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EFFECT OF SELECTED METAL IONS ON THE MYCELIAL GROWTH OF *SCLEROTINIA SCLEROTIORUM* ISOLATED FROM SOYBEAN FIELD IN RONGAI, KENYA.

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ABSTRACT

White mold caused by Sclerotinia sclerotiorum attacks a wide host range of broad-leafed plants which includes soybeans. The effect of twelve metal ions (Hg^{+2}, Co^{+3}, Ag^{+1}, La^{+3}, Cd^{+2}, Cr^{+3}, Cu^{+2}, Zn^{+2}, Mo^{+5}, Sr^{+2}, Sn^{+4} and Ba^{+2}) on the growth of pathogenic fungus S. sclerotiorum was studied. The fungus was isolated from infected soybean plant collected at Rongai, Kenya. The isolate was tested for the tolerance to metal ions at concentrations of 50.0, 100.0, 250.0 and 500.0 ppm amended into the C: N (35:1) glucose peptone prepared using 1.5% (3.75g) agar culture medium. All the investigated metal ions exhibited concentration dependent mycelial growth using disc diffusion test. Of 12 metal cations tested, only copper and zinc stimulated mycelial growth of S. sclerotiorum mycelial in relatively higher concentrations. Higher concentrations of Hg^{+2}, Ag^{+1}, La^{+3} and Cd^{+2} inhibited growth of fungi causing an opaque halo in the medium. FT-IR spectral analysis of culture filtrate reviewed oxalic acid secreted precipitated primarily as oxalate at the periphery of the fungal colony. This work suggests that strong pollution of soil by some heavy metals could be a restrictive factor of development and pathogenicty of S. sclerotiorum fungi in the environment.

Keywords: *Sclerotinia sclerotiorum*, Metal ions, Mycelial growth, Inhibitory activity, Oxalic acid.

Contribution/ Originality

This study contributes in the existing literature that secretion of oxalic acid is the primary cause for proliferation of *S. sclerotiorum.* From this work fabrication of metal based fungicide looks an attractive strategy towards management of the oxalic acid secreted by the fungi during its colonization or growth. Furthermore, the results demonstrates the potential utilization of *S. sclerotiorum* in bioremediation.

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1. INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is the causal agent of disease on more than 400 plant species of 246 genera and 78 families, including soybeans, on which it causes stem rot [1] throughout the world. The management of *S. sclerotiorum* is difficult, inconsistent and uneconomical due to the presence of wide host range and long-term survival of the resting structures. Despite many years of co-evolution and environmental pressure to select for resistance trait, there is no soybean variety known to be completely resistant. Prophylatic methods of disease control such as crop rotation, have not constrained the fungi to acceptable levels. Resulting to reliance on the application of limited number of fungicides available. Lack of effective registered fungicide for *Sclerotinia* control poses huge problem for horticultural industry worldwide. Moreover, reports world wide indicate an increase in incidence, severity of SSR and variation within *S. sclerotiorum.* Indeed, scientists are dedicated to the development and application of management practices that reduces adverse effect of disease on our food. Understanding the factors that trigger the development of plant disease epidemic is essential in order to create and implement effective strategies for disease management.

Despite decades of dedicated efforts, the pathogenesis mechanism is not well understood yet. However, reports indicate that upon establishment, the fungus deploys two main pathogenicity determinants, the secretion of oxalic acid (OA, $C_2H_2O_4$) and a wide array of degradative lytic enzymes such as cellulases, hemicellulases and pectinases, aspartyl protease, endopolygalacturonases and acidic protease released by the advancing mycelium [2]. The mechanism(s) by which oxalic acid functions to aid in pathogenicity are centred on several proposed modes of action; i) Chelation of cell wall Ca^{2+} by the oxalate anion has been proposed both to compromise the function of Ca^{2+}-dependent defense responses and to weaken the plant cell wall [3]. ii) By lowering pH to or below levels for optimum cell wall degrading enzymes activity, oxalic acid may also contribute to an escape from acidic polygalacturonases inhibition by plant defence polygalacturonase-inhibiting proteins [4]. iii) The acid also inhibits the activated free radical-generating oxidase directly or by blocking a signaling step leading to activation of oxidase an oxalic acid degrading enzyme, thus suppressing the oxidative burst, a controlled release of $O_2^{\cdot-}$ and H_2O_2 at the site of pathogen ingress [2].

The management of oxalate ($C_2O_4^{2-}$) or calcium (Ca^{2+}) ions is likely to yield beneficial results and can be a useful approach in the fungal management as fungicides remains as an indispensable mainstay of Sclerotinia management. However, Ca^{2+} ions play crucial role in plant, therefore, the focus should be directed to $C_2O_4^{2-}$ ion. Among the factors that reduce $C_2O_4^{2-}$ ion concentration are low pH and reduction of production of its metabolites. However, earlier studies have shown that the oxidative burst can be suppressed at low pH [5]. Therefore, the possibility of controlling plant pathogen by selectively tagging pathogenic factor or their metabolic precursors looks as an attractive and strategic approach. This tagging might lower the accumulation of pathogenic factor to a level at which the pathogen would not develop or spread. A variety of compounds have been investigated aiming at metabolic precursors, however, most of these compounds have not proved to be effective on-field situation and some of them are not free from the toxic effect.

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Therefore, use of competitor metal ion to tag $C_2O_4^{2-}$ could offer useful advantages to circumvent sequestration of Ca^{2+}. Environment provides an array of metals, some of which are essential and play various roles; serve as micronutrients, used for redox-processes, stabilize molecules through electrostatic interactions, as components of various enzymes, and for regulation of osmotic pressure. Many other heavy metals have no known biological role and are nonessential [6]. These metal ions can either enhance or inhibit the fungal growth rate, sporulation and enzymatic activities, causing morphological and physiological changes and may affect the reproduction [7]. The MIC is defined as the minimum concentration of a heavy metal at which microbial growth is completely inhibited by toxicity of heavy metal ion [8]. Virtually, all metals whether essential or nonessential, can exhibit toxicity to all forms of life above certain threshold concentrations which for highly toxic metal species may be extremely low. Metal toxicity is greatly affected by the physico-chemical nature of the environment and the chemical behaviour of the metal species in question [9].

However, the influence of metal ions on suppression of *S. sclerotiorum* has received little attention, and the potential for utilizing metal amendments for optimizing disease management remains largely unexplored. Studying tolerance and resistance of these fungi to metals as well as testing for their potentials in biomineralisation could be of great significance. The results could provide insight on the possible utilization of metals in fungicide formulation and fungal strains for bioremediation of heavy metals in metal contaminated environments and other industrial purposes. Therefore, the present study was conducted to determine the effectiveness of the following metals; Hg, Co, Ag, La, Cd, Cr, Cu, Zn, Mo, Sr, Sn and Ba at different concentrations to inhibit the mycelial growth of phytopathogenic fungus, *S. sclerotiorum* under laboratory conditions

2. METHODOLOGY

2.1. Source, *in Vitro* Growth and Purification of *Sclerotinia Sclerotiorum*

S. sclerotiorum was obtained from naturally infected soybean plant collected from soybean field in Rongai, Rift Valley province in 2011. For isolation, sclerotia on the pods and stems were surface sterilized by washing them for 1 min in 70% ethanol and with 0.1% (w/v) sodium perchlorate solution for 3 min, then rinsed three times with sterile distilled water. Finally, sclerotia were air dried on sterile filter paper for 10 min and placed on potato dextrose agar plates (PDA) (fortified with 0.1 mg ml^{-1} streptomycin sulphate). Plates were incubated at room temperature. To obtain a pure culture of the pathogen, a 5-mm-diameter mycelial disc from the margin of an actively growing colony (3d old) was taken using sterile cork borer.

Disks were then aseptically transferred to C: N (35:1) medium consisting of 4% glucose and 1% peptone; Sabouraud Dextrose Agar (SDA), these medium was finally selected for the routine culture and experiments in this study. Pure cultures of this pathogen were stored at 4 ^{o}C inside the refrigerator for use throughout the study [10].

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2.2. Effect of Metal Ions on Growth of *Sclerotinia Sclerotiorum*

2.2.1. Preparation of Metal Ions Solutions

Different metal ions stock solution Ag^{+}, Cd^{3+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Hg^{2+}, La^{3+}, Mo^{6+}, Mn^{2+}, Sr^{2+}, Sn^{2+} and Ba^{2+} (1000 ppm) were diluted with double distilled water to other metal concentrations of 5, 10, 20, 50, 100 and 250 ppm. The effect of various metal ions on *S. sclerotiorum* was investigated on optimum growth sterilised modified solid media C:N (35:1) solidfied in 1.5% (3.75 g) SDA, in triplicates for each composition. Positive controls consisted of metal-deficient agar medium inoculated with *S. sclerotiorum* and negative controls consisted of metal-supplemented agar medium without *S. sclerotiorum.* The pH value in the culture media was adjusted to desired values with 0.1 mol L^{-1} HCl or NaOH.

2.2.2. Growth Performance Test

Growth perforfomance test was initiated by transferring a single 5-mm agar-mycelial plug of *S. sclerotiorum* cut from the advancing edge of a 3 day-old SDA plate culture, to a flask of non-shaken metal amended solid SDA culture medium. Growth was evaluated by measuring the colony radial growth diameters of the mycelium after every 3 days up to 12 days post inoculation in two directions at 90° angles. The growth rates (cm day^{-1}) calculated from the linear portions of the curves plotted from these values. For biomass determinations, mycelium (vegetative part of the fungus) from each culture flask was collected by vacuum filtration of molten agar with the embedded fungus through a Büchner funnel containing a pre-weighed Whatman No. 1 filter paper. Collected fungal biomass was oven-dried at 55 °C for 3 days (Hot air oven, Bhattacharya & Co DTC 72S1, Kolkata, India), cooled to room temperature in a desiccator, and then weighed. Biomass formed was expressed as mg dry weight $flask^{-1}$. Mycelial growth of the fungus on solid media was estimated by linear or dry weight measurements, or by both methods. The triplicated data for the fungal growth assay were analyzed using excel to obtain LS means ± standard deviation. The lowest concentration of heavy metal ion that completely inhibited growth after 5 days was termed as the minimal inhibitory concentration (MIC). Culture media were washed with distilled water, filtration followed and the filtrates concentrated at 40 °C. The concentrates were allowed to settle and crystals formed analysed using FTIR.

2.2.3. Metal Tolerance

The torelance results were expressed as tolerance index (TI) based on the dry weights of fungal biomass, was calculated by Equation 1 as follows:

TI = (dry weight of treated mycelium/ dry weight of control mycelium) x 100%.

2.2.4. Spectroscopic Studies

The infrared spectra were recorded on a Shimadzu Fourier transform infrared (FTIR-8400 CE) spectrophotometer scanning over the wavenumber range of 4000-400 cm^{-1} with 4 cm^{-1} optical resolution after background correction. Potassium bromide pellets were used for solid samples while for liquid samples, one drop was placed between two plates of single crystal

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sodium chloride. The drop forms a thin film between the plates. Resonance frequencies were given in cm^{-1} and intensities reported as s - strong, m - medium and w - weak.

3. RESULTS AND DISCUSSION

The isolate of *S. sclerotiorum* used for the experiment was recovered from sclerotia on soybean pod obtained from a soybean field, re-inoculation of mycelium of the isolated fungus on soybean plant showed characteristic symptoms and the disease was confirmed to be *S. sclerotiorium*. The choice of carbon nitrogen C: N (35:1) was made from our earlier work as the most potent growth media for the test in terms of radial growth rate and biomass accumulation [11].

3.1. Influence of Metal Ions on Growth of Sclerotinia Sclerotiorum

The effect of thirteen aforementioned metal ions at different concentations on the growth of *S. sclerotiorum* was evaluated by determining both the extension of a colony and dry weight since the extension of a colony alone does not take into account the density of the fungal mycelium.

Figure-1. Images of antifungal activities of different concentration of metal ions on *Sclerotinia sclerotiorum* after 10 days post inoculation.

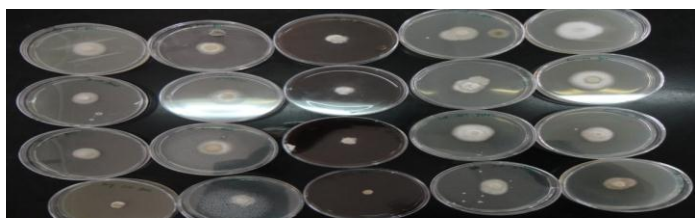

Table-1. Summarizes the growth colony diameter of *S. sclerotiorum* on metal amended media, positve and negative control.

	Concentration (ppm) at pH 4.5						
Metal ion	**Control (0)**	**5.0**	**10.0**	**20.0**	**50.0**	**100.0**	**250.0**
Co^{2+}	44.0 ± 1.2 ^{a}	45.0 ± 1.5 ^{a}	38.5 ± 2.1 ^{b}	30.5 ± 2.1 ^{bc}	28.0 ± 1.4 ^{bc}	23.0 ± 1.4 ^{c}	11.3 ± 2.1 ^{de}
Sn^{4+}	44.0 ± 1.2 ^{a}	42.3 ± 1.5 ^{a}	23.0 ± 4.2 ^{c}	14.5 ± 0.8 ^{d}	11.0 ± 1.3 ^{de}	-	-
La^{3+}	44.0 ± 1.2 ^{a}	32.5 ± 0.6 ^{b}	24.3 ± 1.5 ^{c}	14.3 ± 1.4 ^{d}	-	-	-
Mo^{6+}	44.0 ± 1.2 ^{a}	42.3 ± 1.5 ^{a}	39.0 ± 1.4 ^{a}	41.5 ± 2.1 ^{a}	25.5 ± 0.7 ^{c}	23.5 ± 2.1 ^{c}	9.6 ± 0.7 ^{e}
Zn^{2+}	44.0 ± 1.2 ^{a}	39.0 ± 2.5 ^{a}	24.5 ± 0.7 ^{c}	21.5 ± 2.1 ^{c}	19.5 ± 2.1 ^{cd}	12.5 ± 0.7 ^{de}	10.3 ± 1.1 ^{e}
Ag^{+}	44.0 ± 1.2 ^{a}	34.0 ± 1.4 ^{b}	24.0 ± 1.4 ^{c}	11.5 ± 0.7 ^{e}	-	-	-
Cd^{2+}	44.0 ± 1.2 ^{a}	41.0 ± 1.4 ^{a}	40.0 ± 2.8 ^{a}	21.0 ± 1.4 ^{c}	11.5 ± 2.1 ^{e}	-	-
Sr^{2+}	44.0 ± 1.2 ^{a}	38.5 ± 0.7 ^{b}	30.5 ± 2.1 ^{bc}	23.0 ± 1.7 ^{c}	16.0 ± 0.0 ^{d}	7.0 ± 1.1 ^{f}	-
Hg^{2+}	44.0 ± 1.2 ^{a}	24.0 ± 1.7 ^{c}	11.3 ± 1.4 ^{e}	-	-	-	-
Cr^{3+}	44.0 ± 1.2 ^{a}	38.5 ± 2.1 ^{b}	34.2 ± 1.4 ^{bc}	24.5 ± 0.7 ^{c}	20.5 ± 2.1 ^{cd}	13.5 ± 0.7 ^{d}	11.0 ± 0.3 ^{e}
Cu^{2+}	44.0 ± 1.2 ^{a}	48.5 ± 2.1 ^{a}	44.2 ± 1.4 ^{a}	44.5 ± 0.7 ^{a}	36.5 ± 2.1 ^{ab}	33.5 ± 0.7 ^{bc}	31.0 ± 0.3 ^{bc}
Mn^{2+}	44.0 ± 1.2 ^{a}	44.7 ± 1.3 ^{a}	42.3 ± 4.2 ^{a}	40.5 ± 0.7 ^{a}	37.0 ± 2.3 ^{ab}	36.7 ± 1.1 ^{ab}	32.0 ± 1.7 ^{bc}
Ca^{2+}	44.0 ± 1.2 ^{a}	49.0 ± 2.6 ^{a}	45.3 ± 2.1 ^{a}	42.6 ± 1.1 ^{a}	38.9 ± 0.3 ^{ab}	29.6 ± 0.7 ^{bc}	12.3 ± 1.1 ^{de}
Murtano	44.0 ± 1.2 ^{a}	33. 5 ± 0.7 ^{b}	21.5 ± 2.1 ^{c}	13. 5 ± 0.9 ^{d}	-	-	-

Key: (-) No growth detected; Data were obtained from triplicate assays; data are presented as means ± SDMean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test).

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The growth of *S. sclerotiorum* in all the metal amended media decreased in a concentration dependent manner and displayed direct relationship with nature and metal concentration. At low concentration all metal ions tested exhibited growth. Among the tested samples Hg^{2+}, La^{3+}, Ag^{+} and Murtano were found to be potentially fungitoxic to *S. sclerotiorum* with minimum inhibition concentration (MIC) of 20, 50, 50 and 50 ppm, respectively. This clearly indicates that Hg to be most fungitoxic, however, phytotoxicity and residual toxicity of mercury is widely recognized [12].

The fungus displayed low sensitivity to Cu^{2+}, Cr^{3+}, Mo^{6+}, Zn^{2+}, Ca^{2+}, Mn^{2+} and Co^{2+} at 100 ppm their colony diameter was almost 50 % in comparison to the control. These metal ions are essential in life processes of microorganisms, serve as micronutrients and are used for redox-processes, to stabilize molecules through electrostatic interactions, as components of various enzymes, and for regulation of osmotic pressure [6].

Growth rate and fungal dry weight of *S. sclerotiorum* grown on the metal ions are presented in Table 2, together with positive (Murtano, synthetic fungicide) and negative (unamended) control. These rates are expressed in terms of ratios of colony growth rate in the presence of the metal compound (RM) in relation to control colony growth rate (RC). Similarly, tolerance index was in reference to the control.

Table-2. Effect of metal ions on the growth rate and tolerance of *Sclerotinia sclerotiorum*

Metal ion	**Sn^{2+}**	**La^{3+}**	**Mo^{6+}**	**Zn^{2+}**	**Ag^{+}**	**Cd^{2+}**	**Sr^{2+}**	**Hg^{2+}**
Growth rate (mm/day)	3.7	5.2	6.2	4.2	3.9	4.5	4.2	4.1
R_M:R_C	0.87	0.74	0.96	0.89	0.77	0.83	0.96	0.55
Fungal dry weight (mg)	0.098	0.067	0.171	0.174	0.078	0.113	0.125	0.054
Tolerance index (TI)	52.9	36.2	92.4	94.1	42.2	61.1	67.6	29.2

Metal ion	Cr^{3+}	Co^{2+}	Cu^{2+}	Mn^{2+}	Ca^{2+}	Murtano	Control
Growth rate (mm/day)	3.2	5.6	3.8	4.8	5.2	4.2	5.2
R_M:R_C	0.88	1.02	1.1	1.02	1.00	0.76	1
Fungal dry weight (mg)	0.146	0.136	0.169	0.155	0.170	0.093	0.185
Tolerance index (TI)	78.9	73.5	91.4	83.8	91.9	50.3	100

A RM:RC value of >1.0 indicates that the metal compound stimulated the growth of the fungus and a RM:RC ratio of <1.0 indicates growth inhibition. Rates of growth were calculated for three replicates on 3, 6 and 10 day of incubation at 25 °C. The *S. sclerotiorum* mycelial growth rate was enhanced by Ca, Co, Cu and Mn-amended, but inhibited in presence of La, Ag, Hg, Sn, Cd and Murtano at a pH of 4.5 compared to controls (metal-free).

Metal tolerance based on *S. sclerotiorum* fungal biomass was expressed as tolerance index (TI) and fall in the following increasing order Hg, La, Ag, Murtano, Sn, Cd, Sr, Co, Cr, Mn, Cu, Ca, Mo and Zn. It was interesting to observe that metals with lower tolerance than positive control (Murtano) coincidentally were the same metals that displayed lowest growth rate, that is Hg, La and Ag. Metals that enhanced growth; Ca, Co, Cu and Mn displayed over 70% fungal tolerance

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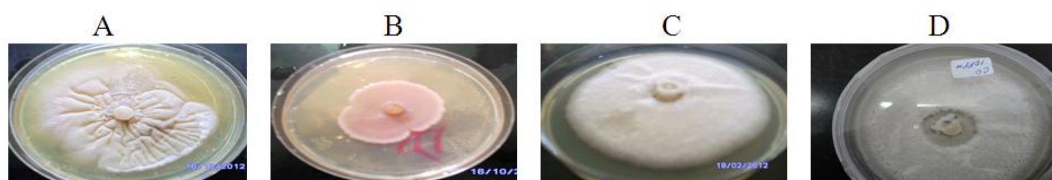
index, supporting inadequate control provided by Mn- and Cu-based fungicides hence unsustainable because of metal accumulation in the soil.

Mycelium of *S. sclerotiorum* grows plainly along the surface of culture media without amended metal ions, and the shape of the whole colony appears to radiate from its central point. However, the mycelial growth in some metal amended media (Hg, Ag, Cd and La) and Murtano was depressed, turned watery after 3 days (Figure 2).

Figure-2. Image of colony of *Sclerotinia sclerotiorum* growing on C:N (35:1) medium; A, Cd amended; B, Murtano amended; C, negative control; and D, Co amended opaque halo surrounding a colony.



The fringe of the colony was convex and irregular and not as smooth as that of the control in non poisoned culture media. This growth pattern was more pronounced with increasing concentration, an aspect of toxic metal tolerance. Roanne and Pepper [13], reported that toxic metal ions can disrupt the cell membrane, inhibit transcription and translation processes, inhibit enzymes activity and cell division, damage DNA and denature proteins of fungi. *S. sclerotiorum* growth inhibition by metal ion was monitored by the production of a clear zone appear underneath the colonies on solid media. La^{3+}, Sn^{2+}, Ag^{+}, Cr^{3+}, Cd^{2+}, Hg^{2+} and Co^{2+} (fig. 2D), showed a distinct opaque halo around the periphery of the colony. The culture filtrate from these medium on settling yielded crystalline solid, whose FT-IR spectra data are shown in Table 3. The infrared spectrum of the ligand (oxalic acid) was compared with those of samples obtained in order to ascertain the coordination sites that may be involved in chelation.

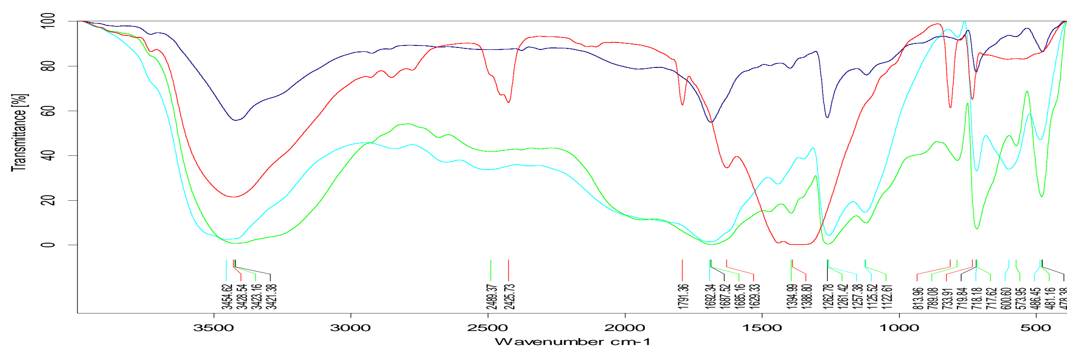


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Table-3. FTIR spectra data (cm^{-1}) of La, Sn, Co, Cr, Cd, Hg and Ag crystalline solid.

La	Sn	Hg	Co	Cr	Cd	Ag	Oxalic acid
3446	3425	3865	3444	3440	3435	3411	3455 vs, br
	2825	3739	2862	2675	2869	2831	
2399		3431		2522	2524	2516	
1629	1662	2923	1641	1687	1693	1622	1692 vs
	1440	2306	1546	1446	1519	1442	
		2005	1514	1400	1444	1348	
		1689	1460	1253	1409		
1384		1535	1380	1126	1350		
1244	1128	1384	1226	916	1255	1253	1257 vs
	721	1240		815	1124	1116	1122 s
828	603	1118		719	721	717	718 m
720	565			567	597	594	592
564	493			503	480	488	478

key: vs, very strong; s, strong; m, medium; w, weak; sh, shoulder

All the complexes displayed broad absorption band in the range 3411-3440 cm^{-1}, characteristic of OH stretching of water or hydroxyl group. In case of complexes a notable peak at 1692 cm^{-1} of ligand shows negative shift and appears in the range of (1689-1622 cm^{-1}). This notable peak is attributed to carboxylate v(C=O) stretching and its shift indicates that in complexes coordination of ligand is through carbonyl oxygen atom. Whereas the O-C-O stretching modes showed intense bands in the range 1257-1240 cm^{-1} and non-ligand bands occured in frequencies of the M-O appeared in the region 586-473 cm^{-1}.

From FTIR spectra data (Table 3) there were additional peaks between 1700-1200 cm^{-1}. The band around 1535-1519 cm^{-1} suggest presence of amide, while band around 1384-1348 cm^{-1} and 1460-1440 cm^{-1} suggest presence of C-N and N-H bond, respectively, supporting Nies, 1999 [14], findings that thiolates and amines as preferred ligands for soft and borderline ions such as Ag^{+}, Hg^{2+}, Cd^{2+} and La^{3+}.

From this analysis, limited growth of *S. sclerotiorum* on synthetic media and the concomitant accumulation of metal oxalate at the colony edge suggest a possible causal relationship between poor growth and oxalate accumulation. Growth stimulation by some metal ions was accompanied by low to lack of crystals. *S. sclerotiorum* demonstrates, ability to chelate toxic metals resulting in the formation of metallo-organic molecules, which may serve as agent for the fungi ability to exploit almost all ecophysiological niches. The formation of water-insoluble metal-oxalate crystals is an efficient way to prevent toxic metal ions entering fungal cells [15].

It is evident from the present study that *S. sclerotiorum* production of oxalic acid provides both protons and an organic anion, the latter capable of forming a complex with the metal cation, and in some cases the subsequent formation of an insoluble oxalate. *S. sclerotiorum* has demostrated the capacity of effectively immobilizing excessive La^{3+}, Sn^{2+}, Co^{2+}, Cr^{3+}, Cd^{2+}, Hg^{2+} and Ag^{+} ions from culture media. This result demonstrates the potential of incorporation of these metals on potential fungicide as well as *S. sclerotiorum* in bioremedation, which is of increasing interest for heavy metal sequestration, and is recognized as a potential alternative to existing technologies for waste purification and the recovery of valuable elements. Furthermore, the

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fungus looks attractive as it is widely available in extensive quantities, easily grown in almost all culture medium and as a low cost technology to solve the problem of heavy metal pollution.

4. CONCLUSION

The fungistate profile of metal amended culture media against *S. sclerotiorum* demonstrated variation with the nature and concentration of metal ions. *S. sclerotiorum* was found to be tolerant to high concentration of Ca^{2+}, Co^{2+}, Cu^{2+} and Mn^{2+} (up to 250 ppm) which indicated that these fungi could survive in these metals polluted environments. Whereas La^{3+}, Sn^{2+}, Cd^{2+}, Hg^{2+} and Ag^{+} appeared to be highly toxic to the fungus, indicating pollution of environment by these metals could be a restrictive factor for development of this pathogen. The toxicity and ability to tag oxalic acid formed by some metal ions forms the basis for potential fungicide fabrication. In biotechnological terms, it is possible that *S. sclerotiorum* fungi could find commercial applications in mediating metal and mineral bioprecipitation.

5. ACKNOWLEDGMENTS

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