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Synergistic Effect of Antifungal Activity of Medicinal Plants with Transition Metal Ferrocyanides Against *Rhizoctonia solani*

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Manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were synthesized and characterized by IR spectra, magnetic susceptibility and elemental analysis studies. The natural antifungal plants such as *Acacia nilotica*, *Emblica officinalis*, *Nerium oleander* and *Withania somnifera* were showed synergistic effect with transition metal ferrocyanides. The natural antifungal plant extracts with metal ferrocyanides complexes were found to be having more antifungal property in comparison to metal ferrocyanides and plants extract alone. Antifungal activities of natural antifungals, metal hexacyanoferrate(II) compounds were tested against *Rhizoctonia solani* causing black scurf in potato. Cadmium ferrocyanide with *Withania somnifera* extract and nickel ferrocyanide with *Acacia nilotica* extract complexes were found to have maximum and minimum antifungal properties respectively.

Key Words: Medicinal plants, Transition metal ferrocyanides, Synergistic effects, *Rhizoctonia solani*.

INTRODUCTION

There are many medicinal plants found in India having antifungal properties. These plants include *Acacia nilotica*, *Emblica officinalis*, *Nerium oleander* and *Withania somnifera* *etc*. studies on these plants have shown that they possess antifungal compounds mainly in their leaves, bark and fruits[1-5].

The medicinal plants are the plants whose parts (leaves, seeds, stem, roots, fruits, foliage *etc*.), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals[6]. The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases.

Extracts of many plants are highly efficient against parasitic as well as microbial infections. The Ayurvedic approach to the prevention and treatment of microbial infection recognizes the emergency use of modern drugs, but recommends traditional herbal combinations and extracts known to balance the individual and improve health, as well as herbs that help to combat or prevent microbial infections.

The most important bioactive constituents of plants are alkaloid, tannin, flavanoids and phenolic compounds[7]. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time to other for medicinal purposes. The use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries[8].

In the present scenario, an emergence of multiple drug resistance in human pathogenic microbes and the small number of antimicrobial classes available stimulated research directed towards the discovery of novel antifungal and antibacterial agents from other sources, such as medicinal plants[9]. The plant botanicals are known to possess medicinal properties and biocidal activity against microbial and other pests and pathogens[10,11].

Experiments of primitive earth conditions, cyanide could have combined with a large number of metal ions present in primeval sea. Consequently, several insoluble metal ferrocyanides of general formula $M_2[Fe(CN)_6]\cdot xH_2O$, where M = Mn, Co, Ni, Cu, Zn and Cd *etc*., could have been formed. It is well established that metal ferrocyanides acts as an adsorbent[12], ion-exchangers[13,14] and photosensitizers[15].

There are few reports on synergistic effect of antimicrobial activity of metal ferrocyanide with botanicals[16]. These complexes have also been reported to adsorb biomolecules[17,18]. There may be the possibility of adsorption of active ingredient at the surface transitional metal ferrocyanides. Thus concentration and shift life of active botanicals may increase and may result in increased activity (biopotentiation). Further studies in view of this attempt were made to study medical application of these complexes are in progress.

Keeping in view the importance of the subject synergistic effect of transitional metal ferrocyanides with medicinal plants were examined for their antifungal activity.

EXPERIMENTAL

Collection of fungal cultures: Fungal pathogen *Rhizoctonia solani* employed in the present investigations have been collected from Central Potato Research Institute Campus, Modipuram, Meerut, India. The pathogen was grown on potato dextrose agar (PDA) medium and incubated at 28 ± 1 ºC. The medium was incubated at 28 ± 1 ºC for 2 days for the *Rhizoctonia solani*.

Collection of plants: The samples of leaves of *Acacia nilotica*, *Emblica officinalis*, *Nerium oleander* and *Withania somnifera* were collected from medicinal garden of Banasthali University Rajasthan India. Plant material was dried in shade at 35 ºC for 15-20 days. The shade dried leaves of each plant spp. were grinded in mixer and stored in airtight containers after grinding. Dry powder of leaves was extracted four to five times with 5 mL methanol solvent/g of plant material. All these extracts were combined and concentrated by flash evaporation at 40 ºC.

Synthesis of transition metal ferocyanides: Manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were prepared following the Kourim's procedure[19]. A solution of potassium ferrocyanide (167 mL, 0.1 M) was added to solution of desired metal salt (500 mL, 0.1 M) with constant stirring at room temperature. A slight excess of metal salt solution markedly improves the coagulation of the precipitate. The reaction mixture was heated on a water bath at 80 ºC for 3-4 h and allowed to stand at ambient temperature for 24 h. The precipitate was filtered under vacuum and washed thoroughly with double distilled water. It was dried in an oven at 60 ºC. The dried product was ground and sieved to 100 mesh sizes. The coloured powers of metal complexes were stable in air. These were characterized on the basis of elemental analysis (carried on Carbo Erba 1108, CHN analyzer), IR spectra (KBR disc on Bio-red FTIR spectrophotometer) and magnetic susceptibility measurement (Sherwood Scientific) (Tables 1-3).

The molecular formula of synthesized metal complexes established on the basis of data obtained from elemental analysis are $Mn_2[Fe(CN)_6]\cdot 3H_2O$, $Co_2[Fe(CN)_6]\cdot 2H_2O$, $Ni_2[Fe(CN)_6]\cdot 5H_2O$, $Cu_2[Fe(CN)_6]\cdot 7H_2O$, $Zn_2[Fe(CN)_6]\cdot 3H_2O$ and $Cd_2[Fe(CN)_6]$, respectively.

Testing the antifungal activity of plant extracts-metal ferrocyanide complexes: Paper disc method[20] was used for initial screening of antifungal potential of metal complexes and plant extracts chosen for present investigations. This method was based on diffusion capacity of test chemical(s) through agar medium. Metal ferrocyanide (5 mg) and antifungal plant extract (5 mg) were placed in sterilized petri dish containing media. The fungal spores were sprayed on the entire bottom of the petri dish using an aspirator. This method was repeated using different extract and metal ferrocyanide complexes.

RESULTS AND DISCUSSION

Antifungal activity of plant extracts only: Antifungal activity of *Acacia nilotica*, *Emblica officinalis Nerium oleander* and *Withania somnifera* were studied. *Withania somnifera* and *Acacia nilotica* were found to have maximum and minimum antifungal property, respectively. The following order of antifungal property was

Withania somnifera > *Nerium oleander* > *Emblica officinalis* > *Acacia nilotica*

Antifungal activity of metal ferrocyanides only: Antifungal activities of manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were studied. Cadmium and nickel ferrocyanides were found to have maximum and minimum antifungal property, respectively. The following order of antifungal activity was observed in metal ferrocyanides.

Cadmium ferrocyanide > copper ferrocyanide > cobalt ferrocyanide > manganese ferrocyanide > nickel ferrocyanide

TABLE-1
ELEMENTAL ANALYSIS DATA OF METAL FERROCYANIDE COMPLEXES

Complexes	Found (%) (calcd.)				
	M	Fe	N	C	H
$Mn_2[Fe(CN)_6]\cdot 3H_2O$	28.56 (29.23)	14.66 (14.86)	22.59 (22.36)	20.67 (19.17)	1.69 (1.61)
$Co_2[Fe(CN)_6]\cdot 2H_2O$	32.12 (32.22)	15.30 (15.27)	21.16 (22.97)	19.65 (19.70)	1.11 (1.10)
$Ni_2[Fe(CN)_6]\cdot 5H_2O$	27.85 (27.93)	13.00 (13.28)	18.79 (19.19)	16.51 (17.14)	2.22 (2.30)
$Cu_2[Fe(CN)_6]\cdot 7H_2O$	27.10 (27.32)	12.10 (12.01)	18.12 (18.07)	14.75 (15.49)	3.13 (3.03)
$Zn_2[Fe(CN)_6]\cdot 3H_2O$	32.84 (32.95)	14.10 (14.08)	20.40 (21.18)	17.74 (18.16)	1.51 (1.45)
$Cd_2[Fe(CN)_6]$	50.12 (51.47)	12.58 (12.79)	20.38 (19.24)	17.71 (16.50)	–

TABLE-2
INFRARED SPECTRAL DATA (cm^{-1}) OF METAL FERROCYANIDE COMPLEXES

Complexes	Adsorption frequencies (cm^{-1})				
	ν(HOH)	ν(C≡N)	HOH bending	ν(Fe-C)	ν(M-N)
$Mn_2[Fe(CN)_6]\cdot 3H_2O$	3701	2070	1631	592	451
$Co_2[Fe(CN)_6]\cdot 2H_2O$	3724	2083	1609	592	465
$Ni_2[Fe(CN)_6]\cdot 5H_2O$	3697	2091	1611	592	463
$Cu_2[Fe(CN)_6]\cdot 7H_2O$	3845	2090	1621	592	503
$Zn_2[Fe(CN)_6]\cdot 3H_2O$	3685	2080	1600	603	496
$Cd_2[Fe(CN)_6]$	3724	2071	1623	590	508

TABLE-3
MAGNETIC MOMENTS OF METAL FERROCYANIDE COMPLEXES

Metal hexacyanoferrate (II)	μ_{calc} (BM)	μ_{eff} (BM)
$Mn_2[Fe(CN)_6]\cdot3H_2O$	5.92	6.21
$Co_2[Fe(CN)_6]\cdot2H_2O$	3.87	4.36
$Ni_2[Fe(CN)_6]\cdot5H_2O$	2.83	2.99
$Cu_2[Fe(CN)_6]\cdot7H_2O$	1.73	2.45
$Zn_2[Fe(CN)_6]\cdot3H_2O$	0.00	0.81
$Cd_2[Fe(CN)_6]$	0.00	0.90

Zinc ferrocyanides did not show any growth of inhibition against *R. solani*.

Synergistic effect of medicinal plant extracts with metal ferrocyanides: Plant extract (5 mg) and metal ferrocyanide (5 mg) and were placed in sterilized petri dish containing media. The fungal spores were sprayed on the entire bottom of the petri dish using an aspirator. This method was repeated using different extract and metal ferrocyanide complexes.

The following order of antifungal activity was observed in natural antifungal with metal ferrocyanide complexes. (i) Cadmium ferrocyanide: *Withania somnifera* > *Nerium oleander* > *Emblica officinalis* > *Acacia nilotica.* (ii) Copper ferrocyanides: *Withania somnifera* > *Nerium oleander* > *Emblica officinalis* > *Acacia nilotica.* (iii) Cobalt ferrocyanide: *Withania somnifera* > *Nerium oleander* > *Emblica officinalis* > *Acacia nilotica.* (iv) Manganese ferrocyanides: *Withania somnifera* > *Nerium oleander* > *Emblica officinalis* > *Acacia nilotica.* (v) Nickel ferrocyanides: *Withania somnifera* > *Nerium oleander* > *Emblica officinalis* > *Acacia nilotica.*

Conclusion

The following conclusion drawn from present studies: (a) antifungal activity of secondary metabolites are enhanced through intraction with metal ferrocynides; (b) cadmium and nickel ferrocyanides were found to have maximum and minimum anti fungal property, respectively; (c) *Withania somnifera* and *Acacia nilotica* were found to have maximum and minimum antifungal properties, respecticely; (d) *Withania somnifera* extract-cadmium ferrocyanide complex and *Acacia nilotica* extract with nickel ferrocyanide complex were found to have maximum and minimum properties, respectively; (e) it may be also concluded from present studies that cadmium ferrocyanid-*Withania somnifera* extract complex may be used as effective antifungal drug for black scurf disease of potato.

The result lends credence to the folkloric use of these transition ferrocyanides in treating microbial infection and shows that ferrocyanides of cobalt, cadmium and copper could be investigate the probable reason of synergistic effect of bioactive fractions of medicinal plants. Studies on surface structure of metal ferrocyanides have been carried out. Scanning electron micrographs shows porous surface structure of metal ferrocyanides.

Synergistic effect of methanol extracts of medicinal plants with metal ferrocyanides against *R. solani* may be due to porous surface structure and adsorption capacity of metal ferrocyanides. These metal ferrocyanides may adsorb active components of medicinal plants on its active sites thus the concentration of active components increases at the surface of metal ferrocyanides at active sites for adsorption as well as inside the pores present at their surface. Their mobility and rate of degradation also decreases, which may result in increased shelf life of fungicidal components of medicinal plants and magnification of inhibition zones against *R. solani* in presence of metal ferrocyanides, exploited for new potent antifungal agents.

The results suggest that these transition metal complexes may be used as fungicides alone or in combination with other plant botanicals fungicidal substances resulting in the development of ecofriendly fungicides for future that can help in safeguarding our environment.

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