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Biomedical and antifungal application of Cu (II) soaps and its urea complexes derived from various oils

Abstract

The antifungal activities of copper soaps and their urea complexes derived from various oils have been evaluated by testing against *Alternaria alternata* and *Aspergillus niger* at different concentrations by agar plate technique and it is found that Cu (II) soaps and their urea complex are very effective on these.

CSo > CSe > CG ≈ CM

CSoU > CSeU > CGU ≈ CMU

Keywords: copper surfactants, anti-fungal studies, edible oils

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Introduction

The deeper understanding of the role of metal ion in bio-system has led to the awareness that metal complexation is useful in the treatment of bacterial, fungicidal and viral diseases. Most of the antimicrobial agents are obviously organic compounds, which behave as good chelating agent. Their pathogenic activity is enhanced on complexing with various transition and toxic metals. The biological effect of these derivatives depends upon the nature and structure of ligands and their metal complexes and also on the presence of particular element.[1–3]

Many Copper compounds are employed as pesticides either alone (as Cu (I) oxide and Cu (II) sulfate) or in mixtures. Examples of the latter are the pesticide Cupronil which contains 35% Cu (II) carbonate hydroxide and 15% zinc N,N-chloride hydroxide and 15% Zineb (zinc ethylene-bis- dithiocarbamate); Bordeaux mixture (Copper sulphate and hydrated lime) was the first fungicide to be used in large scale.[4]

Nitrogenous ligands have been found to be effective against many metal enzymes, bacteria and number of fungi. Large number of compounds containing nitrogen and sulphur atoms in the heterocyclic ring shows different types of activities. Biological, pharmacological and medicinal significance of many co-ordination complexes has been studied by several workers.[5]

Recently, wood has developed metabolic and antagonist theory of drug action and Fields led to the synthesis of several co-ordination compounds, which have been used for antibacterial and anti-tubercular activity.[6] The use of Copper linoleate as heavy-duty wood preservative and many other biological activities of Copper metal containing surfactants also has been studied.[7]

Proprietary brands of cuprous oxide are extensively employed as fungicides and seed dressings. Copper oxychloride has a number of applications, by far the most important being as an agricultural fungicide for which purpose it is extensively employed in formulated form as dusts, wettable powders and pastes. Small quantities of Copper soaps such as Copper stearate, Copper oleate and Copper abietate (from resins), are employed mainly for rot-proofing textiles, ropes, etc. The main applications of Copper sulfate are in the production of proprietary wood preservatives and agricultural fungicides. Copper naphthenate is a widely used, broad-spectrum wood preservative. It has several properties which make it a highly valued commodity, including effective control of decay fungi and excellent control or mitigation of wood destroying insects, including termites, beetles carpenter ants and other host organisms. In the United States, the Environmental Protection Agency also classifies Copper naphthenate as a general use (unrestricted) pesticide. Only limited data are found in the literature describing the preservative efficacy of Copper soaps of other carboxylic acids. Metal soaps of naturally occurring plant-based acids such as oleic, stearic, and tall oil acids were inferior to naphthenates when used to protect cellulosic material. Metal soaps of synthetic acids are frequent substitutes for naphthenate soaps in paint driers and other applications because of their lower cost and higher metal content.[8–10]

All the above studies suggest that the Copper metal play a vital role in fungicidal activities. Our continuing interest in the search for better fungicides and bactericides has led us to synthesis some complexes of Copper (II) soap with nitrogen donor system.

For the preparation of fungicides to be studied, various edible oils like mustard, sesame, groundnut and soyabean were used as raw material. These vegetable oils were particularly chosen as they are commercially easily available and they are biodegradable in nature.

Since Copper metal is toxic in nature, these vegetable oils were converted into Copper soaps. Then these soaps were made to undergo complexation with nitrogen and sulphur containing ligands. To study their micellar characteristics and their applications in various industries and agriculture. For this purpose, we have chosen some fungicidal activities of the synthesized Copper soaps and its urea complexes against easily available fungi *Alternaria alternata* and *Aspergillus niger.*

Experimental

All chemicals used were of LR/AR grade. Oils were procured directly from the seeds of Mustard, Groundnut, Sesame, Soyabean (pure) Oil was taken from the market of a reputed brand. The fatty acid composition of oils was confirmed by sending their methyl esters to RSIC,CDRI,LUKNOW U.P. India. Copper soaps were prepared by refluxing the oils with 2N KOH solution and alcohol for about 3 h (direct metathesis). The excess of KOH was neutralized using 1N HCl. Saturated solution of Copper sulphate was added to it. The Copper soap obtained was filtered, washed with warm water followed by alcohol, dried at 50^{0}C and recrystallized with hot benzene. The metal was analyzed by standard procedure. Copper soap-urea complexes were prepared by taking Copper soap and urea in molar ratio (1:1). 0.005 moles of ligand (urea) was dissolved in 2-3 ml of ethyl alcohol and 0.005 moles of Copper (II) soap derived from various edible oils were dissolved in 10-15 ml of benzene and solution of urea was added in it. The above reaction mixture was then heated for 1.5 hours. Separated solid complex was filtered, washed with hot water and alcohol and dried in vacuum over fused calcium chloride. The formation of copper soap and their urea complexes was confirmed by using IR, UV, NMR technique.

The general laboratory techniques for antifungal studies followed in the course of this investigation are as suggested by Booth and Hawksworth.

Sterilisation of glasswares

Glassware's used in our present study were of pyrex brand. The glassware's viz. conical flask of various volumes, glass rods, test tubes, and petriplates were cleaned with chromic acid. After cleaning with chromic acid the glassware's should be cleaned thoroughly several times with distilled water. After this glassware should be sterilized by keeping them in hot air oven at 160°C for 24 hours before use.

Culture media used

The culture medium used for the growth of the organism in the present study was natural media i.e. P.D.A. The following media was used in the present study

Potatoes 200gm

Dextrose 20gm

Agar 20gm

Distill water 1000ml

pH 6.0-6.5

200gm of potatoes were cleaned, cut into pieces and boiled with 500 ml of water so that the potato tissues are softened. This process takes about 15 minutes. Now this prepared pulp is being sieved through a muslin cloth 20 g of agar and 500ml of water are heated together so that a solution is prepared. Now, both the solutions are mixed, 20 g of dextrose are added to it. Thus, 1000 ml of potato dextrose agar medium (P.D.A.) is prepared.

Preparation of sample solutions

All the Copper soaps and complexes derived from various edible oils have been tested for their antifungal activity. All chemicals used were of LR/AR grade. The calculated amount of the soap and its complexes was weighed in a standard flask and the solutions containing different concentration (10^3 and 10^4 ppm) of soaps and complexes in methanol-benzene mixtures of varying compositions were prepared.

Test organism

The test organism used in the present study was *Alternaria alternata* and *Aspergillus niger* which was isolated from its natural habitat (plants, debris) and then purified, characterized and identified.

Fungicidal testing

The antifungal activity of the Copper soaps derived from various edible oils and its complexes with urea under study were checked by the agar plate technique.

2 ml and 5 ml sample solution of concentration 10^3 ppm and 10^4 ppm of Copper (II) soap and its urea complexes in 40% methanol-benzene solvent was aseptically transferred into sterile petri-plates. Into these plates 20 ml of PDA (media) was poured and was mixed with soap and complex solution by rotating the petri-plates in clock-wise and anti -clock-wise direction and was allowed to solidify. To evaporate the solvent, the petri-plates were kept at 60°c for two hours.

After the solidification of the above medium and evaporation of solvent, single hypha/spore of test organism was aseptically transferred in the centre of the Petri-plates. The petri-plates were wrapped in polythene sheets and put in incubator at 30±1°c for 72 hours i.e. for 3 days. After the period of incubation, the plates were observed for the growth of fungus for different concentration of the soap and complex solution used in the present study. The growth of fungus was measured by recording the total area of fungal colony. The data were statistically analysed according to the following formula:

Fungal growth Inhibition % = (C-T)*100/C

Where C = total area of fungal colony in control plates after 72 hours

T = total area of fungal colony in test plate after 72 hours.

Results and discussion

The antifungal activities of Copper soaps and their corresponding urea complexes have been evaluated by testing them against *Alternaria-alternata* and *Aspergillus-niger* at 10^3 ppm and 10^4 ppm using 2ml and 5ml of these solutions by agar plate technique. The results of the fungicidal screening data are shown in Figure 1 & Figure 2.

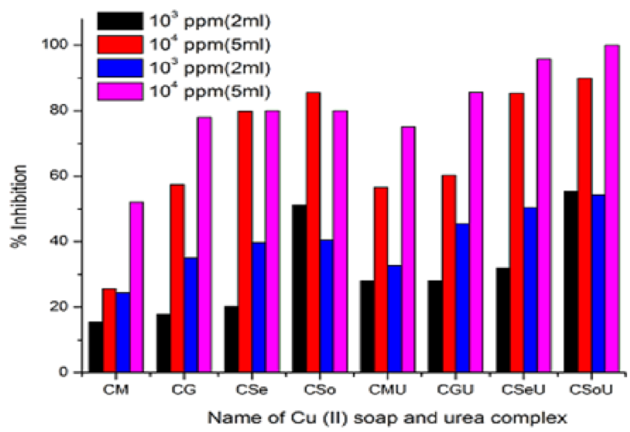

Figure 1 Antifungal activity of copper soaps and their urea complexes for fungi *Aspergillus niger.*

A perusal of Figure 1 & Figure 2 reveals that all the urea complexes of Copper (II) soaps show higher activity than pure soaps, suggesting

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that complexes are more powerful anti-fungal agents: and N, S, O etc. containing compounds are able to enhance the performance of Copper soaps. The organic compounds containing amino group play important role in biology.[11]

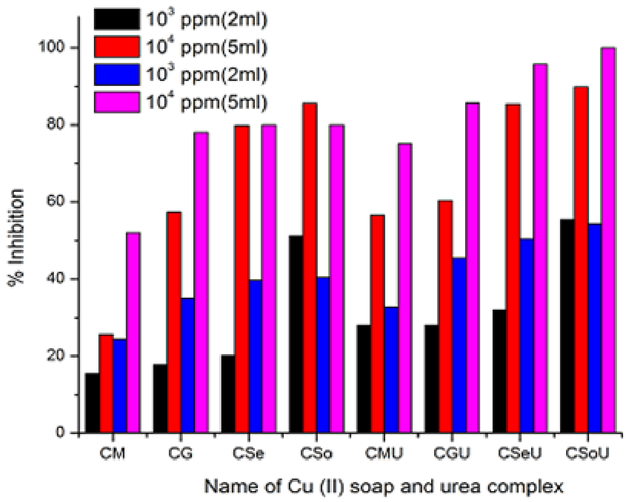

Figure 2 Antifungal activity of copper soaps and their urea complexes for fungi *Alternaria alternate.*

From the Figs it is apparent that all the Copper soaps and their complexes with urea have significant fungi toxicity at 10^4 ppm but their toxicity decreases markedly on dilution (at 10^3 ppm). Their comparative order could be as follow: 1000ppm < 10000ppm. It is evident that their efficiency increases with increase in concentration. Thus concentration plays a vital role in increasing the degree of inhibition. Also, on increasing the amount of solution in Petri-plates from 2ml to 5ml, the % inhibition increases, suggesting that the % inhibition is affected by the increase in the concentration of the active fungicidal moiety in the analysed system.

On the basis of earlier workers[12] observations, it was observed that enhanced activity of complexes may be due to synergistic mechanism i.e. the free soaps are less active but on complexation they show more activity in combination with ligands containing N atom. The evaluation of anti-fungal studies further revealed that fungi-toxicity of the complexes and soaps also depends on the nature of metal ions.

From comparison of the results for both the fungi it is found that Copper (II) soaps and their urea complexes show almost similar behavior for both the fungi.

A perusal of the figs shows that CM soap is the least fungi toxic (% inhibition lowest) where- as CSo is the most toxic against both fungi. The activity (toxicity) of Copper (II) soaps derived from various edible oils is found in the order:

CSo > CSe > CG ≈ CM

And for complexes the order of activity is almost same as soaps.

CSoU > CSeU > CGU ≈ CMU

In general, CSo and its complexes CSoU both are more potent (more toxic) than other soaps and their complexes. The observation suggests that the soap possessing maximum fungicidal activity has lowest molecular weight.[13] The activity of soap and complexes both follows the same order which is just reverse of their average molecular weights suggesting that the soaps and complexes derived from soyabean and sesame oils containing more content of shorter fatty acids and more poly unsaturation are more active than soaps and complexes derived from mustard and groundnut oils, containing longer fatty acid chain content and less unsaturation in their chemical composition.[14]

The data clearly indicates that the inhibition power of soap has been increased on the complexation. Thus it may be suggested that nitrogen, and oxygen containing ligand enhance the activity of the soap molecule for all the referred systems. These studies will play a significant role in selection and promotion of ecofriendly and biodegradable fungicides, pesticides and insecticides.[15]

Conclusion

It has been observed that the antifungal activity increases with the increase in the concentration of the solution. It has been observed that complexes show higher activity than pure soaps, which suggests that complexes are more powerful antifungal agent. All the above studies led to the conclusion that copper soaps and their complexes derived from various oils due to its toxic and its bio degradable nature having fungicidal, herbicidal and many other biological activities. All these activity will play a significant role in their application in various fields of industries, pharmaceuticals, pesticides, wood preservation etc. Some recent studies about antimicrobial activities of newly synthesized 1, 4-benzothiazines possessing thiazolyl / imidazolyl moieties also support our studies

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Conflict of interest

The author declares no conflict of interest.

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