



Fungal Activity of *Xanthium Stumarium* Unsullied Deposit Remove

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Abstract: Plants measure wealthy supply of medication for antifungal properties. These properties are employed properties are employed by plants to protect themselves from foreign pathogens. With the advancement of techniques currently we carry out extracting bio active compounds from plants extract and cure plant and animal infection. The in vitro antifungal activity of binary compound extract from *Xanthium stumarium* plants was also utilized in ancient drugs for the treatment of varied diseases. Extract effectivity was evaluated victimisation the agar well diffusion assay against two fungi i.e. *Aspergillus niger* and *Fusarium oxysporum*.

Keywords: *Fusarium oxysporum*

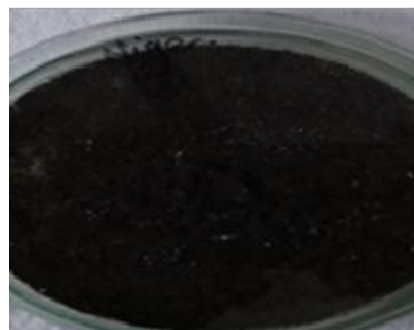
I. INTRODUCTION

A variety of microorganisms as well as fungi, bacteria, viruses, harbour the soil. Out of those microorganisms, few are useful for the soil, however a number of them have the potential to cause major injury to plant growth and reduce the quality of soil. They will result in reduction in plant growth that takes place by obstruction the nutrients that the plant should absorb, or they could cause physical damage that result in uncommon look of the plant successively reducing the market price. Preventive measures taken for the soil to avoid contamination and healthy environmental conditions will be effective for healthy plant growth. Fungi are present and infection caused due to them has become common. These fungal growths can be controlled by fungicides but chemically made fungicides have adverse effects on the plants and on the organism feeding on them. Hence, organic fungicides or naturally available compounds are preferred more to obstruct the growth of fungus on plants.

II. MATERIALS

2.1 Fungi Used

A. *Aspergillus niger*





Isolation - *Aspergillus* culture was obtained by doing agar plate method and the culture was observed under microscope and sub-culturing was done to obtain pure cultures of *Aspergillus niger*. Seven days old culture of fungi was used.

Macroscopic characters - The colony is black in colour and the reverse surface is white to light yellow in colour.

Microscopic characters - Hypha is septate and hyaline. Conidiophore is present and arises from the basal foot of the. Its conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biserial with the phialides borne on brown, often septate metulae. Conidia are globose to sub globose (3.5-5.0 um in diameter), dark brown to black and rough-walled. It is known to create increased amount of pathogenicity in various species of plant.



B. *Fusarium oxysporum*

- **Isolation** - *Fusarium* culture was obtained by placing banana peel on agar which gave pinkish white colonies surrounding the peel. The colony was first identified under microscope and then sub-cultured and *Fusarium oxysporum* pure cultures were obtained. Seven days old culture of fungi was used.
- **Macroscopic characters**- Colonies are initially white in colour and turn pinkish or purplish in colour at maturity. The reverse of the plate shows purplish colour.
- **Microscopic characters**- Hyphae are septate and hyaline. Conidiophores are short and simple (mostly not branched). Conidia may be ellipsoidal, slightly curved in shape.

2.2 Plants used



Xanthium stumarium



It is a summer annual weed in the daisy family that is native to North America belong to family Asteraceae. The plant may have some medicinal properties and has been used in traditional medicine in South Asia and traditional Chinese medicine. In Telugu, this plant is called Marula Matangi. However, while small quantities of parts of the mature plants may be consumed, the seeds and seedlings should not be eaten in large quantities because they contain significant concentrations of the extremely toxic chemical carboxyatractyloside. Native American tribes used the cocklebur medicinally, as a food source (seeds), and in ceremonies. The seed pods may have been used to make a yellow dye.

2.3 Culture Medium Used

A. Liquid Culture Medium

Richard's Broth

Potassium Nitrate: 10g, Magnesium Sulphate: 5g, Ferric Chloride: 0.02g, Sucrose: 50g, Distilled water: 1000ml

All above constituents was added in conical flask. The flask was plugged and autoclaved at the pressure of 15 lbs./sq. inch. at 121°C for 20 minutes. Streptomycin was added before using the broth.

B. Solid Culture Medium

Richard's Agar

Potassium Nitrate : 10g,, Potassium Monobasic Phosphate : 5g, Magnesium Sulphate : 0.25g, Ferric Chloride : 0.02g , Sucrose : 50g , Distilled water : 1000ml , Agar : 18g

All above constituents was added in conical flask. The flask was plugged and autoclaved at the pressure of 15 lbs./sq. inch. at 121°C for 20 minutes. Streptomycin was added before using the agar.

2.4 Chemicals Used

Ethanol - Used as a solvent for extraction of secondary metabolites from *Xanthium stumarium*.

Dimethyl Sulfoxide (DMSO) -Used to dissolved ethanol extract of *Xanthium stumarium*

2.5 Other Requirements

Petri plates, Conical flasks, Micropipettes, cork borer, forceps, nichrome loop, Laminar Air Flow, etc. The entire experiment must be carried out in Aseptic conditions with sterilized glasswares.

III. METHOD

3.1 Ethanolic Extract Preparation of *Xanthium stumarium*:

Plant material (leaf) were carefully washed and oven-dried (120°C for 2 hours) and put in shade and aerated place later for drying completely. The dried leafare ground into a fine powder and the powder is then soaked in Ethanol and kept on shaker overnight (150 rpm for 24 hours). Next day the mixture was filtered using muslin cloth then Whatmann filter paper and the concentrations were made using another organic solvent i.e. DMSO (Dimethyl sulfoxide).

Concentration (%)	Dried powder (gm)	Distilled water(ml)
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5%	10	100
10%	20	100
20%	30	100
30%	40	100
40%	50	100

3.2 Testing of Plant ethanolic Extract of *Xanthium stumarium*:

1. In Liquid Culture Medium: 49 ml of Richard's Broth was added to each conical flask and during the time of experiment 1 ml of plant extract was added to it. The total volume should sum up to be 50 ml in each conical flask. A control flask was maintained to compare the growth and efficacy. The flasks were plugged and autoclaved for sterilization and homogenization of Richard's broth and extract. Preparation of the concentrations of ethanolic extract of *Xanthium stumarium*. After 7 days of incubation, the fungal growth was observed

Concentration g/ml (wt/vol)	Volume of extract(ml)	Volume of broth (ml)
Control	0	50
5	1	49
10	1	49
20	1	49
30	1	49
40	1	49

V. RESULTS

The results indicated that *Xanthium stumarium* was effective as an antifungal extract because it since it indicated a Zone of Inhibition. *Fusarium oxysporum* although was very sensitive to the plant extract as compared to *Aspergillus*

A. On Liquid Culture Medium

Aspergillus showed a considerable reduction in mycelial growth on 40% ethanolic extract of *Xanthium*. It also showed colour changes (brown pigmentation) of *Aspergillus* on Richard's media. The growth of *Fusarium* was very slow in the ethanolic extract and it also showed complete inhibition at 40%. The mycelial mat quantity varied considerably in 20%, 30% of ethanolic extract as compared to the Control used.

B. On Solid Culture Medium



5% shows overgrowth of *Fusariumoxysporum* on the well. 10% onwards there is a major inhibition seen. 40% show maximum inhibitory zone as compared to rest of the concentrations.

VI. DISCUSSION

Both fungi (*Aspergillus niger* and *Fusariumoxysporum*) which fall under the sub division Deuteromycotina, against which antifungal extracts were tested are pathogenic fungi which caused disease of the plants and are most common contaminants of crops worldwide. For testing the efficacy of extracts on pathogenic fungi *A. niger* and *Fusariumoxysporum* two types of methods were performed - 1. Liquid culture media method (Broth method) and 2. Solid culture media method (Agar well-diffusion method). In liquid media ethanolic extract of *Xanthium* showed reduction at 40% in *Aspergillus niger* and complete inhibition in *F. oxysporum* at 40%. On solid media however, *F. oxysporum* showed maximum inhibition zone at 40%. Therefore, the minimum inhibitory concentration is 40%

VII. CONCLUSION

The above investigation has brought us to the conclusion that Ethanolic extract of *Xanthium stumarium* are effective against both pathogenic fungi i.e. *Aspergillus niger* (which causes mold disease) and *Fusarium oxysporum* (which is a wilt causing fungi). *Aspergillus niger* showed more resistance against the plant extracts at most concentrations. *Fusarium* being sensitive to the antifungal compounds present in the extracts has shown considerable decrease in biomass and complete inhibition at the highest concentrations used (40% of the Ethanolic extract). Studies indicate that the higher the concentration of plant extract the higher is effect of inhibition or reduction. Higher concentrations of extract show tremendous reduction in *Aspergillus niger* growth and complete inhibition at a certain percentage for *Fusariumoxysporum*. *Aspergillus niger* does not show inhibition zones even higher concentrations whereas, *Fusariumoxysporum* does.

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