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Synthesis, characterization and antifungal activity of 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene and its complex with iron (III)

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ABSTRACT

3-Hydroxy-3-p-tolyl-1-m-nitrophenyltriazene and its complex with Iron (III) were synthesized and characterized using several physical techniques like elemental and spectral analysis and their antifungal activity was screened. It was observed that 3- hydroxy-3-p-tolyl-1-m-nitrophenyltriazene and its complex with iron (III) showed inhibitory activity at 100 ppm against 3 fungi; Rhizoctonia Solani, Fusarium Solani and Pythium Aphanidermatum. Thus the study has brought about a novel application of this class of analytical reagents as an emerging class of bioactive chemicals.

Keywords: Hydroxytriazene, antifungal activity, Iron (III), *Rhizoctonia Solani, Fusarium Solani* and *Pythium Aphanidermatum.*

INTRODUCTION

Chemistry of co-ordination compounds containing ligands such as hydroxytriazenes[1-2] has received much attention owning to their biological activities. Recently they have also been screened for their biological activity in our laboratory[3-11].Both hydroxytriazenes as well as their metal complexes have been reported for such activities.

We here report antifungal activities of 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene (HTNPT) and its Fe (III) complex. The fungi taken are *Rhizoctonia Solani, Fusarium Solani* and *Pythium Aphanidermatum*. The results obtained have been compared with commercial fungicide Bavistin. 3-Hydroxy-3-p-tolyl-1-m-nitrophenyltriazene (HTNPT) and its iron (III) complex (C) were characterized before screening.

EXPERIMENTAL SECTION

2.1 Synthesis of 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene

***2.1.1 Synthesis of p-tolyl-hydroxylamine*:** In a one litre beaker (0.1 mol) of p-nitrotoluene, 5 gm of NH_4Cl 50 ml water and 50 ml C_2H_5OH were mixed, stirred mechanically and cooled to 0°C by surrounding the beaker with ice salt mixture, 20 gm Zn dust was added in small lots such that the temperature of reaction mixture remained between 50 to 60°C. Addition of Zn dust was completed in 40 min. The reaction mixture was stirred mechanically for another 15 min. The solution was filtered under suction and washed with ice cold water. The filtrate was taken in a beaker and kept in freezer and used as such for coupling with diazotized product.

***2.1.2 Diazotization of m-nitroaniline*:** In a 500 ml beaker 0.1 mol (14.0 mL) of m-nitroaniline was dissolved in warm mixture of 25 mL of concentrated HCl and 25 mL of water. After constant stirring the mixture was kept in a freezer to cool. In another beaker 6.9g of $NaNO_2$ was dissolved in 20 mL of distilled water and kept it in the freezer. The beaker which contained m-nitroaniline solution was put in an ice bath to maintain temperature between 0 to 5ºC. To this The $NaNO_2$ solution was added drop by drop with continuous stirring. The diazotized product so obtained was directly used for coupling.

***2.1.3 Coupling*:** The p-tolylhydroxylamine prepared in step (a) was coupled with the diazotized product of (b) step at 0 to 5°C under mechanical stirring with occasional addition of sodium acetate solution for maintaining the pH close to 5 during coupling process. The compound 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene (HTNPT) was obtained as greenish yellow powder after crystallization from ethanol. Melting points of all synthesized compounds were taken in open capillaries and are uncorrected. C H N analysis corroborated the purity of compound. The compound was subjected to IR spectral analysis and following bands were observed. IR (KBr) cm-1: 3249 (O-H str.), 3078 (C-H str. Ar), 2981 (C-H str., CH_3), 1632 (N=N str.), 1419 (N-N str.).The spectra showed the compound to be in pure state. IR spectra (KBr) were recorded on FT IR RX1 Perkin Elmer Spectrometer. Physical and analytical data are given in Table 1 and the structure of HTNPT has been given in Figure 1.

Table 1: Elemental analysis of 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene (HTNPT)

Molecular Formula of (HTNPT)	Molecular Weight	M.P. ºC	Elemental Analysis			
				C%	N%	H%
$C_{13}H_{12}N_4O_3$	272.26	143	Th.	57.35	20.58	4.44
			Exp.	56.93	19.25	4.05

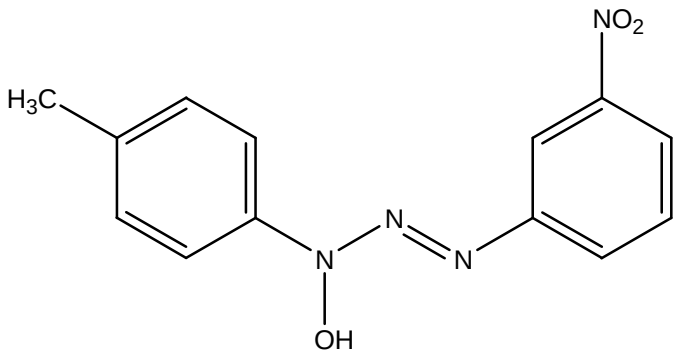

Figure 1:Structure of 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene

2.2 Preparation of iron (III) complex with HTNPT

It has been established that Fe (III) forms instantaneously dark brown complex with 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene (HTNPT) in acidic medium (pH=3.1-4.2).The composition of Iron complex with HTNPT has been established by Job's and Mole Ratio methods. The composition of Fe (III) complex has been found to be 1:3(Fe: A). The requisite quantity of 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene (HTNPT) (2.72gm) was dissolved in minimum quantity of ethanol. Similarly requisite quantity of A.R. grade Fe $(NO_3)_3.9H_2O$ (1.33gm) was dissolved in double distilled water. pH of the solution as well as HTNPT solution were adjusted in the range 3.1-4.2 by using tris buffer or perchloric acid. Both the solution was warmed. This was followed by addition of hot solution with continuous mechanical stirring. The ratio in which HTNPT and Iron were mixed was 1:3 (Fe: HTNPT). After complete addition of iron solution, the reaction mixture was further stirred for five minutes and kept aside for ten minutes. The dark brown complex formed was filtered under suction and washed with double distilled water to remove un-reacted metal ion. It was then dried and recrystallised with alcohol. Complex formation has been verified by elemental and spectral analysis. Physical and analytical data are given in Table 2. The tentative structure of 1:3 complex of Fe (III) with HTNPT has been given in Figure 2

Table2: Elemental analysis of 1:3 complex of Fe (III) with 3-hydroxy-3-p-tolyl-1-m-nitrophenyltriazene

Molecular Formula of Complex	Molecular Weight	M.P. °C	Elemental Analysis				
				C%	N%	H%	Fe%
$Fe[C_{13}H_{11}N_4O_3]_3$	869.60	172	Th.	53.87	19.33	3.82	6.44
			Exp.	53.69	19.19	3.71	6.31

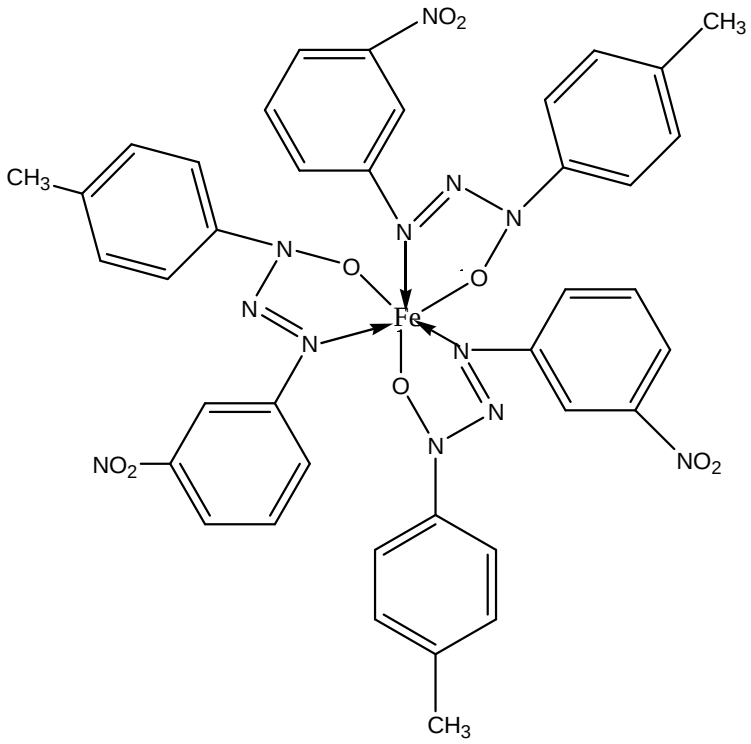

Figure 2: The tentative structure of 1:3 complex of Fe (III) with 3-hydroxy-3-p-tolyl-1-m-nitro phenyltriazene

2.3Bioassy

Following method was used to determine the antifungal activity.For fungal bio-assay Potato Dextrose Agar (PDA) medium was used.

2.3.1 Cleaning and Sterilization:

Corning and Borosil glass wares were used for the experiment. Cleaning was done by dilute chromic acid followed by Teepol. Glasswares were thoroughly washed with distilled water and dried before being autoclaved in hot oven at 180º C for 2 hrs. Throughout the experiment the media (PDA) was sterilized by autoclaving at pressure of 1.045 kg/cm2 for 20 minutes. Further, polyethylene bags were sterilized using 5% formalin.

2.3.2Preparation of fungal bioassay medium

For fungal bioassay, potato Dextrose agar medium (PDA) was used. Potato (250gm) was cut into pieces and boiled in 1 lt. distilled water. It was sieved through muslin cloth and volume was again made to 1 lt. After mixing 20 gm of agar-agar powder and 20gm of dextrose, it was boiled again and then filtered through muslin cloth. Medium was then transferred to sterilized conical flasks which were again sterilized by autoclaving at 15 lbs/cm^2.

2.3.3Pathogenecity test

Each of chemical i.e. HTNPT or its respective Fe (III) complex was studied at 100 ppm invitro against the individual pathogens using poisoned food techniques. The test compound was weighed and dissolved in acetone or alcohol as per solubility of compound. They were mixed with desired quantities of sterilized PDA medium under aseptic condition and were then transferred to pre-sterilized Petri plates. On the other hand only medium without test compound was kept to observe control. A 5mm disc of 7 day old culture of each fungus was inoculated at center of each Petri plate. The inoculated Petri plates were incubated in BOD incubator at 30±° C. the treatment was repeated thrice. Observation were recorded when control plates were full of mycelial growth. Mean was calculated for the values obtained.

RESULTS AND DISSCUSION

The table-I shows the result of antifungal studies.

Table-I Effect of HDNPT and its complex with Fe (III) on mycelia growth rate along with percent inhibition at 100ppm on different fungi

Fungi	HTNPT		Fe(III)-HTNPT Complex		Bavistin	
	Mycelial growth	PI	Mycelial growth	PI	Mycelial growth	PI
Rhizoctonia Solani	51.83	42.40	65.00	27.74	00	100
Fusarium Solani	64.83	27.96	38.66	57.03	00	100
Pythium Aphanidermatum	88.33	01.85	88.33	1.85	00	100

** PI = percent inhibition*

Perusal of results in case of test compounds reveal that HTNPT has PI value 42.40% against *Rhizoctonia Solani* where as its Fe(III) complex is far more efficient showing PI values 27.74%. In case of *Fusarium Solani* results reveal that reagent HTNPT shows PI value 27.96% while its complex has PI 57.03%. In case of *Pythium Aphanidermatum* the reagent HTNPT has almost negligible activity even its complex is effective to same extent.

CONCLUSION

In the present study it seems that although the ligand HTNPT is active against the fungi inhibition, its complex is more effective. This is certain that HTNPT as well as its metal chelate can be suitably used as antifungal agents.

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