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Nematicidal Activity of Cu(II) and Fe(II) Schiff Base Ligand Complexes: Synthesis and Characterisation

[1]*M.U. Adaji, [2]R.A. Wuana, [2]A.U. Itodo, [2]I.S. Eneji and [2]M.S. Iorungwa
[1]Department of Chemistry, Kogi State University, PMB 1008, Anyigba, Nigeria
[2]Department of Chemistry, Federal University of Agriculture, PMB, 2373, Makurdi, Nigeria
*Correspondence E-mail: adajimed@gmail.com

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Abstract
The Schiff base *(E)* – N[1]- (2 -hydroxybenzylidene)nicotinohydrazide and its metal complexes of Cu(II) and Fe(II) have been successfully synthesized and characterized physiochemically and spectroscopically. The ligand and the complexes were obtained in good yield ranging from 74% - 76%. The compounds were solid; air and moisture stable with melting point 231 - 245°C. The FT IR spectroscopic data shows appearance of the azomethine (C=N), carbonyl group (C=O) and C-O of phenolic group peak at 1614 cm^{-1}, 1644 and 1357 cm^{-1} respectively in the spectrum of Schiff base and lower by 52 – 29 cm^{-1}, 45 cm^{-1} – 30 cm^{-1} and 67 cm^{-1} – 59 cm^{-1} in the spectra of the complexes respectively, signifying tridenticity of the Schiff base upon interaction with the metal ions. The molar conductivity data of the Schiff base was found to be 4.0 $\Omega^{-1}cm^2mol^{-1}$ indicates non electrolytic nature of the ligand whereas that of the complexes ranges between 142 -162 $\Omega^{-1}cm^2mol^{-1}$ indicating that the complexes were weak electrolyte in DMSO. On the basis of magnetic and electronic spectral data, octahedral geometry was proposed for all the complexes. The powdered x ray diffraction data suggests orthorhombic crystal system for the complexes and the thermal behaviour (TGA/DTA) of the synthesized complexes shows Cu(II) complexes having four stages of decomposition while Fe(II) complexes showing three stages of decomposition with the first stage showing lost of one lattice of water molecule. The nematicidal activity was studied in terms of egg hatch and mortality; and the compounds exhibited nematicidal properties, with hatching rate ranging from 3.0 to 48% and mortality rate ranging from 6.5 to 95 (%). The results revealed that the hatching rate increases with decrease in concentration while the mortality rate increases with increase in concentrations and time. This is evident that the nematicidal activity is time and dose dependant.
Keywords: Nematicidal activity, Schiff base, hatching and mortality rate

Introduction

Meloidogyne incognita is a major plant – parasitic nematode affecting the quantity and quality of the crop production in many annual and perennial crops [1 -3]. Infected plants show typical symptoms including root galling, stunting and nutrient deficiency, particularly nitrogen deficiency. Phytonematodes occur throughout the world, infect all major and minor crop plants and cause substantial reductions in crop yield and quality of produce [4-7]. Estimate overall average yield loss of the world's major crops due to damage by plant parasitic nematode is 12.3%. It is estimated that 50% crop losses are caused by all kinds of pests together and nematodes' share in this may be about 5%. The nematode populations levels present in soil are directly correlated with damage to cereal crops [8]. Environmental concerns in research and industry are increasing with the increasing pressure to reduce the amount of pollutants. This requires a new approach, which will minimize or eliminates the dispersion of harmful chemicals in the environment in a way that enhances the industrially benign approach and meets the challenges of green chemistry [10-12]. To this end, there is need to synthesize more active and eco – friendly Schiff base complexes to control the root knot nematodes that affect the plants [13-15].

Schiff base ligands can coordinate with many different metal ions and stabilize various oxidation states, they have numerous application such anticancer, antibacterial, antiviral, antifungal, and other biological properties [16-19]. The study was initiated to understand the characteristic nature and applications of

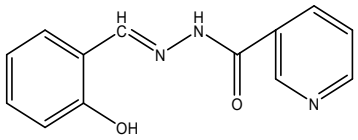
the nematicidal active complexes of nitrogen and oxygen donor Schiff base ligand and its metal complexes.
In view of the above, we have undertaken the synthesis, characterization and nematicidal activity of Cu(II) and Fe(II) complexes of Schiff base.

Materials and Methods

***Reagents*:** All chemicals used were of analytical grade and procured from Sigma – Aldrich chemical Ltd, and used without further purification. Nicotinic acid hydrazide (97%), Salicylaldehye (98%), Copper (II) sulphate pentahydrates (98%), Iron (II) sulphate heptahydrates (97%) and Calcium chloride (98%)**,** Absolute ethanol, Methanol, Dimethylsulfoxide (DMSO), Dimethylformamide (DMF) and Acetone

***Equipment*:** routine laboratory apparatus/equipments such as beakers, conical flasks, test tubes, desiccators, thermostatic magnetic stirrer, weighing balance, water bath, eflux, thermometer, incubator, Sieves, centrifuge, pipette and microscope.

***Synthesis of* ligand:** The Schiff base *(E)* – N[1]- (2 - hydroxybenzylidene) nicotinohydrazide (HNH) (Fig. 1) was synthesized according to the literature [20, 21]



(*E*)-*N*'-(2-hydroxybenzylidene)nicotinohydrazide

Fig.1: Structure of Schiff base ligand *(E)* – N[1]- (2 - hydroxybenzylidene)nicotinohydrazide

***Preparation of metal* complexes:** The complexes were synthesized by the method reported in the literature [20 - 22]. A warm ethanolic solution [0.005 mol] of (HNH) Schiff base ligand was added slowly to the hot ethanolic solution of the corresponding metal salts (sulphate) (0.005 mol) with continuous stirring. The resulting solution was placed on placed on a magnetic stirrer with a constant stirring for about 10 -15 min and left to cool. The crystals obtained was filtered and dried over $CaCl_2$ in the desiccators and weighed.

Determination of the metal content of the complexes

Known amount (0.25 g) of complexes was decomposed with concentrated nitric acid. This process was repeated till the organic part of the complexes got completely lost. The excess nitric acid was expelled by evaporation with concentrated sulphuric acid. The Cu(II) and Fe(II) contents of the complexes were determined per the procedure available in literature [23]

***Physical* measurements:** Elemental analysis (C, H, N and O) was performed using Perkin Elmer CHNO analyser. FTIR spectra of the ligand and their complexes were recorded on Agilent Technology FTIR spectroscopy within the range of 4000 – 650 cm^{-1} using KBr disc. The electronic absorption spectra of the ligand and their complexes using JASCO V-670 spectrophotometer from 250 – 800 nm. Magnetic susceptibilities of the complexes were measured using Guoy balance at room temperature, by making diamagnetic corrections using pascals constant. Thermal studies of the complexes were carried out on Perkin Elmer diamond TGA instrument at a heating rate of 10° C and a nitrogen flow rate of 20 mL/min. The x ray patterns of the complexes were recorded on Xpert pro X – ray diffractometer with Cu kα radiation ($\lambda = 1.5406\ A°$). The diffraction data were integrated using the trial and error methods

***Nematicidal activity*:** Root knot nematode, *Meloidgogyne incognita* is major plant parasitic nematodes affecting the quantity and quality of the crop production in many annual and perennial crops. *Meoidogyne nematode* can develop galls and lesions in the roots [24], thereby causing stunted growth of the plants. Some chemicals such as aldicarb and oxamyl can be used to control nematodes but found to be toxic to the eco system.
Nematicial activity of the complexes was carried out on *Meloidogyne incognita*. Fresh egg masses of *Meloidognyne incognita* are collected from stock and maintained on tomato (*Lycopersiscon esculentum*) root tissues and kept in water for egg hatching. The eggs suspensions were poured on a cotton wool filter paper and incubated at 30°C to obtain freshly hatched second juveniles (J_2). Juveniles collected within 48 h were used for screening nematicidal activity of the compound.
The compounds were initially dissolved in dimethylsuloxide (DMSO) and then in distilled water to make dilutions of 250, 200, 150, 100 and 50 µg/mL. Experiments were performed under laboratory conditions at 30°C. About 100 freshly hatched second stage juveniles were suspended in 5 mL of each diluted compound and incubated. Distilled water with nematode larvae was taken as control. The dead nematodes were observed under an inverted binocular microscope. After an incubation of 24 h and 48 h, percentage of mortality was

calculated. Nematodes were considered dead if they did not move when probed with a fine needle [25]

Results and discussion

Formation of the Complexes

All the complexes precipitated as powders, coloured, stable and hygroscopic in nature. The complexes were insoluble in common organic solvents but soluble in DMF and DMSO. The elemental analysis showed that the complexes have 1:2 stoichiometry of the type $[M(HNH)_2]SO_4.nH_2O$. Molar conductance of the complexes was measured in DMSO. The conductance values, which are presented in the Table 1, indicate that the complexes are weak electrolyte [26]. The melting points of complexes were determined and the results presented in Table 1 show the physiochemical properties and elemental analysis of the complexes and the melting points was within the range of 231 ℃ - 245℃.

Table 1: Physiochemical Properties and Elemental Composition of Metal Complexes

	Complex	Colour	Yield (%)	m.p ^{0}C	Molar conductance ($\Omega^{-1}cm^2mol^{-1}$	Elemental Composition (%)				
						C	H	N	M	SO_4^{2-}
	$C_{13}H_{11}N_3O_2$	Light yellow	85	205	4	64.93 (64.73)	4.66 (4.56)	17.68 (17.43)	-	-
SCu	$C_{26}H_{24}N_6O_9$	Dark blue	76	246	142	47.79 (47.30)	3.75 (3.64)	12.74 (12.74)	9.11 (9.64)	14.12 (14.56)
SFe	$C_{26}H_{24}N_6O_9$	Dark brown	74	231	162	47.38 (47.86)	3.66 (3.68)	12.54 (12.89)	8.23 (8.57)	14.64 (14.73)

Electronic Absorption Spectra and magnetic moment: The electronic spectra of data Cu(II) and Fe(II) complexes along with magnetic moment values shown in Table 2. The electronic spectra of Cu(II) complexes in DMSO show bands at 15151 and 17123 cm^{-1} assignable to a $^2E_g \rightarrow {}^2T_{2g}$ and $^2B_{1g} \rightarrow {}^2A_{1g}$ transition respectively. The observed magnetic moment of 1.74 B.M. was found in Cu(II) complex which in addition to the electronic spectra data suggest the possibility of an octahedral geometry for Cu(II) complex. Fe(II) complex exhibit bands at 15128, 15649 and 20408 cm^{-1} assignable to $^6A_{1g}$ (F) $\rightarrow$ $^5T_{1g}$ (G)(ν_1), $^6A_{1g}$ (G) $\rightarrow$ $^5T_{2g}$ (G) transition and charge transfer respectively. The observed magnetic moment of 5.23 B.M. along with electronic transition corresponds to an octahedral geometry.

Table 2: Electronic absorption spectra data and Magnetic Data for Cu(II) and Fe(II) complexes

Compound	μeff (B.M)	Absorption (cm^{-1})	Assignment	Geometry
$C_{26}H_{24}N_6O_9SCu$	1.74	15151	$^2E_g \rightarrow {}^2T_{2g}(\nu_1)$	Octahedral
		17123	$^6B_{1g} \rightarrow {}^2A_{1g}(\nu_2)$	
$C_{26}H_{24}N_6O_9SFe$	5.23	15128	$^6A_{1g} \rightarrow {}^5T_{1g}$ (G)(ν_1)	Octahedral
		15649	$^6A_{1g} \rightarrow {}^5T_{2g}$ (G)(ν_1)	
		20408	Charge transfer	

Infrared Spectra of the Ligand and Metal (II) Complexes

The data of the FT - IR spectra of the ligand and its metal complexes are listed in Table 3. The IR spectra of the complexes were compared with those of the free ligand in order to determine the involvement of coordination sites in the complexes. Characteristic peaks in the spectra of the ligand and complexes were considered and compared. The band in the spectra of the ligand at 1644 cm^{-1} have been assigned to υ(C=O). These bands were lowered by 30 to 45 cm^{-1} (1614 - 1599 cm^{-1}) on the

metal complexes indicating the involvements of the carbonyl group in the bonding to metal [27].
The imino group i.e υ(C=N) band at 1614 cm $^{-1}$ for the free ligand which are found to be shifted to lower frequency region around (1585 – 1562 cm $^{-1}$) on complexes suggest the involvement of the nitrogen atom

of the υ(C=N) azomathine nitrogen in the bonding with the metal (II) ions [28].
A high intensity band was present around 1357 cm $^{-1}$ region in the spectrum of the ligand, which was assign to phenolic υ(C-O) vibrations and shift towards lower frequency region around 1298 – 1290 cm $^{-1}$ on complexion and it indicative of bonding through phenolic oxygen [29].
The appearance of broad band at 3141 cm $^{-1}$ in the Schiff base ligand may be due to υ(OH) and the broadening of the same was also due to intermolecular H – bonding between OH groups. These bands were missing in the metal complexes indicating that the phenolic character of the ligand has been lost upon complexation [30]

The various observation bands in the regions around 1197 – 1153 cm $^{-1}$ may be assigned to SO_4^{2-} [31].
In the FT-IR spectra, the band due to N– H stretching in the free ligand occurs in the 3476 cm^{-1} region which may be due to hydrogen bonding probably of the type NH…N type in the ligand which account for the appearance of the υ[N-H] band around 3078 – 3048 cm^{-1} in the complexes. The stretching υ(NH) of the ligand is not much altered in the spectra of the complexes indicating the nonparticipation of nitrogen atom of NH_2. Hence it is concluded that the compound (HNH) acted as a neutral tridentate ligand [32].
The υ(M-N) stretching bands were not observed in the spectrum of the ligand but observed in the spectrum of the complexes around 758 – 724 cm^{-1} probably because there is no (M-N) coordinated in the ligand [33]. Similarly, there is no υ(M-O) stretching band in the ligand because, there was no metal coordinated to oxygen meanwhile observed in the spectrum of the complexes around 937 – 903 cm^{-1}, this is in line with the study of [34].

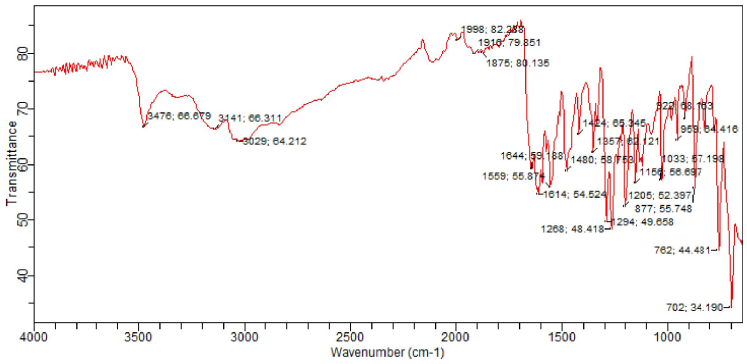


Fig 2: FT – IR Spectrum of the HNH Schiff base

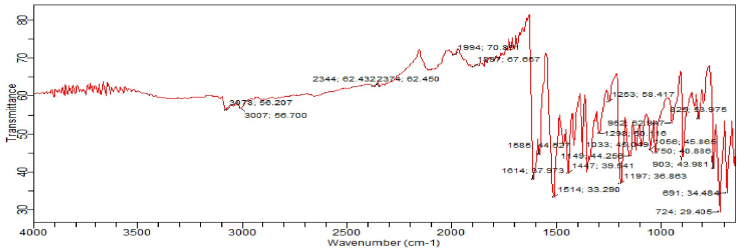


Fig 3: FT – IR Spectrum of $[CuHNH)_2]SO_4H_2O$

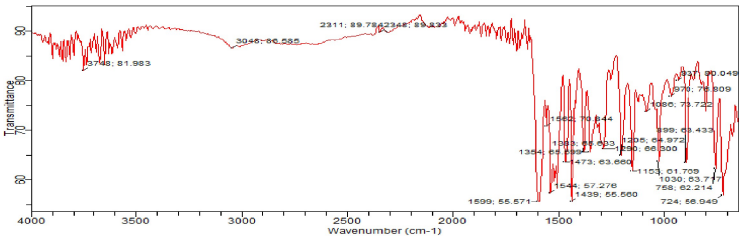


Fig 4: FT – IR Spectrum of $[Fe(HNH)_2]$ $SO_4.H_2O$

Table 3: Infrared Spectra Data for the Ligand and Complexes

Molecular formulae	Vibrational frequencies (cm^{-1})								
	C=N	N-N	N-H	C=O	M-O	M-N	SO_4^{2-}	(OH)H_2O	C-O
HNH	1614	1357	3476	1644	-	-	-	3141	1294
$[Cu(HNH)_2].SO_4.H_2O$	1585	1298	3078	1614	937	724	1153	-	1253
$[Fe(HNH)_2].SO_4.H_2O$	1562	1354	3048	1599	903	758	1197	-	1290

Thermo gravimetric analysis (TGA)

The temperature range, weight loss found and calculated mass loss percentages of the complexes are illustrated in Table 4. The TG curve of Cu(II) and Fe(II) complexes is shown in Fig. 5 - 6. Cu(II) complex follow four stages of decomposition while Fe(II) complex follow three stages of decomposition. $[Cu(HNH)_2]SO_4H_2O$ takes place in four stages. The first stage account for a mass loss of 2.2 % (2.73 %) was attributed to the loss of one molecule of water takes place between 30 – 190 °C. The maximum rate mass loss was indicated by the DTA peak at 95 °C. The remaining complex begins to decompose from temperature range of 191- 400 °C. This range corresponds to decomposition of $C_6H_5ON_3SO_4$ with DTA peaks observed at 250 °C which clearly shows that at least unstable intermediates are also formed during this degradation stage. The observed mass loss was 36.8 % (calculated 35.03 %). The third stage was decomposition of $C_{20}H_{17}N_3O$ in temperature range of 401 °C to 550 °C with the DTA peak observed at 420 °C. The observed mass loss was 41.0 % (calculated 50.18 %). The overall mass loss observed was 88.6 % and it was compared with theoretical mass loss value. The end product was estimated to be CuO which decomposed above 551 °C with observed mass loss of 8.6 % (calculated 12.06 %) and the observed mass loss and calculated mass were overall within the range.

The thermal dehydration of the compound $[Fe(HNH)_2]SO_4H_2O$ take place in three stages. In the first stage of the thermal dehydration of the complex takes place in a single step that is between 30 -240 °C with mass loss of 3.0 % (calculated 2.76) corresponding to loss of one molecule of water. The maximum rate of mass loss was indicated by DTA peak at 55 °C.

In the second stage which occurs in the temperature range of 241 – 460 °C with DTA peak observed at 290 °C, this range corresponds to the decomposition of $C_{26}H_{22}O_3N_6SO_4$ and the observed mass loss was recorded as 69.0 % (86.34 %). The third stage was the decomposition of FeO residue in the temperature above 461°C and the observed mass loss recorded was 8.4 (11.02 %). The overall mass loss observed was 76.4 % and it was compared with theoretical mass loss value which was found to fall within the range.

Table 4: Thermal Analytical data of Metal Complexes

Name of complexes	Tempt. (°C)	DTA (°C)	Stage	Mass loss(g) found (%Calc)	Assignment
$[Fe(HNH)_2].SO_4.H_2O$	30 – 240	55	I	3.0 (2.76)	Dehydration of H_2O
$Fe(C_{13}H_{11}N_3O_2)_2SO_4.H_2O$	241 - 460	290	II	69.0 (86.22)	$C_{26}H_{22}O_3N_6SO_4$
	>461	495	III	8.4 (11.1)	FeO Residue
$Cu(HNH)_2\ SO_4.H_2O$	30 – 190	95	I	2.2(2.73)	Dehydration of H_2O
$Cu(C_{13}H_{11}N_3O_2)_2SO_4.H_2O$	191 - 400	250	II	36.8 (35.03)	$C_6H_5O_2N_3SO_4$
	401- 550	420	III	41.0 (50.18)	$C_{20}H_{17}O_2N_3$
	> 551	560	IV	8.6 (12.06)	CuO Residue

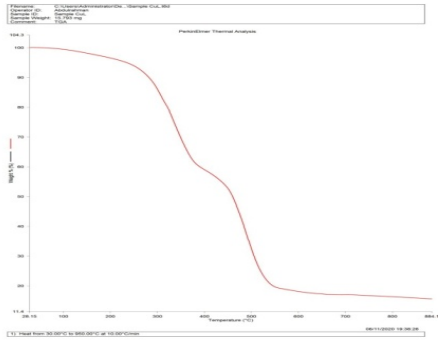


Figure 5: TGA thermograph of $[Cu(HNH)_2]SO_4.H_2O$

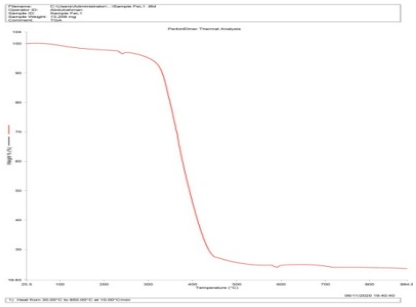


Figure 6: TGA thermograph of $[Fe(HNH)_2]SO_4.H_2O$

Powder XRD Studies of the complexes

Powder XRD patterns of all the complexes were recorded over $2\theta = 10 - 50°C$ range and all metal complexes display sharp crystalline peaks indicating theri crystalline nature. It is also one of the evidences about the structure of the ligand and its complexes. The XRD patterns of the ligand and its complexes are shown in Fig 6 and 7. Unit cell parameters were calculated by using trial and error methods as follows: all the compounds are orthorhombic with the unit cell parameters for Cu- HNH: a = 12.68A°, b = 18.61A°, c = 13.01A° and V= 3070.03 A^3; Fe-HNH: a = 10.94A°, b = 10.00 A°, c = 9.74 A° and V = 808.03 A^3. The average crystallite sizes of the complexes were calculated from Scherer's formula [35]

$$D = \frac{0.94\lambda}{\beta Cos\theta} \qquad (1)$$

Where λ is the wavelength of the X ray radiation, β is the full width at half maximum of diffraction line and θ is the diffraction angle and were found to be 8.68 and 119.7 for Cu(II) and Fe(II) complexes respectively suggesting that the complexes are nanocrystalline phase. This is in agreements with findings obtained elsewhere **[36]**

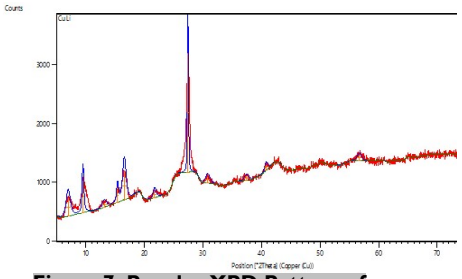


Figure 7: Powder XRD Pattern of $[Cu(HNH)_2]SO_4.H_2O$

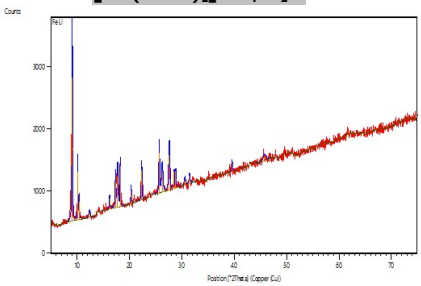


Figure 8: Powder XRD Pattern of $[Fe(HNH)_2]SO_4.H_2O$

Nematicidal Activity

The nematicidal activity of the complexes is depicted in Tables 5 - 10**.** From the results, the complexes showed effective nematicidal activity against root knot nematode by inhibiting egg hatching and enhancing mortality. The metal complexes showed a better nematicidal activity compared to Schiff base ligand, which may be due to complexation [37- 40]. All the metal complexes exhibited moderate activity with hatching rate ranging from 3.0% to 48% and mortality rate ranging from 6.5% to 95% respectively at varied time of 24 h to 120 h and concentrations 50, 100, 150, 200, and 250ppm. However, the activity of the metal complexes depends on concentrations and time [41 - 45], that is, activity was higher at higher concentrations and increased with time, in terms of percentage mortality. These results agreed with earlier studies [46 - 48]. Meanwhile, in terms of hatching, the nematicidal activities increases with decrease in percentage of hatching and increase in time.

Table 5: Hatching (%) of Nematodes in HNH of at Different concentrations

Compound	Duration (h)	Percentage (%) Hatching at Different concentrations (ppm)				
		250	200	150	100	50
HNH	24	4.6	7.7	10.6	13.7	16.7
	48	8.3	11.5	14.6	17.8	20.9
	72	13.6	16.8	24.8	30.4	32.8
	96	15.4	19.9	27.2	30.9	41.9
	120	23.3	25.3	34.3	38.4	43.2

Table 6: Hatching (%) of Nematodes in Cu-HNH at Different Concentrations

Compound	Duration (h)	Percentage (%) hatching at different concentrations (ppm)				
		250	200	150	100	50
Cu-HNH	24	1.5	3.0	4.6	6.1	10.6
	48	3.0	4.7	6.3	9.4	18.8
	72	4.0	4.8	7.2	13.6	20.0
	96	5.1	5.9	8.8	20.6	24.3
	120	8.2	8.9	12.3	21.2	26.7

Table 7: Hatching (%) of Nematodes in Fe-HNH at Different Concentrations

Compound	Duration (h)	Percentage (%) hatching at different concentrations (ppm)				
		250	200	50	100	50
Fe-HNH	24	4.6	6.1	12.1	21.4	22.7
	48	7.3	10.4	17.1	23.9	30.2
	72	10.4	12.0	23.2	24.8	32.0
	96	18.4	19.1	24.3	27.2	41.9
	120	20.6	21.2	26.0	30.8	43.8

Table 8: Mortality (%) of Nematodes in HNH at Different Concentrations

Compound	Duration (Days)	Percentage (%) Mortality at different concentration (ppm)				
		250	200	150	100	50
HNH	1	15.0	12.1	9.3	7.3	6.2
	2	27.7	21.6	13.3	10.9	9.2
	3	47.3	36.8	27.2	13.1	13.1
	4	71.3	49.1	43.7	29.1	29.1
	5	83.2	60.1	47.0	34.1	34.1

Table 9: Mortality (%) of Nematodes in Cu-HNH at Different Concentrations

Compound	Duration (Days)	Percentage (%) mortality at different concentrations (ppm)				
		250	200	150	100	50
Cu-HNH	1	10.9	7.21	6.7	5.2	4.5
	2	38.9	30.1	19.9	18.4	16.6
	3	62.9	44.7	41.4	27.8	27.2
	4	69.5	62.6	49.6	41.9	31.2
	5	90.5	65.6	57.1	43.3	38.6

Table 10: Mortality (%) of Nematodes in Fe-HNH at Different Concentrations

Compound	Duration (Days)	Percentage (%) Mortality at different concentration (ppm)				
		250	200	150	100	50
Fe-HNH	1	21.8	19.7	14.4	12.7	9.8
	2	42.7	33.9	25.9	23.0	18.2
	3	66.9	49.6	38.3	34.5	27.8
	4	91.2	66.3	52.1	47.2	38.6
	5	91.5	75.6	56.8	50.0	39.7

Conclusion

In this study, Cu(II) and Fe(II) complexes were prepared from (*E*) – N^1- (2 - hydroxybenzylidene)nicotinohydrazide Schiff base and characterized using different spectral techniques. The IR spectral data reveals that the Schiff base ligand coordinates through the azomethine nitrogen, carbonyl oxygen and phenolic oxygen atoms in a tridentate manner. Magnetic and electronic specta studies revealed octahedral geometry for all the complexes. Thermal data provided the number of lattice water molecules in the complexes and also shows the decomposition trend. Powder XRD results show that the ligand and its complexes are crystalline in nature and are of orthorhombic system satisfying the condition a≠b≠c for a typical orthorhombic crystal system.

Declaration of conflicting interests The authors declared no potential conflicts of interest

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