0.8 gm) dissolved in minimum amount of aqueous methanol (10 ml) was added and stirred overnight. The white compound formed was filtered and thoroughly washed with methanol and diethyl ether, and was desiccated over calcium carbonate.

B. **Synthesis of Metal Complexes M(acpdte)$_2$ where M = Mn(II), Fe(II), Co(II), Ni(II) and Cu(II)**

The transition metal complexes, M(acpdte)$_2$ were conveniently obtained in high yields by the addition of methanolic solution (25 ml) of hydrated metal chloride MCl$_2$·nH$_2$O (5 mmol) and K(acpdte)(10 mmol ) in the same solvent (15 ml). The desired metal complexes were precipitated immediately. However, the stirring was continued additionally for 6 hours in order to ascertain complete precipitation. The precipitate was filtered, washed successively with
methanol and diethyl ether. The purity of the resulting complexes was checked using silica gel TLC.

C. Procedure for Antifungal Activity

The *in vitro* antifungal activity of the free ligand, K(acpdtc) and its transition metal complexes were tested against *Fusarium sp.* and *Sclerotina sp.* by agar well diffusion method by the following procedure:

*Sabourad dextrose agar plates:* A homogeneous mixture of glucose-peptone-agar (40:10:15) was sterilized by autoclaving at 121 °C and 15 lb/cm² for 20 min. The sterilized solution (25 ml) was poured in each sterilized petridish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plate. These plates were inverted and kept at 30 °C in incubator to remove the moisture and to check for any contamination.

*Antifungal assay:* Fungal strain was grown in 5 ml sabourad dextrose broth (Glucose: Peptone; 40:10) for 3-4 days to achieve $10^5$ CFU/ml cells. The fungal culture (0.1 ml) was spread out uniformly on the sabourad dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5-10 min so that culture is properly adsorbed on the surface of sabourad dextrose agar plates. Now small wells of size (4 mm × 2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8 % soft agar to prevent the flow of test sample at the bottom of the well. The test solutions 50 µl, 100µl, 200 µl, 400 µl of stock solution (10 mg/ml) were loaded into the wells of the plates. DMF was also loaded as control. The plates were kept for incubation at 30 °C for 3-4 days and then the plates were examined for the formation of zone of inhibition.
III. RESULTS AND DISCUSSION

The transition metal complexes were readily obtained by the substitution reaction of metal halide with bidentate ligand, K(acpdtc) in 1:2 molar ratio (Fig. 5.1 and 5.2) forming complexes of the type M(acpdtc)$_2$ as shown below:

$$\text{MCl}_2 \cdot \text{nH}_2\text{O} + 2 \text{K(acpdtc)} \rightarrow \text{M(acpdtc)}_2 + 2 \text{KCl}$$

where M = Mn(II), Fe(II), Co(II), Ni(II), Cu(II), acpdtc = 1-acetylpiperazinyl- dithiocarbamate. Microanalytical data (CHNS), FT-IR, UV-visible spectroscopy, $^1$HNMR, and TGA/DSC were used to characterize the complexes and are consistent with the proposed formulation (Table 5.1). The room temperature conductivity measurement ($10^{-3}$ M) in DMSO indicated them to be non-electrolytes$^{22}$. All the complexes are non-hygroscopic and stable in air, but are insoluble in common organic solvents and hence there crystal structures were not determined.

A. IR Spectra

The prominent IR bands are listed in Table 5.2. The infrared spectrum of dithiocarbamato complexes consists of two types of bands which are of direct structural significance. The first lies in the region 950–1050 cm$^{-1}$, which is characteristic of the nature of binding of the dithiocarbamato moiety$^{23}$ while the second lies in between 1450-1600 cm$^{-1}$ and is termed as thioureide band$^{24}$. This thioureide band may be considered as an intermediate in between v(C-N) and v(C=N) and its position predicts the shift of electron density towards the coordinating metal ion. According to the criterion laid down by Ugo and Bonati, the presence of a solitary band in the 950–1050 cm$^{-1}$ region is due to symmetrical bidentate coordination of the dithiocarbamato group while the splitting of this band within a narrow range of 20 cm$^{-1}$ is due to the unsymmetrical monodentate nature of the dithiocarbamato group$^{25}$. In
the present study we have observed a single sharp band at 994 ± 10 cm⁻¹ implying the symmetrical bidentate coordination of the 1-acetylpiperazinyldithiocarbamate leading to the formation of two four membered rings on either side of the metal atom as shown below:

\[
\begin{array}{c}
\text{H} \\
\text{C} \\
\text{N} \quad \text{N} \\
\text{C} \quad \text{S} \quad \text{M} \\
\text{S} \\
\text{S} \\
\text{C} \quad \text{N} \quad \text{N} \\
\text{O} \\
\end{array}
\]

Similarly, the thioureide band allows us to predict the shift in electron cloud of NCSS bond upon coordination with the positive metal center. In the present study, broad NCSS band was observed at 1454 cm⁻¹ in case of ligand and was found to be shifted appreciably (~30 cm⁻¹) in case of metal complexes owing to the increase in double bond character²⁶ of the NCSS bond (Table 5.2). This effect may be accounted due to the electron delocalization toward the metal center. The metal coordination is further ascertained by the presence of new medium to weak intensity bands in the far-IR region which may be assigned to \(\nu(M-S)\)²⁷. Hence, the band observed in the range 478-519 cm⁻¹ may be assigned to \(\nu(M-S)\) which is in accord with the observations made by other authors²⁸.

Another interesting feature is the appearance of a strong band at 1660 cm⁻¹ in case ligand, K(acpdtc) corresponding to the carbonyl group, and remains unaltered in complexes implying the non-involvement of carbonyl group in coordination²⁹. Similarly, a slight variation is observed in the characteristic skeleton vibrational and deformation modes of the ligand as compared to its complexes which may be attributed to a distortion of the chair conformation upon coordination³⁰.
B. \textit{\textsuperscript{1}H-NMR spectra}

The \textit{\textsuperscript{1}H-NMR} spectra of the ligand exhibits a triplet at $\delta = 2.34$ ppm due to the methylene protons of the piperazine ring. This peak was found to be shifted slightly downfield ($\delta = 2.55-2.62$ ppm) in case of complexes implying the shift of electron cloud towards the positive metal center\textsuperscript{31}. This effect is similar to that found in the IR spectra, where a blue shift was observed in the thioureide band. However, a singlet at 7.81 ppm was observed for the hydroxyl proton, remains unaltered on going from free to complexed state implying its non-involvement in coordination.

C. \textbf{Electronic Spectra and Magnetic Moments}

The free ligand, \textit{K(acpdtc)$_2$} exhibits only two intense bands at 36800 and 39480 cm$^{-1}$ which may be attributed to the intraligand charge transfer of the \textit{R$_2$NCS$_2$} group\textsuperscript{32}. The pale yellow solution of \textit{Mn(acpdtc)$_2$} complex in DMSO exhibits strong charge transfer bands at 32884 and 30750 cm$^{-1}$ characteristic of tetrahedral Mn(II) ion besides an additional band at 19230 cm$^{-1}$ assigned to $^{4}T_1 \leftarrow ^{6}A_1$ transition. The magnetic moment (5.44 B.M.) also supports a tetrahedral geometry for the Mn(II) ion\textsuperscript{33}.

The \textit{Fe(acpdtc)$_2$} complex shows only one spin allowed d-d band at 22880 cm$^{-1}$ corresponding to $^5E \leftarrow ^5T_2$ transition in addition to charge transfer band at 32880 cm$^{-1}$. Generally the magnetic moment for tetrahedral Fe(II) complexes lies between 5.0-5.5 B.M. corresponding to four unpaired electrons and we have obtained a value of 5.38 B.M. which is consistent with the tetrahedral nature of Fe(II) ion\textsuperscript{34}.

For the \textit{Co(acpdtc)$_2$} complex we have observed two bands at 14500 and 21470 cm$^{-1}$ corresponding to $^4T_2$(F) $\leftarrow ^4A_2$(F) and $^4T_1$(F) $\leftarrow ^4A_2$(F) transitions, respectively, in addition to a charge transfer at
28500 cm\(^{-1}\). Although three spin-allowed electronic transitions are expected to occur for tetrahedral Co(II), but the visible spectrum is actually dominated by the highest energy transition, \(^4T_1(F) \rightarrow \(^4A_2(F)\). This visible transition actually envelopes a large number of transitions originating from doublet excited states occurring in the same region. These transitions acquire some intensity by means of spin–orbit coupling which is one of the interesting feature of Co(II) tetrahedral complexes. Generally, the magnetic moment of tetrahedral, Co(II) complexes lies in the range 4.2 to 4.7 B.M. while for octahedral Co(II) it falls between 4.4 to 5.5 B.M. The observed magnetic moment (4.38 B.M.) and the ligand field spectrum suggest a tetrahedral geometry for Co(II) ion\(^{35}\).

In case of Ni(acpdtc)\(_2\) complex two high intensity d-d transitions are observed at 16503 and 21990 cm\(^{-1}\) corresponding to the \(^1A_2g \rightarrow ^1A_{1g}\) and \(^1B_{1g} \rightarrow ^1A_{1g}\) transitions, respectively supporting a square-planar structure\(^{36}\). The possibility of square-planar geometry was further strengthened by a 0 magnetic moment obtained in the present case.

The greenish Cu(II) complex shows two d-d absorption bands at 20410 and 14614 cm\(^{-1}\) corresponding to \(^2A_{1g} \rightarrow ^2B_{1g}\) and \(^2E_g \rightarrow ^2B_{1g}\) transitions, respectively and a charge transfer band at 22500cm\(^{-1}\). The absence of bands below 10000 cm\(^{-1}\) discards the possibility of any tetrahedral or pseudo-tetrahedral geometry around the Cu(II) ion\(^{37}\). Moreover, it is well known that for square-planar Cu(II) complexes the magnetic moment value falls in 1.82-1.86 B.M. while for tetrahedral Cu(II) ion, the \(\mu_{\text{eff}}\) is slightly higher (1.92-2.00 B.M.). Hence, the magnetic moment value obtained in the present case, 1.71 B.M is quite typical of mononuclear Cu(II) compounds with a \(S = 1/2\) spin state\(^{38}\).
D. Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)

A substantial amount of studies have been reported on the thermal behavior of transition as well as group(IV) element complexes. Generally, the metal dithiocarbamates obtained from aliphatic amines decompose to respective metal sulfides involving metal thiocyanate as the main intermediate whilst those generated from cyclic amines decomposes in a direct manner without involving the thiocyanate intermediate\(^{39}\). In the present study, the ligand, potassium 1-acetylpiperazinyldithiocarbamate and its transition metal complexes were heated under an inert atmosphere in the temperature ranging from 50 to 650 °C. The ligand was found to decompose in two distinct stages while the metal complexes exhibit three concurrent stages. The thermolytic cleavage of the ligand starts from 95 °C and ends at 110 °C with 13.01 % loss of weight corresponding to the evolution of carbon monoxide gas (Table 5.4). Such a behavior is quite expected as carbonyl group lies on the periphery of the molecule (Fig. 5.1). The second pyrolytic stage ranges in between 115 to 250 °C after which a straight line is observed. The whole organic moiety decomposes in this step and no residue is found in the crucible. This behavior is similar to that reported for the decomposition of ammonium pyrrolidinedithiocarbamate\(^{40}\). The DSC profile of the ligand also shows two well defined endotherms implying that the evolution of CO and decomposition of the organic moiety are accompanied with the absorption of heat.

The first thermal decomposition of metal complexes onsets from 118 °C with the evolution of carbon monoxide, akin to the ligand but with a higher initial decomposition temperature, IDT implying the greater thermal stability than its precursor\(^{41}\). The second decomposition
stage ranges from 145 to 268 °C leading to the pyrolysis of the whole piperazine molecule (Table 5.4). However, the last stage of decomposition is consistent with the formation of respective metal sulfide as the end product, a feature common to nearly all metal dithiocarbamates.

The DSC curves of the metal complexes exhibit a sharp endotherm at about 124 °C while the broad endothermic hump obtained between 200-250 °C is due to pyrolysis of the piperazine moiety implying that both processes involve absorption of heat. The last process of formation of metal sulfide is exothermic in nature and on further increasing the temperature, a straight line is obtained in all the DSC curves implying no further change.

E. Antifungal Activity

The antifungal study was conducted against *Fusarium* sp. and *Sclerotina* sp. using agar well diffusion method. Table 5.5 represents the results of antifungal activity of metal complexes, \( M(acpdtc)_2 \), at different loading volume. The bidentate ligand \( K(acpdtc) \), did not show any activity (Fig.5.3) while all the metal complexes show strong antifungal activity at higher loading (200 µl and 300 µl) of test solution. The zone of inhibition (37mm) was maximum in case of nickel complex (Fig.5.4) at 300 ml of loading. The antifungal activity of metal dithiocarbamates may be attributed to their ability to chelate with metal ions. When these metal complexes penetrates lipid barriers in the fungal cell and may be ultimate toxicant or alternatively it may be converted into free dithiocarbamate ions, which complexing with metal trace metals and thus depriving the cell of the needed metal ion, and therefore causes death of fungal cell.
Fig. 5.1: Synthesis of the ligand K(acpdte)

Fig. 5.2: Synthesis of the complexes, $M(acpdte)_2$ where $M = \text{Mn(II), Fe(II), Co(II), Ni(II) and Cu(II)}$. 
Fig. 5.3: Fungicidal behavior of bidentate ligand, $K(acpdtc)$ on *Fusarium* sp.

Fig. 5.4: Fungicidal behavior of $Ni(acpdtc)_2$ on *Fusarium* sp. & *Sclerotina* sp.
Table 5.1: Analytical data and physical properties of the complexes.

<table>
<thead>
<tr>
<th>Compounds (F.W)</th>
<th>Colour</th>
<th>Yield (%)</th>
<th>Molar Conductance (ohm$^{-1}$mol$^{-1}$cm$^2$)</th>
<th>Analysis, (%) Found (calcd).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>K(acpdte)</td>
<td>White</td>
<td>72</td>
<td>-</td>
<td>31.29 (31.55)</td>
</tr>
<tr>
<td>Mn(acpdte)$_2$</td>
<td>Brown</td>
<td>55</td>
<td>18</td>
<td>33.01 (33.25)</td>
</tr>
<tr>
<td>Fe(acpdte)$_2$</td>
<td>Brown</td>
<td>62</td>
<td>24</td>
<td>32.93 (33.18)</td>
</tr>
<tr>
<td>Co(acpdte)$_2$</td>
<td>Green</td>
<td>67</td>
<td>17</td>
<td>32.65 (32.94)</td>
</tr>
<tr>
<td>Ni(acpdte)$_2$</td>
<td>Green</td>
<td>70</td>
<td>31</td>
<td>32.68 (32.96)</td>
</tr>
<tr>
<td>Cu(acpdte)$_2$</td>
<td>Brown</td>
<td>68</td>
<td>19</td>
<td>32.29 (32.60)</td>
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Table 5.2: Diagnostic IR bands of the ligand, its mononuclear and trinuclear complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>v(C=O)</th>
<th>v(C=N)</th>
<th>v(C=S)</th>
<th>Piperazine ring vibrations</th>
<th>v(M-S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(acpdtc)</td>
<td>1631 s</td>
<td>1454 s</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn(acpdtc)_2</td>
<td>1630 s</td>
<td>1480 s</td>
<td>991 s</td>
<td>759 s</td>
<td>500 w</td>
</tr>
<tr>
<td>Fe(acpdtc)_2</td>
<td>1638 s</td>
<td>1482 s</td>
<td>1004 s</td>
<td>763 s</td>
<td>478 m</td>
</tr>
<tr>
<td>Co(acpdtc)_2</td>
<td>1637 s</td>
<td>1484 s</td>
<td>987 s</td>
<td>760 s</td>
<td>524 w</td>
</tr>
<tr>
<td>Ni(acpdtc)_2</td>
<td>1646 s</td>
<td>1481 s</td>
<td>984 s</td>
<td>760 s</td>
<td>519 w</td>
</tr>
<tr>
<td>Cu(acpdtc)_2</td>
<td>1635 s</td>
<td>1484 s</td>
<td>987 s</td>
<td>762 s</td>
<td>516 w</td>
</tr>
</tbody>
</table>

Table 5.3: Magnetic susceptibility, electronic spectra and ligand field parameters of the complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Magnetic moment (B.M.)</th>
<th>Electronic bands (cm⁻¹)</th>
<th>Log ε (l mol⁻¹ cm⁻¹)</th>
<th>Possible assignments</th>
<th>10 Dq (cm⁻¹)</th>
<th>B (cm⁻¹)</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(acpdtc)_2</td>
<td>5.44</td>
<td>19230</td>
<td>2.5</td>
<td>⁴T₁ ← ⁶A₁</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe(acpdtc)_2</td>
<td>5.38</td>
<td>22800</td>
<td>3.3</td>
<td>⁵E ← ⁵T₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Co(acpdtc)_2</td>
<td>4.38</td>
<td>14500 21470</td>
<td>3.2  4.1</td>
<td>⁴T₂(F) ← ⁴A₂(F) ⁴T₁(F) ← ⁴A₂(F)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ni(acpdtc)_2</td>
<td>Diamagnetic</td>
<td>16503 21990</td>
<td>3.8  2.9</td>
<td>³A₂(F) ← ³T₁(F) ³T₁(P) ← ³T₁(F)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu(acpdtc)_2</td>
<td>1.71</td>
<td>14614 20410</td>
<td>2.9  4.2</td>
<td>²A₁g ← ²B₁g ²Eg ← ²B₁g</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
**Table 5.4: Thermal degradation of various fragments of ligand and its complexes**

<table>
<thead>
<tr>
<th>Complex</th>
<th>First decomposition stage</th>
<th>Second decomposition stage</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fragments</td>
<td>Temp. Range (°C)</td>
<td>Mass loss data, found (calcd.) %</td>
</tr>
<tr>
<td>K(acpdtc)</td>
<td>CO</td>
<td>95-110</td>
<td>13.01 (12.26)</td>
</tr>
<tr>
<td>Mn(acpdtc)$_2$</td>
<td>2CO</td>
<td>118-145</td>
<td>13.39 (12.92)</td>
</tr>
<tr>
<td>Fe(acpdtc)$_2$</td>
<td>-do-</td>
<td>-do-</td>
<td>12.41 (12.89)</td>
</tr>
<tr>
<td>Co(acpdtc)$_2$</td>
<td>-do-</td>
<td>-do-</td>
<td>12.68 (12.80)</td>
</tr>
<tr>
<td>Cu(acpdtc)$_2$</td>
<td>-do-</td>
<td>-do-</td>
<td>13.28 (12.67)</td>
</tr>
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</table>
Table 5.5: Fungicidal activity of ligand and metal complexes M(acpdte)\textsubscript{2} by agar well diffusion method

<table>
<thead>
<tr>
<th>Test Solution (µl)</th>
<th>Complex</th>
<th>Zone of Inhibition(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z</td>
<td>K(acpdte)</td>
</tr>
<tr>
<td>Fungus Sp. 50</td>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>Fungus Sp. 100</td>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>Fungus Sp. 200</td>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>Fungus Sp. 300</td>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>A</td>
<td>0</td>
</tr>
</tbody>
</table>

Where A- *Fusarium* sp. and B-*Sclerotina* sp.
IV. REFERENCES


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