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Evaluation of Nano-formulated Heteroleptic Metal Complexes as Potential Antifungals against *Fusarium oxysporum*

Gumber K[1*], Sidhu A[1], Sharma VK[2]

[1]*Department of Chemistry, Punjab Agricultural University, Ludhiana, India*
[2]*Department of Plant Pathology, Punjab Agricultural University, Ludhiana, India*

ABSTRACT

The established antifungal moieties viz. 1,2,4-triazole, dithiocarbamate and phosphorous were brought in a single molecule to give a series of metal complexes of cobalt, copper and iron. The synthesized derivatives were further improvised with an essence of nanotechnology for the better application of the non-soluble molecules to get nano-sized dispersion in water. The formulated target compounds were evaluated for their fungicidal potential against Fusarium oxysporum, by poisoned food technique. The overwhelming results were indicated with compound 5 being the best (EC_{50} value 4.80 ppm).

Keywords: Antifungal, *Fusarium oxysporum*, Nano-formulation, Triazole, Dithiocarbamate, Triphenyl phosphine, Heteroleptic, Metal complexes

INTRODUCTION

Organophosphorous, dithiocarbamates and 1,2,4-triazoles are bioactive moieties used worldwide, in agrochemicals, pharmaceuticals and other industrial fields [1-3]. Dithiocarbamates and 1,2,4-triazoles are the heterorganic moieties with moderate toxicity [4,5]. Organophosphorus also forms a widely used class of pesticides which are still needed at the time of sudden and severe outbreak of diseases. But their toxicity and tendency to develop resistance, demands their redesigning, so as to use them as resistance measure as and when required [6,7]. On the other hand, complexation of heterorganic ligands with bioactive metals has an immense potential to portray an augmented bioactivities to them, which is further influenced by the design and bioactivities of the ligands along with the type of the metal ions used [8].

Most of the organic and inorganic molecules fail to reach the target site due to their poor solubility leading to the poor applicability of the bioactive molecules [9]. The essence of nanotechnology, i.e. preparing the nano-formulations of the bioactive molecules seems to provide solution to the problem of water insolubility. The nano-formulations also impart diversified topological parameters which are responsible for improved bioactivity than the molecules in bulk providing additional benefits with greater effectiveness at low doses [10].

In the multi-component regime for the synthesis of bioactive molecules, we endeavoured to design complexes of Cu (II), Co (II) and Fe (III) with heteroleptic ligands *viz.* 1,2,4-triazole-dithiocarbamate, triphenyl phosphine and isothiocyanate, in variable ratios. The synthesized complexes were converted to nano aqua formulations for evaluation of their antifungal potential against the test fungi *Fusarium oxysporum*, using Tilt (Propiconazole) as positive control. The *in silico* toxicity analysis of the prepared complexes has been done for rationalization of the results.

MATERIALS AND METHODS

All the reagents and solvents were commercially available, analytical grade materials and were used as supplied, without further purification. Deionized water was used for preparation of all the aqua formulations. The antifungal activity of the nano-dispersions was checked by established method against the phytopathogenic fungus of rice, *F. oxysporum.*

General Method for the Synthesis of Heteroleptic Metal Complexes (1-12)

1,2,4-Triazole/4-amino-1,2,4-triazole (0.015 moles) was dissolved in 10 ml of methanol and potassium carbonate (1.5 g) was added to it. The reaction mixture was cooled by keeping in ice bath and carbon disulphide (1.5 ml, 0.02 mol) was added to the stirring mixture. The mixture was stirred till the appearance of reddish yellow coloration. Triphenyl phosphine (0.01/0.02 mol) dissolved in small amount of chloroform and sodium thiocyanate (0.01/0.00 mol) dissolved in distilled water was dropwise added to the same flask under vigorous stirring followed by the addition of aqueous solution of metal chloride (0.01 mol) in the same reaction mixture with formation of reddish brown precipitates. The reaction mixture was stirred for additional 1 h and the precipitates so formed were separated and washed with distilled water followed by

chloroform to obtain the pure solid, which was dried under vacuum and stored for further use [11].

Standardization and Synthesis of Nano-formulations of Metal Complexes

The synthesized metal complexes (1-12) were finely grounded using a pestle mortar. Different amounts *viz.* 1.0, 0.5, 0.2 and 0.1 g of the metal complexes were mixed with 0.2 g of PVP and the mixture was slowly dispersed in 100 ml of distilled water containing 0.2 g of SDS, while ultrasonication. The sonication was continued for 20 minutes and the nano-dispersion was allowed to age for 1 hour. The formulation with clear appearance containing maximum amount of solid complex by weight were analysed by TEM micrographs to get the optimum amount required to prepare 100 ml of nano-formulation. All the other nano-formulations were prepared by the same standard method and were diluted to 500 ppm on active ingredient basis, and stored as stock solution for antifungal evaluation.

Antifungal Assay

The *in vitro* antifungal activity of all the compounds were performed by Poisoned food technique [12,13] against phytopathogenic fungi, *F. oxysporum* in comparison with the standard fungicides Tilt (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole). The isolates of phytopathogenic fungi were provided by the Plant Pathology Department of the Punjab Agricultural University and the standards, which served as the positive control was obtained from their respective manufacturers.

***In silico* Toxicity Analysis**

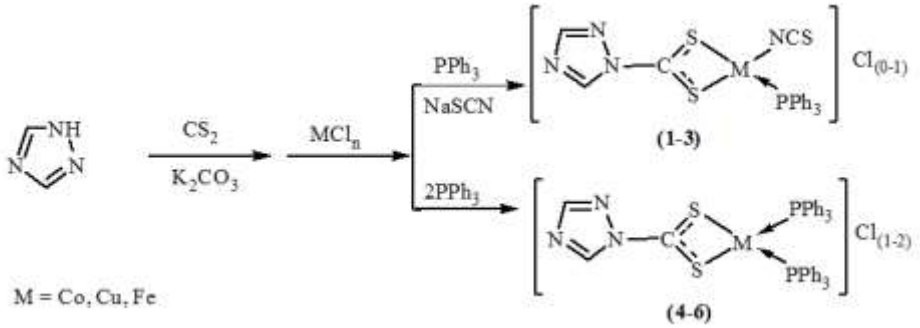
Toxtree v2.6.6 is an open-source software application that places chemicals into categories and predicts various kinds of toxic effect by applying different decision tree approaches. Toxtree was developed by Idea Consult Ltd. (Sofia, Bulgaria) under the terms of an ECB contract. The software is made freely available by ECB as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity. The new module with the revised list of SAS includes also structure–activity relationships (SAR) models that enable the toxicity evaluations for a number of chemical classes to be fine-tuned.

In order to find out the toxic hazards of all the synthesized compounds, two dimensional models of the compounds were first converted into its simplified molecular-input line-entry system (SMILES format) using an online SMILES translator. Then simply putting the SMILES code into the Chemical identifier row available in the Toxtree software we can easily get the toxic characters.

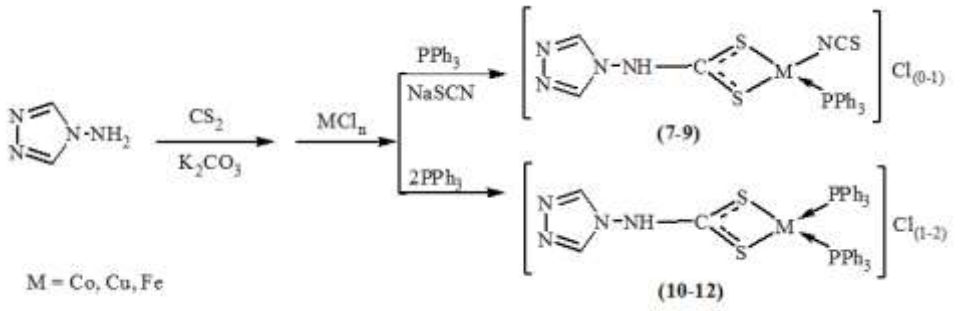
RESULTS AND DISCUSSION

Chemistry

The heteroleptic complexes were prepared according to the synthetic procedure shown in scheme 1 and scheme 2. Dithiocarbamate ligands were first prepared by reaction of 1,2,4-triazole and 4-amino-1,2,4-triazole with carbon disulphide in basic medium followed by addition of metal salt and triphenyl phosphine along with the addition of sodium thiocyanate in two different molar ratios to yield heteroleptic metal complexes (1-12).



Scheme 1: Synthesis of metal complexes of 1,2,4-Triazole



Scheme 2: Synthesis of metal complexes of 4-Amino-1,2,4-Triazole

The complexes formed were stable but insoluble in most of the solvents leading to its poor applicability as antifungal agent. Thus, the essence of nanotechnology was added to make the biologically active molecules water dispersible with help of surfactant SDS and stabilizing them by coating with PVP. The synthesized nano-aqua formulations at different concentrations were prepared, and evaluated for their larvicidal potential (Figures 1 and 2).

Figure 1: Nano aqua formulations of Complexes of Cobalt and Iron with heteroleptic ligands

Antifungal Assay of the test compounds

As shown in Table 1, many of the title compounds showed good control efficacy against *F. oxysporum*, tested at variable concentration *viz.* 500, 250, 100, 50, 25 and 10 µg/ml. Most of the test formulations inflicted the excellent fungitoxicity with EC_{50} values less than 60 µg/ml with some of the complexes (2, 3, 4, 5, 6 & 9) overpowering the existing triazole standard, Tilt (EC_{50} value, 10.79 µg/ml) against the test fungi. Notably, the comparison between the 1,2,4-triazole and 4-amino-1,2,4-triazole analogues showed the edge of the former over the latter. The presence of triphenyl phosphine in higher molar ratio in the complex molecules also inflicted the synergistic results. For example, compound 4 and 5 displayed excellent inhibition with EC_{50} values better than the standard Tilt. The change in bio-efficacy of the tested metal complexes was attributed to the variable ligand system attached to the different metal centre. The enhanced activity was ascribed to the nano-sization of the metal complexes [14]. The better nature of copper and iron complexes in comparison to the cobalt analogues were favoured by the earlier reported work that supported the excellent fungitoxicity of the iron complexes with the polydentate dithiocarbamates ligand against *Phomopsis viticola* [15].

Table 1: Antifungal Assay of nano-formulated metal complexes at different concentrations against *Fusarium oxysporum*

S. No.	Concentration/Percentage inhibition							EC_{50} (µg/ml)
	500	250	100	50	25	10	5	
1	80.25	72.50	56.45	48.63	43.92	35.45	24.25	38.57 (20.00-66.85)
2	95.63	88.75	77.45	72.49	67.85	60.25	50.35	6.21 (2.45-11.17)
3	94.35	82.50	77.50	73.28	65.45	56.25	48.75	7.12 (2.61-13.19)
4	95.68	90.65	83.75	77.84	68.42	59.35	52.75	5.48 (3.24-8.13)
5	97.50	90.90	80.25	75.25	70.46	62.45	55.45	4.80 (1.69-9.03)
6	86.25	80.05	75.45	69.45	62.15	52.50	40.65	9.76 (5.78-14.53)
7	83.75	75.15	62.50	45.25	28.65	20.45	10.56	57.77 (41.89-71.02)
8	95.24	86.25	80.25	74.65	68.42	45.35	35.43	11.71 (8.41-15.40)
9	100	90.45	77.50	69.25	63.85	56.25	48.95	8.28 (2.83-15.50)
10	92.25	77.50	53.25	45.25	38.45	32.68	25.20	38.95 (19.65-69.86)
11	90.24	73.75	56.25	45.80	36.42	25.85	15.26	48.82 (32.24-72.19)
12	95.24	90.20	76.25	67.25	58.42	45.35	32.50	15.17 (11.41-19.36)
Tilt	100	100	100	100	90.35	45.62	10.23	10.79 (9.72-11.97)

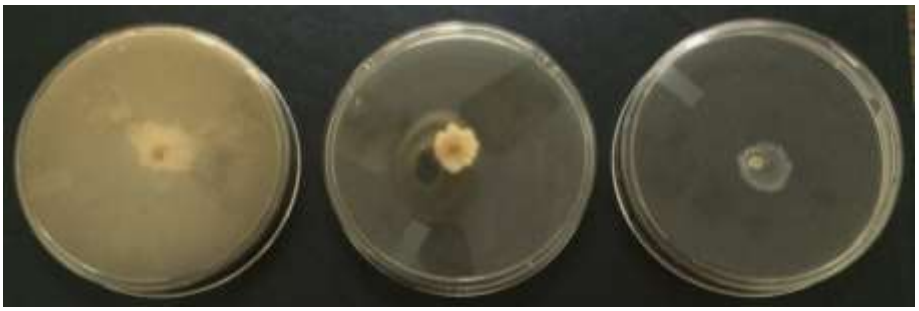


Figure 2: Zone of inhibition for complex 5 at 100 and 50 ppm along with the control set against *Fusarium oxysporum*

CONCLUSION

The heteroleptic metal complexes and their nano-formulations can be considered as a better alternative due to lower toxicity and water dispersibility. Further tailoring and alteration of the effective moieties is recommended to get more effective and eco-friendly solutions. Studies are currently underway to optimize and enhance the activity of the complex derivatives.

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