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ANTIMICROBIAL PROPERTIES OF MATERIALS: HEAVY METALS EFFECTING THE GROWTH OF *Aspergillus niger*

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ABSTRACT

Many research studies have revealed that materials like heavy metals have bacteriostatic and fungicidal properties. Copper and some heavy metals alloy surfaces can inhibit the growth of microorganisms. Our present study was focused on studying the fungal growth inhibitory properties of heavy metals. Heavy metals tested (Copper, Zinc and Magnesium) by surface contact method could inhibit the growth of Aspergillus niger, a common fungus involved in food contamination. This study helps in identifying antifungal heavy metal materials. These metals can be used for water storage and cooking purpose which can reduce the food contamination by fungi. By the usage of antimicrobial materials for the purpose of water supply systems, Air conditioning systems, Surfaces, Door knobs etc. fungal infections in hospitals, laboratories and in our homes can be reduced.

KEY WORDS

Heavy metals, bacteriostatic, fungicidal study.

INTRODUCTION:

Metal ions have disinfecting properties and are being used alone or as complex mixtures for treating solids, liquids and tissues (1). Antimicrobial properties of copper: Copper is being used as sanitizing agent for purifying drinking water, sterilizing wounds also in medicine since ancient times. The idea of using copper vessels to store drinking water is still practiced in many countries. Many recent publications also showed that microorganisms are rapidly killed on metallic copper surfaces by 'contact killing' mechanism (4). Excessive generation of Copper ions can damage the Cell membrane. Currently, there is an intense interest in the use of, Mechanisms of antibacterial action of copper. The antimicrobial properties of copper are still under active investigation. Magnesium and Silver have antimicrobial properties. Silver is being used for purposes like eating food and drinking water. It can eliminate bacterial contamination. Silver ions can inhibit bacterial growth. Silver ions having antibacterial activity have a wide range of applications in medical field like dental work, catheters and burn wound healing. Antimicrobial properties of Zinc: Soluble zinc species or ZnO particles were proved in having the antimicrobial properties. Our present study is designed to study the anti-fungal properties of heavy metals which is under active investigation. Fungus *Aspergillus niger* belonging to the genus Aspergillus is one of the common food contaminants causing black mould in fruits, vegetables and nuts such as grapes, onions and peanuts. Black

conidia can be seen on the contaminated food and vegetables (2 and 3). Some strains of this fungus produce potent mycotoxins called ochratoxins. Humans may get a lung infection called as aspergillosis, up on inhalation of the fungal spores. A. niger can also cause otomycosis, an ear infection. Usage of metals having antimicrobial properties for cooking and storage can reduce the fungal contamination in food. Thus, reducing the chances of getting infection. The study is significant in modern days where the usage of plastic is increasing polluting the environment. The present study aims in encouraging the usage of metals like copper in daily life.

MATERIALS AND METHODS:

Collection of Heavy metals

Required heavy metals for experiments were purchased in the local market of Kadapa, Andhra Pradesh. Autoclaving of the heavy metals was done at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents.

Maintenance of fungal culture: fungal cultures were maintained on potato dextrose agar PDA.

PDA was prepared by boiling 200 g sliced unpeeled potatoes in 1 lit of distilled water for 30 min. Filtered through cheesecloth, saving effluent, which is potato infusion, Mixed with Dextrose, Agar and Water fallowed by autoclaving for 15 min at 121°C. Using this sterilized media PDA plates were prepared. *Aspergillus niger* was allowed to grow on PDA for 2 to 3 days. The well grown colonies after incubation were identified as *Aspergillus niger* based on black colored colony growth, a smear was prepared flooded with lactophenol cotton blue and observed under microscope for morphological identification of *Aspergillus niger.*

Testing the inhibitory effect of heavy metals on Fungi:

Approximately 1 cm of well grown pure culture of fungal mat was taken from the PDA plate and mixed with 1 ml of sterilized distilled water. After mixing well the supernatant containing fungal spores was separated with micropipette. From this fungal spore culture 0.1 ml was taken and spread on PDA medium to get a uniform growth. On these plates one gram of Sterilized heavy metals was taken with sterilized forceps and placed by maintaining distance between each heavy metal tested. The plates were incubated at 37°C for 36 hrs and observed for clear zones around the heavy metals which indicated the zone of fungal growth inhibition.

RESULTS:

Maintenance of fungal culture and identification under microscope:

Aspergillus niger was maintained on PDA. Yellow to white hyphae grow with in 24hrs, turning black with the formation of conidia starts after 36hrs and results in black mould growth. After 36hrs fungal smear treated with lacto phenol cotton blue was observed under microscope. Asexual conidiophores were identified which were long and globose. At the tip conidia, spores were arranged as chains were observed.

Testing the inhibitory effect of heavy metals on Fungi:

Clear zones around the heavy metals indicated the zone of fungal growth inhibition. *Aspergillus niger* growth was inhibited by treating with Copper, Zinc and Magnesium. Whereas other heavy metals Iron, Silver and Aluminium had no inhibitory effect on fungal growth.

Table 1: Zone of fungal growth inhibition on treating with heavy metals

	Mg	Fe	Alu	Cu	Ag	Zn
Inhibitory effect of heavy metals	Inhibition of fungal growth	No inhibition of fungal growth	No inhibition of fungal growth	Inhibition of fungal growth	No inhibition of fungal growth	Inhibition of fungal growth
zone of fungal growth inhibition (cm)	2.1	0	0	1.3	0	2.5

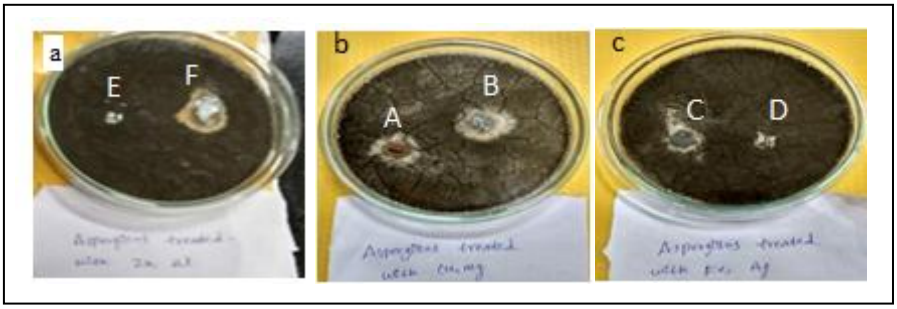

Fig 1: Inhibitory effect of heavy metals on growth of Aspergillus niger on PDA media

(a): Aspergillus niger treated with E- Aluminium, F- Zinc

(b): Aspergillus niger treated with A- Copper, B- Magnesium

(c): Aspergillus niger treated with C- Iron D- Silver

DISCUSSION AND CONCLUSION:

Copper's antimicrobial action and mechanism is complex which takes place on surface and inside the microbial cells. Copper can damage the structure of proteins and enzymes resulting in inactivation of bacteria and viruses. Copper reacting with lipids can damage the cell membrane by creating holes and leaking of cell content leads the cell death. Copper generates super oxide radicals which can damage bacterial cell at many sites. The cell membrane gets damaged and these radicals can act with their individual pathways. Recent works have investigated the activity of copper oxide nano particles (CuO and Cu_2O), which were toxic to *E.coli*. Heavy metal nanoparticles have cytopathic and cytotoxic effects Magnesium oxide nanoparticles (MgO) have stable structure, surface properties and antibacterial effects useful in food industry for eliminating the food borne pathogens like *Escherichia coli* and *Salmonella Enteritidis*. MgO can damage bacterial cell membrane, induce oxidative stress leading to cell death. Silver nanoparticles have medicinal properties thus using silver in daily life is considered good and included in Hindu tradition. To prevent wound infections with bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus* special wound dressings were designed with silver nanoparticles and tested on mice which could reduce the infection. Similarly, the effect of silver ions generated electrically was tested on S. *aureus* and *E. coli*. Silver ions were found reducing the growth of these bacteria by damaging the cell membranes. The activity of silver nanoparticles is shape dependent. Silver nano particles disrupt cell lines. Soluble zinc species or ZnO particles were proved in having the antimicrobial properties. In one study the heavy metal ions were able to decrease the fungal growth. Similarly, in our present study copper, zinc and Magnesium exhibited significant antifungal properties by surface contact. The materials proved fungal growth inhibitory and the mechanism could be the toxic effect on fungal membranes. Usage of copper for cooking, storage of food and water can reduce the fungal contamination. Usage of copper in water supplying pipelines and air conditioning systems can reduce fungal spores' growth thus reducing throat, skin, ear and lung infections. The present study is an evidence for encouraging the usage of metals like copper in daily life.

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