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Research Article

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SYNTHESIS, CHARACTERIZATION AND BIOCIDAL EVALUATION OF AZOLE-BASED LIGANDSMETAL COMPLEXES

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Abstract

Different metal complexes of the azole-based ligands have been synthesized and characterized based on the solubility, percentage yield, melting points and conductivity a well as the antimicrobial evaluations on the selected fungi species of plant pathogens. The studies revealed that solid metal complexes were soluble in 80% water and 20% (DMSO) dimethylsulphuroxide and the percentage yields were of appreciable high while the conductivity results showed that metal complexes were non-electrolytes. The solid complexes were also screened against the fungi species: *Rhizoctonia solani, Pythium aphaindermatum, Rhizoctonia cerealis, Sclerotium rofisil, Phyphotoria palmivora* (causative agent of black pod diseases) and *Benlate* a commercial anti fungi agent (as control).The results of the present studies confirmed that metal complexes had good inhibitory actions on the growth of the fungi species and metal complexes appeared to be more proactive on the tested organisms than the free ligands.

Key words: Synthesis metal complex, azole-based ligands, anti-fungi activities

Introduction

Azoles are parts of the larger family of sulphur and nitrogen containing organic compounds and metal their complexes which display a broad range of biological activity, finding applications ant –tumor, antibacterial, antifungi and antiviral agents. 1,2,4-triazole is also exhibiting excellent bioactivities have particularly multifarious uses in agriculture, medicine and industry (Shen, 2001).

Triazoles are five–membered rings which contains three nitrogen atoms and two carbon atoms at non-adjacent positions in the system (Finar, 1998).The chemistry of these azoles becomes increasingly different from that of pyrrole as the number of nitrogen atoms increases.(Barluenga*et al.*, 2006).Benzimidazole is a alicyclic compound having imidazole ring containing two nitrogen atoms at some non- adjacent position fused to benzene(Finar, 1989).

Transition metals have varying utility and interesting chemistry coordination compounds are important due to their roles in biological and chemical systems in various ways. It has been observed that metal complexes with appropriate ligands are chemically more significant and specific than the metal ions and original (Godard*et al.*, 1994; Jarrehpour, 2004).

Metals complexes of biological important ligand are sometimes more effective than free ligand (Ahuja et *al.*, 1997). Metal ions plays vital roles in a vast number of widely differing biological processes. They have a considerable effect on biological processes and depending on their concentration, they either contribute towards the health of an organism or causes toxicity (Roos and Williams, 1997).

The concentration of biocides has been deemed to be the most important factor that affect its efficacy (Russell and McDonell, 2000).In the case of bacterial boifilm, the biocide concentration and consequently the bacterial susceptibility, is affected by the reduced diffusion of active molecules through the bio film (Anderson O Toole, 2008). Some Schiff bases having heterocyclic residues which possess biological activities, such as analgesic, antiviral, antifungal and anticancer (Godard *et al*., 1994). Among the ligand attracting the largest interest for their roles in binding metals in metalloproteins are the imidazole derivatives. Inspection of mercaptobenzimidazole indicates that it can exist in two tautomeric forms with potential metals binding sites between the N and S atoms (Bouwmann *et al*., 1995).

In Nigeria and other African countries various formulation of copper including Bordeaux mixture(copper sulphate)

Cuprous oxide and copper oxy chloride under field conditions had been used and is currently being used for the chemical control of*Phyphotoriapalmivora*, the causative agent of cocoa pod rot diseases.(Gorenz and Okaisobor,1971).

This present work aims at synthesis, characterization and evaluation of antifungal activities of Azole-based metals complexes in water and methanol media.

Experimental

All chemicals used were of analytical grade and used without further purification. The reagents used include 1, 2,3-triazole, benzimidazole, (ligands) and metal salts are $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, $NiSO_4.6H_2O$ and $ZnSO_4.7H_2O$. Others are distilled water, methanol, DMSO etc.

Instrumentation

Melting points were determined using open capillary tube method, molar conductance of the solid complexes in water –DMSO (80% -20%). Antimicrobial studies were carried by Agar disc diffusion (poison) principle

Synthesis of benzimidazole metal complexes in water and methanol media.

Benzimidazole metal complexes were synthesized as follow:

Copper(II)benzimidazole complex was synthesized in water medium by dissolving 0.5g $CuSO_4$ $.5H_2O$ and 0.476g ligand benzimidazole (0.1M) separately in water and mixed directly on a magnetic stirrer with hot plate for 3hours until a green precipitate solution formed .

Cobalt (II) benzimidazole followed the same procedure but cobalt (II) salt dissolved in water and heated to 60^0c before the ligand was added. Zinc (II) benzimidazole was prepared in similar way as well as Nickel (II) benzimidazole.The same procedure was followed in methanol.

The different precipitation solution of metal complexes were washed in distilled water followed by methanol ,filtered via suction pump, dried over a silica gel until constant weights obtained.

Synthesis of triazole metal complexes were as follows:

Various metal complexes were prepared by direct mixing of a homogenous solution of 0.1M (0.5g/20ml) $CuSO_4.5H_2O$,(0.476g/20ml) $CoCl_2.6H_2O$, (0.526g/20ml) $NiSO_4.H_2O$ and(0.574g/20ml) $ZnSO_4.7H_2O$) and 0.2mol ligand(1,2,3-triazole) 0.276g/20ml in distilled water medium . The mixture was agitated magnetically on a hot plate for 2hrs and no precipitate formed until a 0.1mol. NaOH dissolved in distilled water added, stirring continued for another 1hr until a precipitate solution of different colour formed for the respective metals.

The same procedures were adopted in methanol solution. The products precipitated as solid were filtered, washed with suitable solvents, dried over silica gel for 5 days in a desiccator until constant weight achieved.

Biological activity of the complexes

Table 1: The results of the physical properties of Azole-based metal complexes

Compound	Medium	Colour	% yield	Solubility	MeltingPoint	Conductivity
Copper (II) benzimidazole	H_2O	Green	49.5	DMSO-H_2O	238-240	4.13
	Methanol	Deep green	73.5	„	178-180	3.8
Cobalt (ii) benzimidazole	H_2O	Purple	67	“	253-255	1.0
	Methanol	PurplePink	71	“	255-25	1.1
Nikel (ii) benzimidazole	H_2O	Green	56.0	“	238-240	4.2
	Methanol	Light green	59.60	“	239-241	4.0
Zinc (ii) benzimidazole	H_2O	White	43	“	233-235	4.2
	Methanol	White	58.5	“	257-259	4.7
Copper (ii) 1, 2, 3, triazole	Water	Light green	36.5	“	240-242	2.8
	Methanol	green	47.6	“	245-247	4.2
Cobalt (ii) 1,2,3, triazole	Water	dull green	23.13	“	230-232	3.2
	Methanol	Dull green	26.46	“	240-241	7.5
Nickel (ii) 1,2,3, triazole	Water	Green	36.00	“	250-252	4.6
	Methanol	Green	53.33	“	253-255	7.0
Zinc (ii) 1,2,3, triazole	Water	White	55.06	“	255-257	1.0
	Methanol	White	66.17	“	260-261	1.6

Antifungal evaluation of the Complexes:

Five organisms screened for this work were*Pythiumaphanidermatum, Rhizotonia cereals, Sclerotiumrofsil, Rhizotoniasolani and phyphotoniapalmirora* (causative agent for cocoa pod disease). The 1,2,3-triazole and its metal complexes were directly added to the growth media in varying concentrations (2.5% and 5.0%).5ml of each prepared sample was measured into a conical flask (50ml),25ml of sterile Potato Dextrose Agar was added and mixed properly before pour plating and allowed to set at ambient temperature. A sterile 5mm diameter cork borer was used to inoculate the fungal isolates grown over a period of 72hrs at the centre of the plate. The plates were incubated at $27^0C \pm 2^0C$ for 5-7days.The radial growth of the mycelia of the fungi isolates were measured for every24hrs. The diameter of the zone inhibition produced by the complexewas compared with the benlate(Onifade,1998; Mistra , 1995).

Results and Discussion

The solid complexes were also screened against the fungi species namely *Rhizoctonia solani, Pythium aphaindermatum, Rhizoctonia cerealis, Sclerotium rofisil, Phyphotoria palmivora* (causative agent of black pod diseases) and *Benlate* a commercial anti fungi agent was used as control.The results of these studies are shown in Table-1; Fig-1 to 8 which confirmed that metal complexes had good inhibitory actions on the growth of the fungi species and metal complexes appeared to be more proactive on the tested organisms than the free ligands.

Different metals complexes were synthesized at different concentrations and media. The % yields of the metal complexes were of reasonable yields especially in methanol medium benzimidazole metal complexes have better yield than 1,2,3-triazole due to its larger size (Vogel, 1998) The colours of the metal complexes in both ligands have shown that ligands have much effect on the colour formation. The colours ranged from green to white. The zinc complexes in both benzimidazole and the 1,2,3-triazole gave white this is largely due to the complete filled $3d^{10}$ configuration in which the promotion of elections on d-d transition is prohibited (Lee , 1997; Wilkinson, 1998).

Solubility of the metal complexes appeared to follow the same pattern as they were all soluble in 80% water and 20% DMSO, showing that water increases the salvation energies of the metal complexes, this is no doubt, improves their diffussibility in the cell walls of the microorganisms.

Melting points determined by open capillary tube were sharp, indicating that metal complexes are pure for both the benzimidazole and 1,2,3-triazole in water and methanol media .The results of the conductivity tests on the metal complexes of benzimidazole and 1,2,3-triazole have shown that they were non-electrolytes as the molar conductance in ohm^{-1} cm^2 mol^{-1} in water -DMSO were observed below 20 Ohm^{-1}`cm^2 mol^{-1}, the low values obtained suggest that ligands are monomeric, with a loss of proton or hydrogen which is being replaced by another hydrogen from hydrogen of low conductivity i.e. the conductance decreases owing to the replacement of the hydrogen ion of high conductivity by another cations of low conductivity (vogel, 1998).

The ligands (azole) benzimidazole and 1,2,3-triazole,benlate the commercial anti fungi agent as control and the metal complexes with Cu(II), Co(II), Ni(II) and Zn(II) in both water and methanol media were screened for their antifungal activities against *Pythiumaphanidermatum, Rhizotonia cereals, Sclerotiumrofsil, Rhizotoniasolani and phyphotoriapalmirora* (causative agent for cocoa pod disease), by agar diffusion method at concentration levels of 2.5% and 5.0% wlv medium (DMSO-H_20). The discs after incubating for a period of 24,48,72,96 hours (Figures 1-8) measured with calipier in centimeter and converted to percentage as the % inhibition using Onifade ,1998; Mistra*et al*., 1995 methods). Results were compared with commercial antifungal agent (benlate).

The result of the metal complexes of benzimidazole and 1,2,3-triazole showed great promising activity as antifungal agent although their performances were not as good as the commercial antifungal agent (benlate) where 100% inhibition were recorded on the selected fungi of plant pathogens over a period of 72hrs interaction, it was envisaged that the mycelia growth become insignificant at 72-96hrs.

Copper complexes of benzimidazole did not much inhibition on *Pythiumanphanidermatum ,Rhizoctonia cereals and Sclerotiumrofsil*. These three fungi showed resistant to the effect of copper benzimidazole complexes in both water and methanol but performed better on *Rhizoctonia.solani and Phytophotorapalmvora* which is a well known curative agent for black pod disease in cocoa tree .

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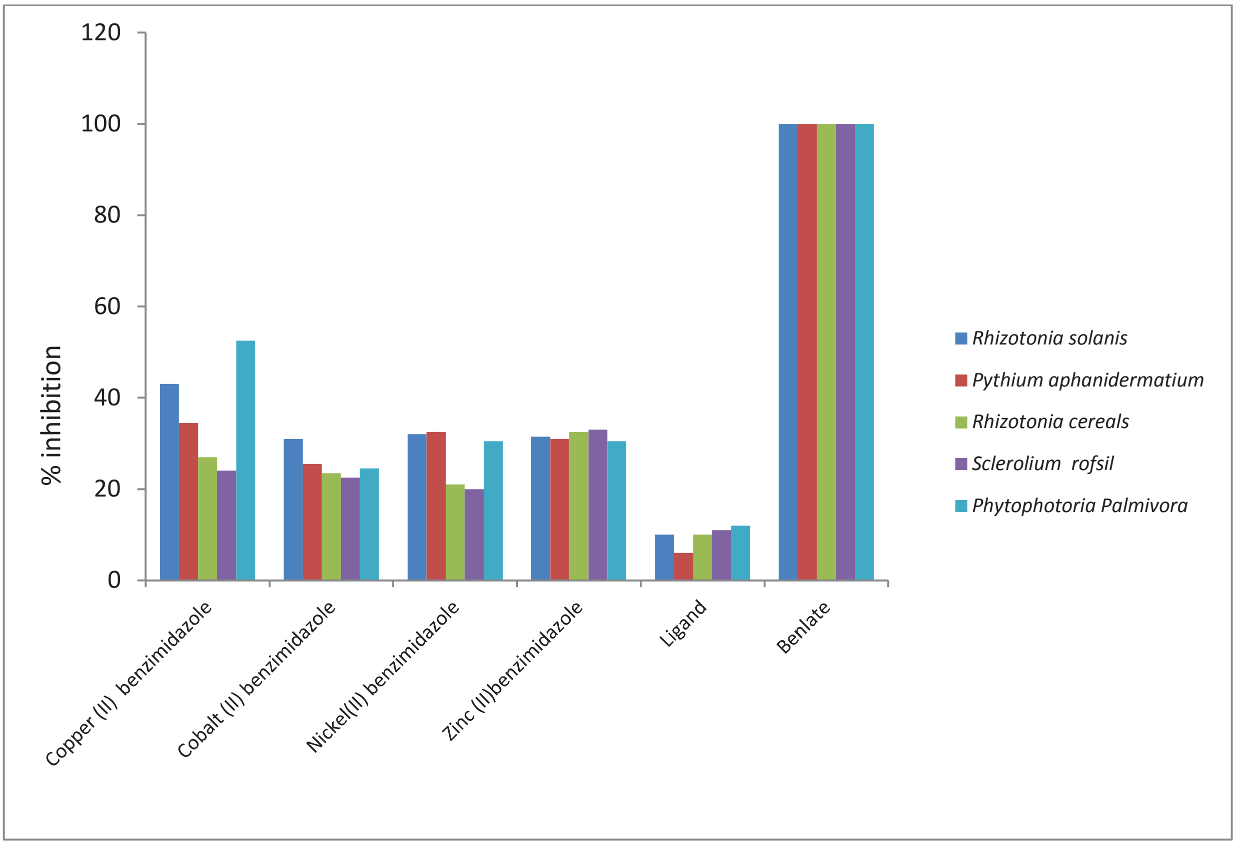

Fig. 1: Antifungal activities benzimidazole metal complexes 25% in water media

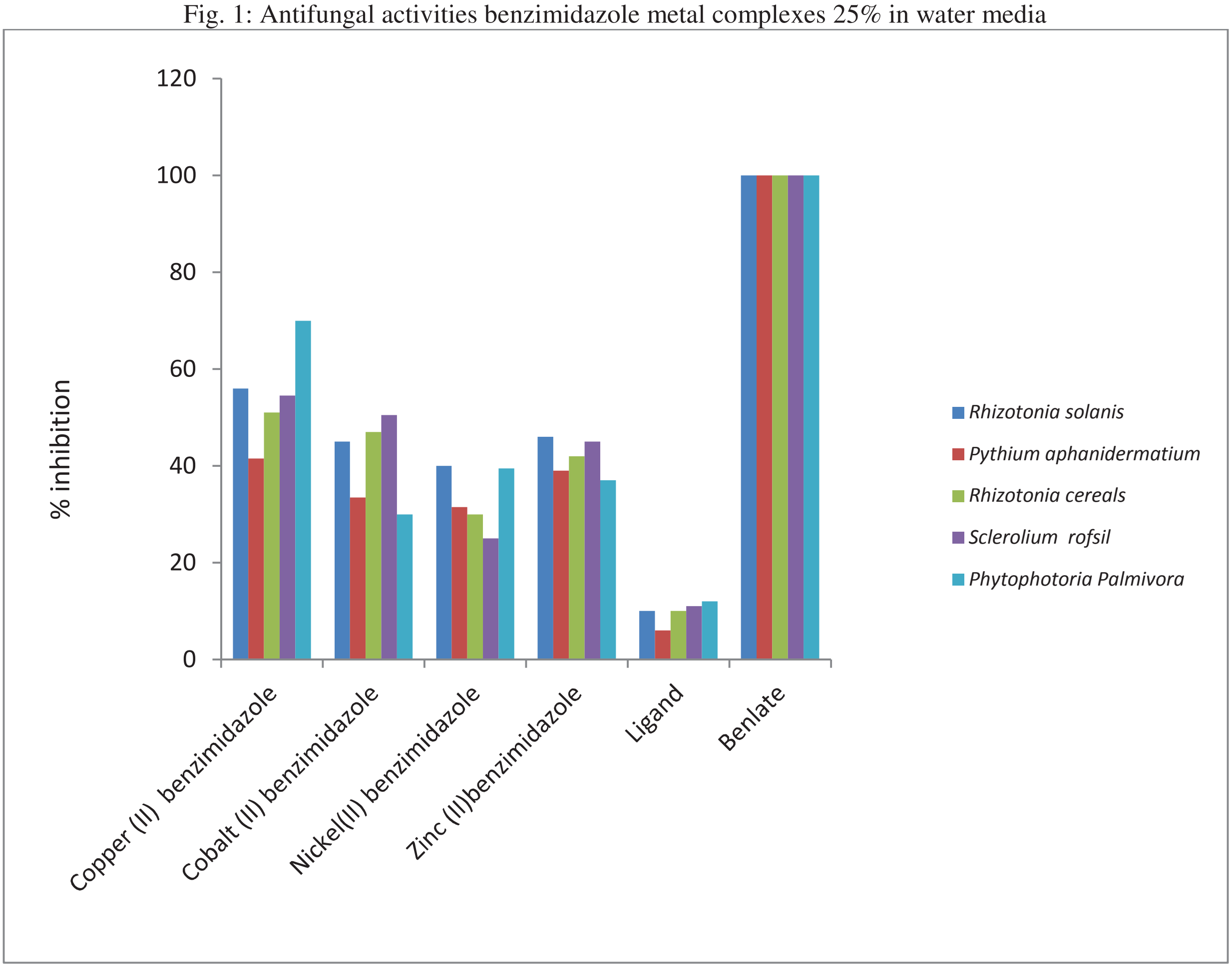

Fig. 2: Antifungal activities benzimidazole metal complexes 5.0% in methanol media

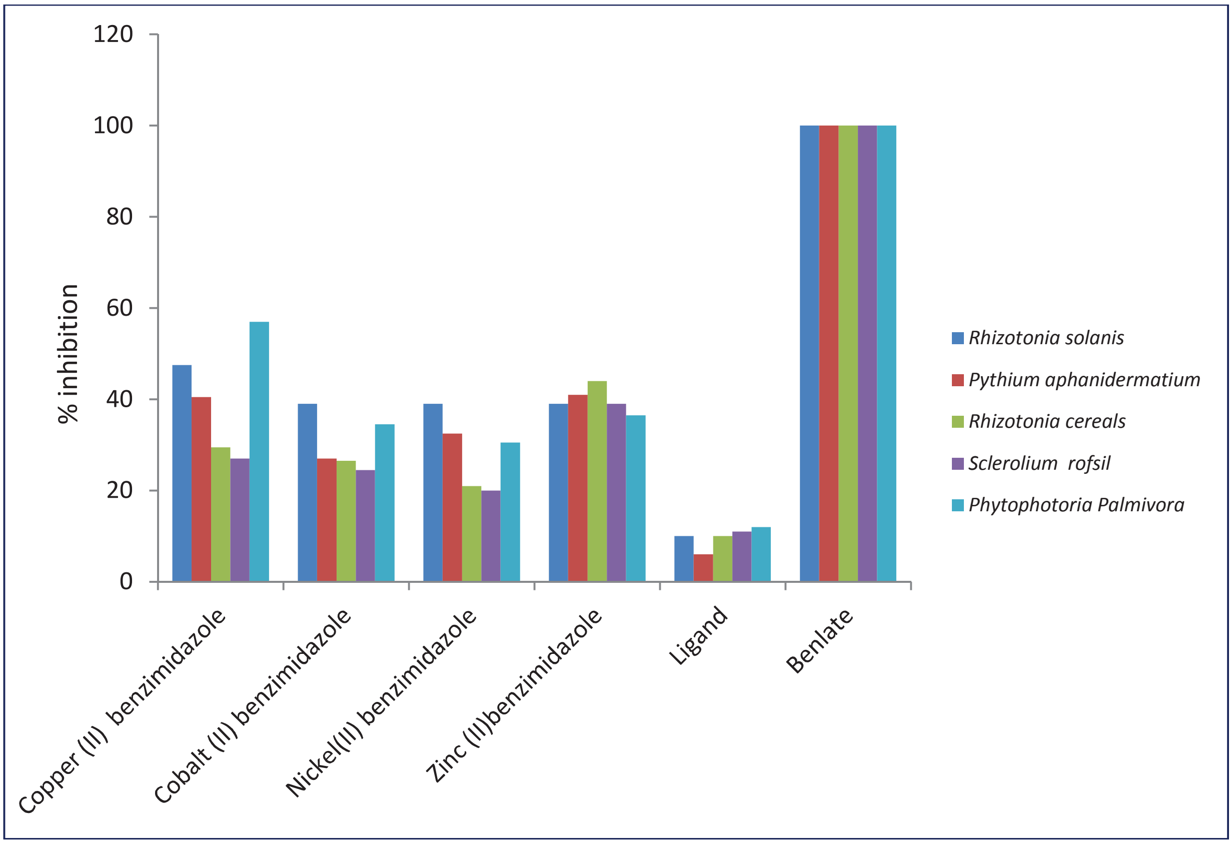

Fig. 3: Antifungal activities benzimidazole metal complexes 2.5% in methanol media

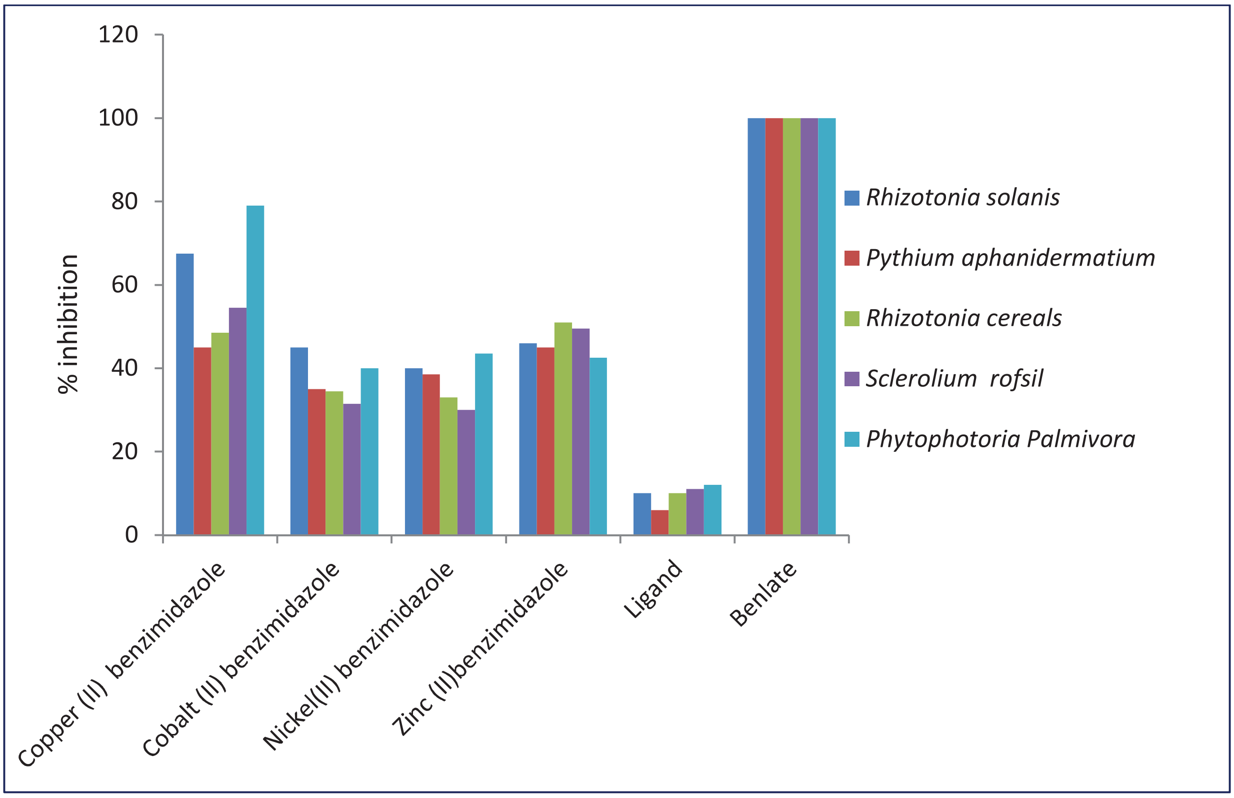

Fig. 4: Antifungal activities benzimidazole metal complexes 50% in methanol media

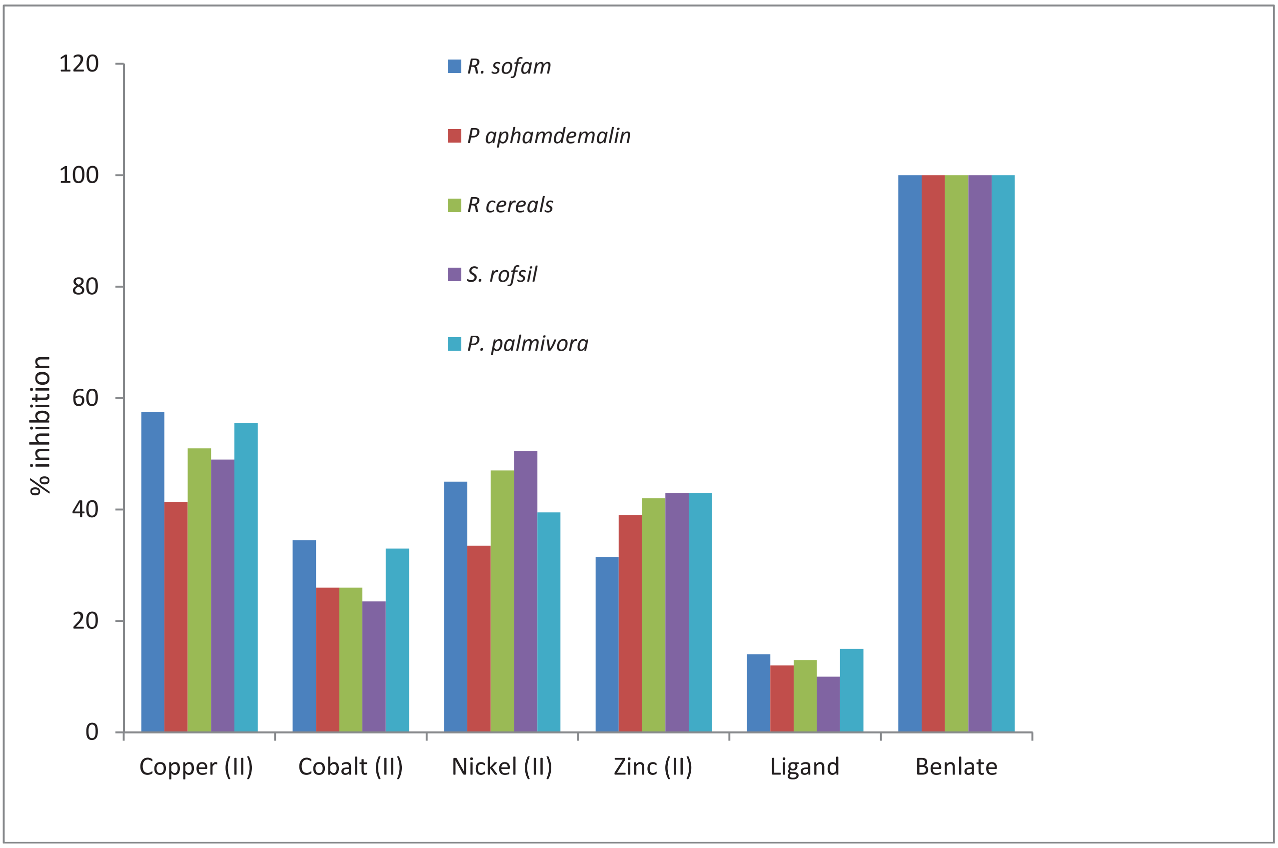

Fig. 5: Antifungal activities of 1,2,3-triazole metal complexes (2.5 %) in water medium

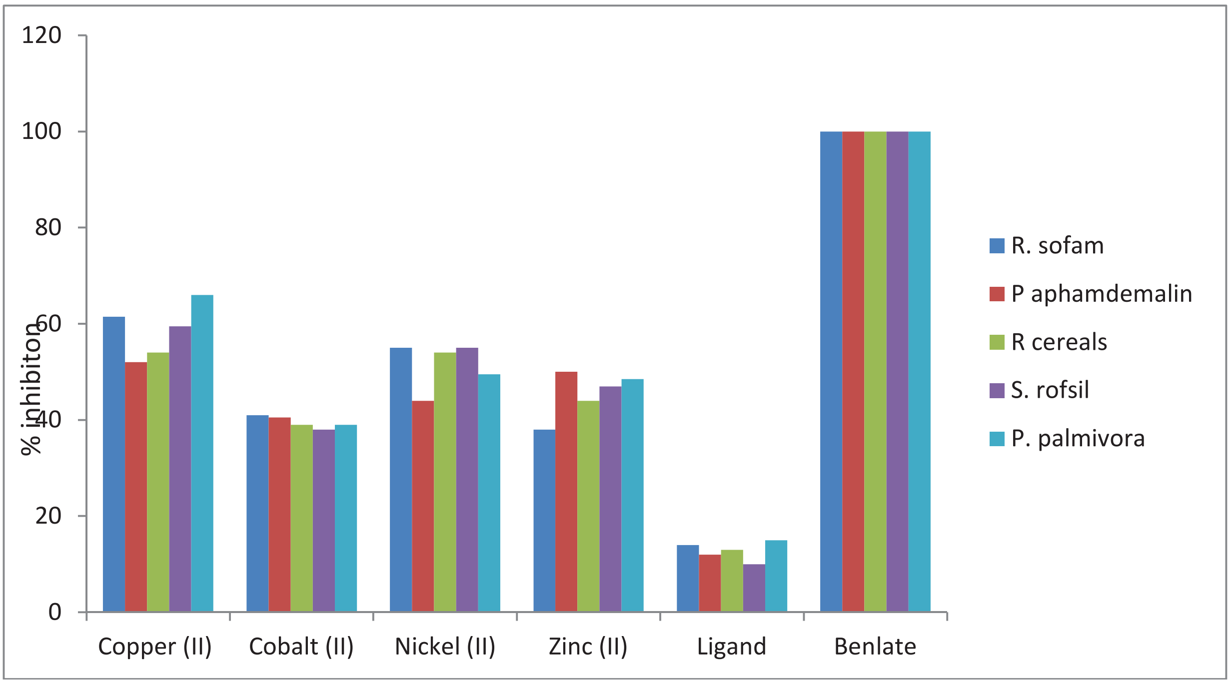

Fig. 6: Antifungal activities of 1,2,3-triazole metal complexes (5.0 %) in water medium

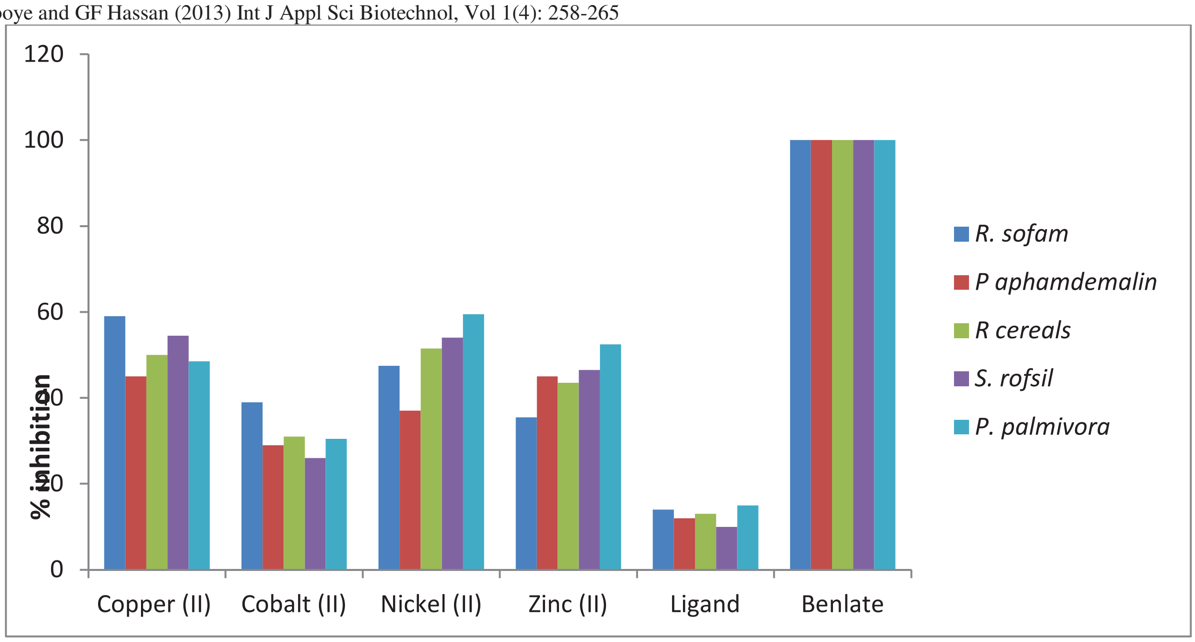

Fig. 7: Antifungal activities of 1,2,3-triazole metal complexes (25 %) in methanol medium

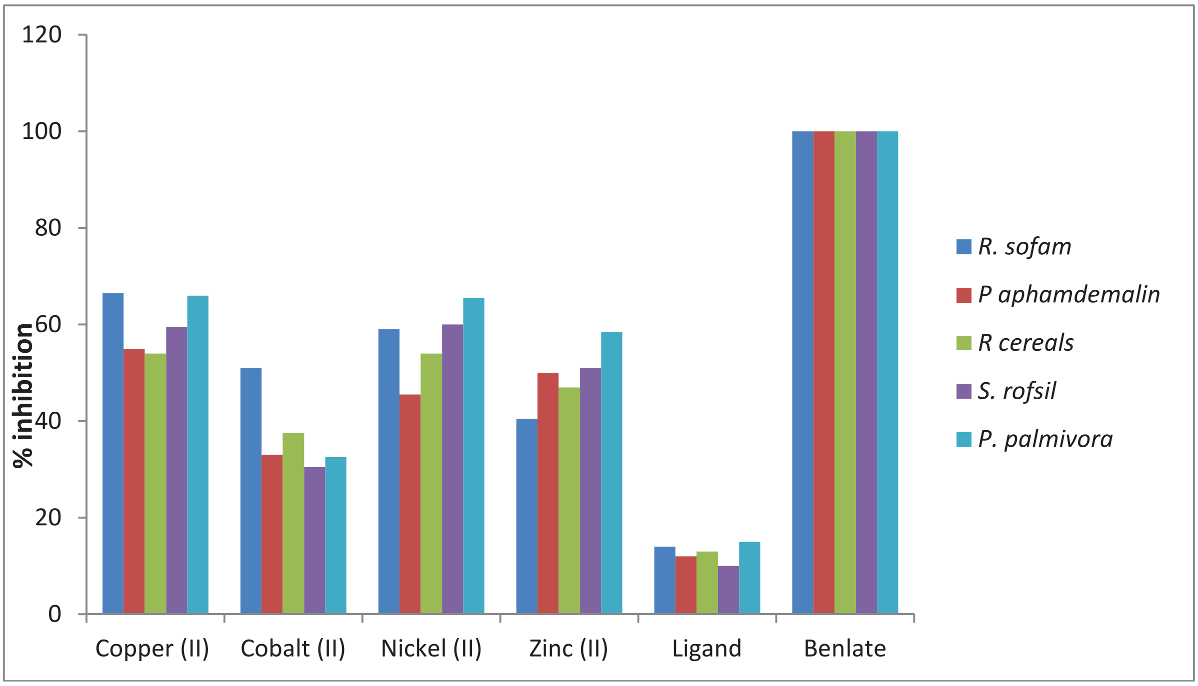

Fig. 8: Antifungal activities of 1,2,3-triazole metal complexes (50%) in methanol medium

Chloro complexes in both benzimidazole and 1,2,3-triazole showed least inhibition. Comparatively metal plays a major role in the inhibition activity. It can be seen that although the sulphate group were found in Cu(II),Ni(II) and Zn(II), copper gave the best inhibition activity. Increased activities were also noted in all the fungi species with an average performance of 45 – 55% inhibition and bioactivities improved in thesulphur containing metal complexes. (Iqbal *et al.*, 2006). This could also be attributed to the ability of sulphur oxides from sulphates to form acids which increase the antimicrobial activities.It is also observed that the performances of the metal complexes increase in methanol than water medium

Benzimidazole and triazole metal complexes of Cu(II), Ni(II), Co(II) and Zn(II) as screened against*phytophorapalmirora*, a causative agent for black pod diseases in cocoa tree. The results of the metal complexes have indicated that Cu(II) benzimidazole and 1,2,3-triazole performances were highly enhanced or impressive on the *phyphotorapalmivora* for a period of 48-96hrs with 86-90% inhibition as it has been well established that Cu(II) salts have shown high potency against cocoa black pod disease (Petal *et al.*, 2010).The performance of 1,2,3-triazole metal complexes were more pronounced than the benzimidazole metal complexes because of the nitrogen atoms since the acidity of the azole increases with the number of nitrogen atoms and 1,2,3-

triazole has more nitrogen than benzimidazole(Gilchrist, 1987).

Conclusion

The results of this present works have revealed that synthetic fungicides of benzimidazole and 1,2,3-triazole were of reasonably high, non-electrolytes and relatively soluble in water and could be useful as a curative agents for black pod diseases in cocoa trees and other citrus fruits/plants shown the symptoms of fungicide within the dosage range of 2.5% and 5.0% concentrations. The metal complexes had strong inhibitory effects on the mycelia growth of the fungi species than the free ligands (azole), indicating that metal plays an active role in the fungi toxicity of the metal complexes.

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