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Synthesis, Characterization and Biocidal Profiles of BHNOPDA and its Fe²⁺ and Mn²⁺ ions complexes against *Meloidogyne incognita*

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Abstract

Two metal complexes have been synthesized from Mn (II) and Fe (II) chloride and ligandbis-2-hydroxy-I-naphthaldehyde-Ophenylenediamine (BHNOPDA) using the microwave assisted synthesis and were characterized using electronic spectra (ultra-violet visible spectroscopy), Fourier Transformed infrared spectra (vibrational spectroscopy), molar conductivity measurement, solubility test and melting point determination. The metal complexes and ligand were insoluble in distilled water, but the complexes were completely soluble in methanol, ethanol, DMSO and DMF Fe²⁺ complex was insoluble in ethers and chloroform. Mn²⁺ and Fe²⁺ complexes were insoluble in acetone, chloroform and ethers. The melting point of both the ligand and the metal complexes showed they are thermally stable. The conductance data showed that the ligand is non-electrolytic but the metal complexes are electrolytic. The Schiff base and their metal complexes were have also been screened for their nematicidal activity against root knot nematode, *Meloidogyne incognita*. Metal complexes showed better nematicidal activity than Schiff base.

Keywords: synthesis, Complexes, Abelmoschus esculentus, Meloidogyne incognita

Introduction

For a long time, coordination chemistry of Schiff base ligands has been a subject of great interest. Schiff bases are capable of forming coordinate bonds with many metal ions via azomethine and Phenolic groups, so they have been used for the synthesis of metal complexes due to their easy formation and strong metal binding ability [1]. Synthesis of complexes of Schiff base having novel structural features and unusual physio-chemical properties have considerable importance in biological processes and constitute an active area of research in modern coordinationchemistry [2].

Azomethine compounds show a wide range of biological activities such as antimalerial, anticancer, antibacterial [3] antifungal, antitubular [4] anti-inflammatory [5], antiviral, antifertility and antipyretic activities [6]. They also show nematicidal activity [7], but could not be exploited to their potential in pest control due to long period of time required for completion of the reaction and are not stable compounds as these get easily disintegrated when exposed to moisture. To overcome this problem, their metal complexes were showed better activity than their synthesized which corresponding ligands Combination of metal ions with polydentate ligands form ring structure, where the metal ion is a part of the ring, is called chelation. By using catalyst, the time taken by the reaction in conventional method can be reduced and the product can be utilized upon industrial level has become cost effective [8].

It is fact that iron, manganese and cobalt are essential metallic elements and show great biological activity when combined with certain metal protein complexes, participating in oxygen transport, electronic transfer reactions or the storage of ions which has created immense interest in the study of system containing these metals. The interaction of transition metal complexes with DNA has been widely studied in the development of new tools for nanotechnology. Antioxidants

are extensively studied for their capacity to protect organism and cell from damage that is induced by oxidation stress [9]. On the other hand, the root knot nematode species, Meloidogyne incognita, is the most widespread and prevalent almost certainly the most serious plant parasitic nematode pest of tropical and subtropical regions throughout the world [10]. It occurs as a pest on wide range of crops and affects the crop quantitatively and qualitatively. Endo parasitic nematodes are more damaging and agriculturally important than other groups. Many crops grown as vegetables such as tomatoes, okra, cucumber, melon, carrot, gourds, lettuce and peppers particularly are susceptible to the nematode. The disease is manifested by the formation of galls in the root accompanied by stunted growth, chlorosis and loss of vigor of the plant [11]. An estimated annual yield loss in the world's major crops as a result of plant parasitic nematodes is about 12.3 % [8]. Plant parasitic nematodes, the hidden enemy of crops, affect the production and economy of crop in diverse ways such as reduction in quality and quantity of crops, need of additional fertilizer and water, application of nematicides and impediment of production and trade by phytosanitaryregulations[11]. During the last decade, emphasis has been put on a wide range of control options, such as green manures, soil amendments, crop rotations, bio-fumigations, soil solarization, steam resistant varieties, grafting, mycorrhizae, ozone treatments, biocidal plants or their derived products and biological control agents. Efforts have been made to search newer chemicals to achieve the effective control of nematodes [12]. But more effort is needed to search for newer chemical which are stable to moisture and can be synthesized within a shorter period of time thereby reducing the industrial cost of production.

This research undertook the synthesis of Schiff base ligand BHNOPDA and Fe²⁺ and Mn²⁺ complexes, their characterization and applicability as nematicides against *Meloidogyne incognita*.



Materials and Methods

Apparatus/Reagents

The apparatus and reagents used include: Routine laboratory apparatus/equipment and machines; Barnstead electro thermal BI900 melting point apparatus with digital thermometer, microwave oven, pH/Conductivity series 510 conductivity meters, pH meter, Carry 630 FT-IR spectrophotometer, Shimadzu 1800UV-Visible spectrophotometer, SB160 heatstirrer, PW 184 weighing balance, 98-I-B temperature regulating heating mantle, and DHG-9053A dry oven.

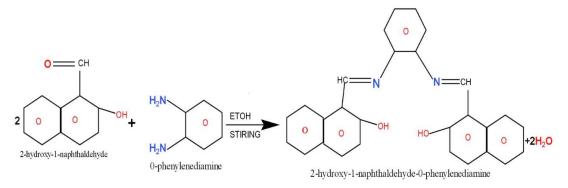
Ethyl alcohol, 2-hydroxy-I- naphthaldehyde , O-phenylenediamine, Methanol, Diethyether, Petroleum ether, Dimethyl sulphoxide, Dimethyl formamide, Acetone , Concentrated Sulphuric acid, Sodium hydroxide, Chloroform, Cobalt (II) Chloride, manganese (II) Chloride. All reagents used

were analytical grade and were used without further purification.

Synthesis of ligand

The Schiff base Bis(2-hydroxy)-I-naphthaldehyde-O-phenylenediamine (BHNOPDA) was synthesized as reported by [13][14] and as represented on Scheme I

Bis-2-hydroxy-I-naphthaldehyde-O-The ligand phenylenediamine was synthesized by condensation of 2hydroxy-I-naphthaldehyde (6.04g, 2mmol) and 0phenylenediamine (3.50 g mmol) respectively, in2:1 molar ratio were added to 5 mL ethanol with continues stirring until complete solubility. The mixture was then exposed to microwave irradiation at 120W power for 15 minutes. The solution was then concentrated under reduced pressure, which on cooling gave crystalline precipitates. These were washed with ethanol and recrystallized in the same solvent, and dried in an oven at 45°C.



Scheme 1: Synthesis of Bis(2-hydroxy)-1-naphthaldehyde-O-phenylenediamine (BHNOPDA)

Synthesis of metal complexes

The metal complexes were synthesized using method adopted by [15]. Exactly 2.0g of Schiff base was mixed with 25 mL methanol containing 2.0g of metal salt with continues stirring until complete solubility. The mixture's pH was adjusted to 7-8 by adding 1.0 M NaOH solution and then placed in a Microwave oven for 15 minutes for the complexation reaction to complete. The precipitated solution was removed and dried in an oven at 45°C.The crystals were collected and the yield was obtained in grams [13, 14].

Characterization

The physio-chemical and spectrophotometric characterization tests on the synthesized compounds were as follows:

Solubility test

The prepared Schiff base alongside their complexes was added to 10 mL portions of each of the solvents (distilled water, methanol, ethanol, dimethylsulfoxide, dimethylformanide, chloroform, diethylether and acetone) and was shaken vigorously. The entire solutes dissolved to give a homogenous mixture after shaking the sample (CS). However, some sample were slightly soluble (SS) and some were insoluble (NS).

Melting point determination

Each sample of the Schiff base with the metal complexes were put in separate capillary tubes and each was inserted into the heating block and was heated one after the other and the temperatures at which each of the sample melt was read from the digital screen.

The molar conductivity measurement

The molar conductivities of the Schiff base along with the respective complexes were obtained from DMSO using pH/Conductivity series 510 conductivity meter.

Infrared and electronic spectra data

The infrared spectra data of the synthesize Schiff base alongside with the complexes obtained by using proRamman-L-785-BIS FT-IR spectrophotometer. The prepared ligand along with the complexes was placed on the slide and the slides were placed on the sample chamber of the spectrophotometer so that the distance between the slide and the lens will be about 7 mm apart. The spectrophotometer was instructed to scan the sample and suitable peaks were obtained.

Ultraviolet-Visible spectroscopy

The ultraviolet spectral measurement of the synthesized ligand alongside the metal complexes was obtained from the solution of the compounds by using UV Spectrophotometer to check the electronic transition state of each of the synthesized compound. The UV spectra of the compounds were obtained



using DMSO as solvent from Shimadzu 1800 UV-Visible spectrophotometer.

Magnetic susceptibility

Magnetic Susceptibility measurements of the complexes in the solid state was determined by the Gouy balance at room temperature using Cu(II) acetate monohydrate as the calibrant.

Nematicidal activity

Two experiments were carried out to check the nematicidal activity of the synthesized compounds. The experiments were mortality test and hatching test.

Hatching Test

The nematicidal studies were conducted using Schiff bases and its metal complexes against *Meloidogyne incognita* (root knot nematode). The compounds were tested for hatching and mortality of root knot nematode at four different concentrations alongside with control.

Raising of root knot nematodes

Roots of the heavily infected okra (Abelmuschusesculentus) were collected from Baga farm in Pankshin LGA of Plateau State. Egg masses were separated from the heavily infected okro (Abelmuschus esculentus) plants and washed under running water. After cutting the roots into smaller pieces, 1% of sodium hypochloride was added, shaken, and then sieved. Then the eggs masses of nematodes were obtained.

Hatching of nematodes

Exactly 20 masses eggswere picked and placed in 100.0 mL of 10, 5, 2.5 ppm concentrations of the ligand and the metal complexes, alongside with control (distilled water) to monitor the hatching within 5 days. A regular time interval of 2,4,6,8 and 10 days were employed in monitoring the hatched larvae of the nematodes, which the number of unhatched nematodes was observed and recorded within the time exposure. These were observed under an inverted binocular microscope. This method adopted is as described elsewhere [13, 14, 16, 17].

Mortality test of nematodes

For mortality, eight (8) freshly hatched juveniles (J2) were taken into different concentrations of the ligand and its metal complexes. The number of sacrificed nematodes was observed with the time interval of 2,4 and 6h respectively. For each treatment, a control was also formed in distilled water. After 6h of exposure, revived juveniles were counted and sacrificed nematodes were confirmed. Nematodes were considered sacrificed when they could not move.

Results and Discussion

Physical properties of ligand and metal complexes The results of the physical properties, molar conductivity data and infrared spectral bands of the Schiff base and metal complexes are presented in Tables I to 3 respectively while Tables 4 and 5 presents the solubility data and electronic and magnetic moment data for the Schiff base and the metal complex respectively.

Table I: Some Physical Properties of the Schiff Base Ligand and its Metal Complexes

	Concentration (Moldm ⁻³)	Specific Conductance ohm ⁻¹ cm ⁻¹	Molar conductance ohm ⁻¹ cm ² mol ⁻¹	Remark
BHNOPDA	1.0×10 ⁻³	26.40×10 ⁻⁶	26.40	Non-electrolytic
Mn(II)BHNOPDA	1.0×10 ⁻³	49.09×10 ⁻⁶	49.09	Electrolytic
Fe(II)BHNOPDA	1.0×10 ⁻³	59.60×10 ⁻⁶	59.60	Electrolytic

Table 2: Molar Conductivity Data of the Schiff base Ligand and its Metal Complexes in DMSO

Ligand/Complexes	Colour	Melting point (°C)	%Yield	рН
BHNOPDA	Yellow	159.20 – 161.60	92.12	7.65
Mn (II)BHNOPDA	Redish-brown	146.90 – 149.70	91.01	7.15
				7.80
Fe(II)BHNOPDA	Dark- brown	245.00 – 452.00	83.2	5.5



Table 3: Important Vibrational frequencies (cm-1) of the Ligand and its Metal Complexes

Ligand/Complexes	0-H	C – N	C - O	C - C	M - O	M – N
BHNOPDA	3471.91	1610.09	1243.02	1176.15	469.97	396.99
Mn(II)BHNOPDA	3373.39	1599.93	1242.86	1161.31	468.76	397.89
Fe(II)BHNOPDA	3354.69	1592.05	1359.89	1092.89	498.58	396.51

Table 4: Solubility Data for the Schiff Base Ligand and its metal complexes in different solvents at room temperature

Ligand/Complexes	Distilled Water	Methanol	Ethanol	Acetone	Ether	Chloroform	DMSO	DMF
BBHNOPDA	NS	CS	CS	CS	CS	CS	CS	CS
Mn(II)BHNOPDA	NS	CS	CS	NS	NS	NS	CS	CS
Fe(II)BHNOPDA	NS	CS	CS	CS	NS	NS	CS	CS

NS – Not Soluble; SS – Slightly Soluble; CS – Completely Soluble

Table 5: Electronic and Magnetic Moment Data for the Schiff base Ligand and its Metal Complexes

Ligand/Complexes	λmax (nm)	Assignment	Magnetic Moment μ_{eff} (B.M)
BHNOPDA	426.00	m - m*	
Mn(II)BHNOPDA	423.00	⁴ E _g - ⁶ A _g	2.0
Fe(II)BHNOPDA	400.00	${}^{4}T_{g}^{2}(G)$	3.75

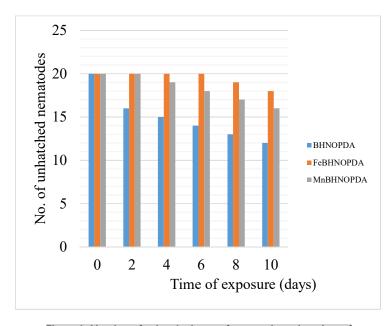


Figure 1: Number of unhatched eggs of nematode against time of Exposure at concentration of 5 ppm

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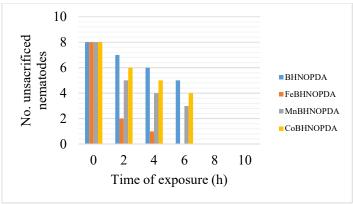


Figure 2: Number of unhatched eggs of nematode against time of exposure at concentration of 10 ppm

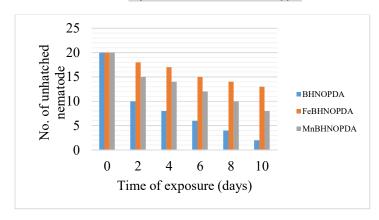


Figure 3: Number of unhatched eggs of nematode against time of exposure at concentration of 2.5 ppm.

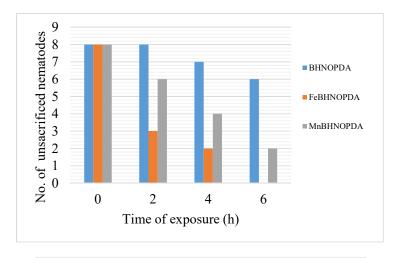


Figure 4: Number of un-sacrificed nematodes against time of exposure at concentration of 10 ppm



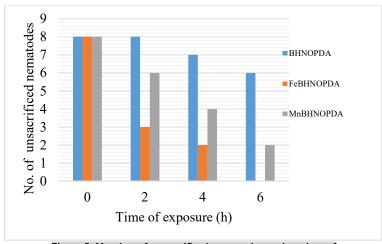


Figure 5: Number of un-sacrificed nematodes against time of Exposure at concentration of 5 ppm

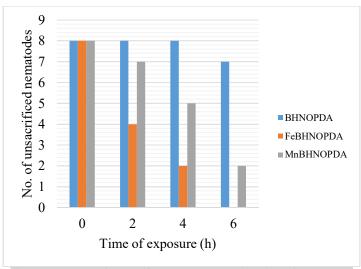


Figure 6: Number of un-sacrificed nematodes against time of exposure at concentration of 2.5 ppm.

Physicochemical characterization of the ligand and its metal complexes

All the metal complexes and the ligand were coloured after the synthesis as seen in Table I. That could be because one of the characteristics attributes of the transition metals is their ability to form coloured complexes. The pungent smell of the complexes is as the result of the 0-phenylenediamine which is the starting material in the reaction.

The reaction between Bis-2-hydroxy-I-naphthaldehyde-Ophenylenediamine (BHNOPDA) and manganese (II), Iron(II) and ions produced metal complexes which are crystalline and coloured, with high percentage yield, which ranged from 83.2-91.01 percent, at a pH ranged between 7-8 (Table I). This indicates that the reaction occurred in a neutral medium. The molar conductivity of the complexes was found to be in the range of 49.09-59.60 Ohm-I cm² mol-I, showing electrolytic nature of the complexes (Table 2). The complexes were found to melt in the temperature I46.90-452°C, showing a fairly stable complex compounds which may require small amount of energy to break the bond between the metal and the ligand.

The complex compounds are insoluble in water but soluble in organic solvents such as; methanol, ethanol, chloroform, acetone, dimethylsulphoxide (DMSO) and dimethylformaldehyde (DMF) (Table 3)

FTIR spectra of the ligand and its metal complexes

The relevant IR spectra assignment is shown on Table 4. The FTIR spectrum of the ligand was compared with that of the metal complexes so as to identify the coordination sites of the ligand. The FTIR spectra of the synthesized ligand and the complexes of Mn(II), Fe(II) are recorded in the region of 500-4000 cm⁻¹. A strong band was observed for the ligand around 1610.09 cm⁻¹, characteristic of the azomethine (C=N) stretching vibration. In the metal chelates, the band characteristic of the azomethine group was shifted to around 1614.79. cm⁻¹ from 1610.09 cm⁻¹ in the ligand, suggesting coordination of the azomethine nitrogen atom to the metal ion [17, 18].

The spectrum also showed broad bands in the region of 3373.75 and 347.9cm $^{-1}$ which may be due to $\upsilon(OH)$ and the



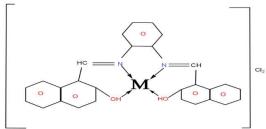
broadening of the same was due to intermolecular H-bonding between OH groups [19]. These bands were missing in the metal complexes indicating that the OH character of the ligand has been lost upon complexation. The various absorption bands for the ligands and the complexes in the region 1176.15-1161.31 cm⁻¹ may be assigned to $\upsilon(C-C)$ aromatic structure. Vibrations in the regions around 432-469.97 cm- have been assigned to $\nu(M-O)$ and 396.5.-396.99 cm⁻¹ $\nu(M-N)$ bands. The bands may be due to the established coordination of the ligand to the respective metal ions in each complex compound. These agreed with the result obtained elsewhere [16,17,18]. Finally, the ether group, v(C-O) has bands around 1207.94-1282.36 cm 1 and has a shift to lower frequencies probably due to the conjugation with the bond and partly because of resonance effects and this also confirms the participation of the ether group or the aromatic ring in the coordination of the metal and the ligand.

Electronic spectra and magnetic moment of the ligand and its metal complexes

A useful tool for the evaluation of results provided by other methods of structural investigation is the electronic spectra also known as the ultra-violet visible spectra. It is used to designate or assign the stereochemistry of metal ions in a complex as shown by the positions and numbers of *d-d* transition peaks. The ligand and the metal complexes absorb in the visible region due to charge transfer processes from the energy in the visible light and hence, provide evidence that they are all coloured compounds. The structure of the complexes was elucidated by the absorption bands they exhibited. The electronic spectra are

represented in Table 5. The most useful transitions for analysis are the intense $\pi \to \pi^*$ transitions and the weaker, but lower energy, $n \to \pi^*$ transitions. The electronic spectrumof the ligand BHNOPDA showed a band at 426.00 nm due to the $\pi \to \pi^*$ transition of the chromophore (-C=N-), suggestive of a tetrahedral geometry [17].

The UV-visible absorption spectra of all the metal complexes showed similarities, which indicates similarity in their structures and generally showed the characteristic bands of the free ligands with some changes both in frequencies and intensities. Upon complexation, the absorption bands of the complexes are to some extent shifted to shorter wavelength compared to those of the ligand. These modifications of the shifts and intensity of the absorption bands indicates the coordination of the ligand to the metal ion. In the UV-region, Mn(II) complex showed absorption band at 423.00 nm, which may be assigned to ⁴E_g-⁶A_Ig transitions, and the magnetic moment of Mn(II) complex was 2.0.B.M. Both the electronic spectra and the magnetic values suggested a tetrahedral geometry for Mn(II) complex. The electronic absorption of Fe(II) complex showed one band at 400.0 nm. This band is assigned to a ${}^{4}T^{2}_{\sigma}(G)$ transition [19], and the magnetic moment of Fe(II) complex was 3.75 B.M. These results suggested a tetrahedral geometry for Fe(II) complex -4A2 transition, [20]. The magnetic moment Mn(II) and Fe(II) complexes also indicated their paramagnetic nature, [20]. On the basis of the above observations and spectral data, it is suggested that all the metal complexes show tetrahedral geometry structures as presented on scheme 2.



Scheme 2: Proposed general struture of the metal complexes

Nematicidal studies of the ligand and its metal complexes

Hatching of root knot nematode at different concentrations

The hatching values of root knot nematode of different compounds at different concentrations and at different intervals of exposure were studied and presented in [22]. This analysis was carried out for 10days and the observations were made after every 2days.

Generally, the amount of unhatched nematodes was found to decrease as the time of exposure increased for both the ligand and all the metal complexes. And this was found to be more effective than the metal complexes. In case of metal complexes, Mn(II) metal complex showed a steady decrease in the unhatching as the time of exposure increased. While Fe(II) complex was found to be extremely effective from the other metal complexes, having the highest number of unhatched nematodes. Generally, it was found that at lower concentrations hatching was more effective as maximum hatched count was observed in Schiff base ligand. This

observation is in agreement with other findings [16, 17, 21]. The rank order of unhatched eggs as a function of chelated metal ion follows the order Fe>Mn ions. The hatching rate of the metal complexes signifies that the ligand failed to cause significant nematicidal activity than the metal complexes

Mortality of root knot nematode at different concentrations

The mortality values of root knot nematode of different compounds at different concentrations and at different intervals as shown in Figures I to 4.The mortality values of root knot nematode of the synthesized compounds at three different concentrations and at different interval of exposure were recorded and presented in Figures 5 to 6. This test was done using the synthesized Schiff base ligand and its metal complexes at three (3) different concentrations each, and the analysis was completed within 6h in which the observations were made every after 2 h. In each of the treatment, exactly eight (8) second juveniles were placed and their mortalities were observed and recorded.



From the results of the analysis, it was generally observed that the number of unsacrificed nematodes decreases as the time of exposure increased for both the ligand and all the metal complexes. And that for the ligand was found to be less effective than the metal complexes. And for the metal complexes, Fe²⁺ complex possessed the highest activity than Mn²⁺. It was also observed that the number of unsacrificed nematodes was higher at lower concentrations, but lower at higher concentrations. This implies that mortality rate of nematodes increases with increase in concentration and decrease with decrease in concentration. This analysis was carried out for 10days and the observations were made after every 2days. Nematode mortality attributed to the Schiff base was found to be less effective compared to the mortality attributed to the

metal complexes. This is as a result of the synergistic effect arising from complexation between the ligand and the metals [17, 18]. As the time of exposure increased there was a decrease in mortality rate recorded. At higher concentrations there was increase in mortality rate of the nematodes, while at low concentrations there was decrease in the mortality rate. Results of the nematicidal activity reflected that the metal complexes are more toxic to root knot nematodes as compared to the Schiff base ligand, and the metal complex made up of iron has the highest toxicity than manganese.

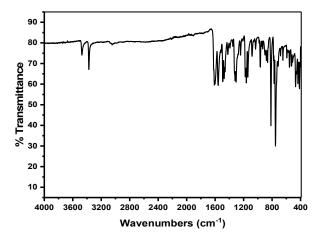


Figure 9: FTIR spectrum for BHNOPDA

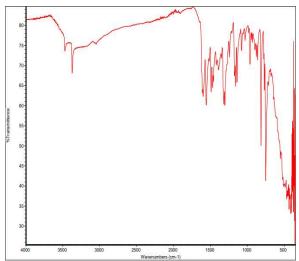


Figure 10: FTIR spectrum for Mn (II) BHNOPDA



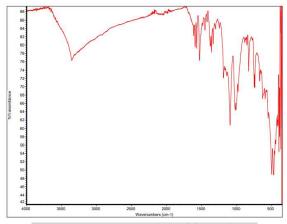


Figure II: FTIR spectrum for Fe (II) BHNOPDA

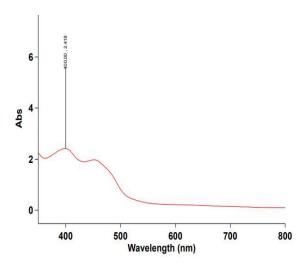


Figure 12: UV-Visible Spectrum for Fe(II)BHNOPDA

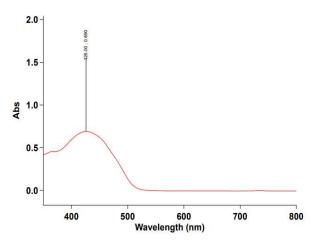


Figure 13: UV-Visible Spectrum for BHNOPDA

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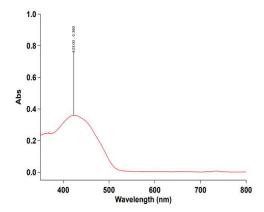


Figure 14: UV-Visible Spectrum for Mn(II) BHNOPDA

Conclusion

The Schiff base BHNOPDA and its corresponding metal (II) complexes of Mn(II) and Fe (II), were synthesized and characterized successfully. The high molar conductance value indicates that the complexes are electrolytic in nature. Based on the physiochemical and spectral studies, a tetrahedral geometry have been suggested for all the complexes. The Schiff base and its metal complexes were insoluble in water, but soluble in most organic solvents. In the present studies, all the synthesized compounds had shown nematicidal activity against root knot nematode (Meloidogyne incognita), by inhibiting egg hatch and causing second stage juveniles mortality and the nematicidal activity of these compounds was found to be dose and time dependent. Egg masses of the nematodes were

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obtained from the roots of heavily infected okra (Abelmoschus esculentus) for studies. The hatching was observed after every one day interval until a complete hatch rate was observed. And same was maintained for mortality, but at an interval of 2 h until a complete mortality was observed. All the compounds showed complete hatch inhibition and mortality at concentration above 10 ppm, whereas the compounds at low concentrations showed less hatch inhibition and mortality. The results indicated that egg hatch inhibition and mortality increased with increase in concentration of the compound, but decreased as the time of exposure increases.

Declaration of conflicting interests

The authors declared no potential conflicts of interest.

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