IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Study of Invitro anti Fungal Activity of *Xanthium Stumarium* Fresh Plant Extract against *Aspergillus niger* and *Fusarium oxysporum*

Udaybhan Yadav[1], Kunal Thakur[2], Lalit Rawat[3], Karina Jaiswal[4]
Co-ordinator, Microbiology ID, ZSCT's Thakur Shyamnarayan Degree College, Mumbai, India[1]
Assistant Professor, Microbiology ID, ZSCT's Thakur Shyamnarayan Degree College, Mumbai, India[2]
Students, Microbiology ID, ZSCT's Thakur Shyamnarayan Degree College, Mumbai, India[3,4]

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

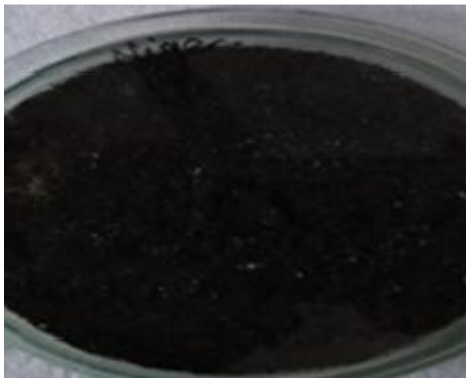
I. INTRODUCTION

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

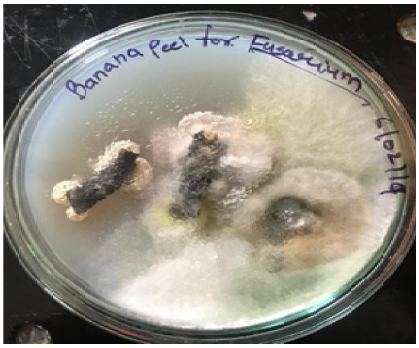
A. *Aspergillus niger*



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 fungiwas used.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

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 biseriate 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. *Fusariumoxysporum*

- **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 fungiwas 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 maybe 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.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

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 *leaf*are 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)
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

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Biomass Estimation

The flasks filtrate was separated by filtration with Whatmann no. 1 filter paper. The weight of filter paper was taken prior. After complete filtration, the mat left on the filter paper was dried completely in oven/incubator (180°C for 2 hours). The dry weight was taken and biomass was calculated.

Observations are recorded and given below.

2. On Solid Culture Medium:

The antifungal activity on Solid medium was studied using Agar well diffusion method. In Agar well diffusion method, sterilized Richard's agar is poured into the petri-plates aseptically. Then the media was allowed to cool. About 5 mm diameter well (reservoir) was made in the centre of the petri-plate using a sterilized cork borer. The plates were then inoculated with fungal discs at equidistant radii. About 100 µl of the plant extract of respective concentration was filled in the well by using sterilized micropipettes. A control petri-plate was maintained without extract for comparison purpose.Petri-plates were incubated for 7 days to observe the zone of inhibition. The observations and pictures are given below.

IV. OBSERVATIONS AND RESULTS

1. In Liquid Culture Media

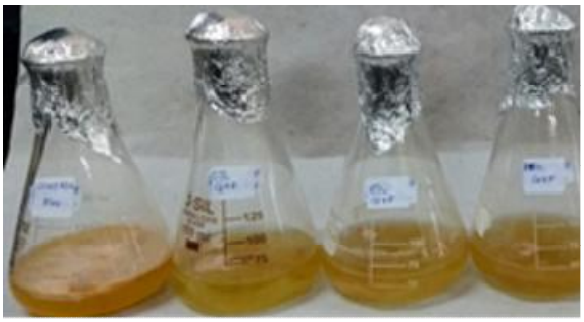
Concentration of Extract	Weight of Whatmann filter paper (g)	Weight of paper + Biomass (After drying)	Weight of Biomass (g)	Visual Fungal growth in Richard's Broth
Control	0.730	1.789	1.059	+++++
5%	0.737	1.728	0.991	++++
10%	0.731	1.650	0.919	+++
20%	0.722	1.642	0.920	+++
30%	0.718	1.548	0.830	++
40%	0.716	1.083	0.367	+

Aspergillus niger grown on Richard's Broth + *Xanthium stumarium* ethanolic extract

Concentration of Extract	Weight of Whatmann filter paper (g)	Weight of paper + Biomass (After drying)	Weight of Biomass (g)	Visual Fungal growth in Richard's Broth
Control	0.896	1.199	0.303	++++
5%	0.886	1.102	0.216	+++
10%	0.866	0.982	0.116	++

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

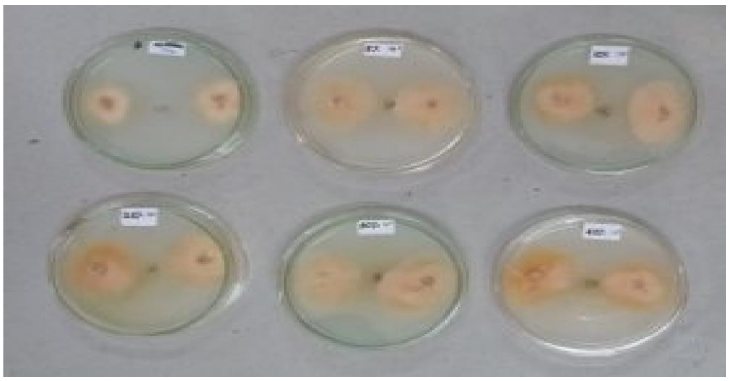
20%	0.889	0.967	0.078	+
30%	0.857	0.925	0.068	+
40%	0.861	0.898	0.037	-



Fusarium oxysporum grown on Richard's Broth+ *Xanthium stumarium* ethanolic extract

2. On Solid Culture Media

Concentration of Extract	Inhibition zone observed on the 8th day (cm)		
	I	II	Average
Control	0.5	0.7	0.6
5%	0.2	0.2	0.2
10%	0.1	0.2	0.15
20%	0.2	0.1	0.15
30%	0.2	0.1	0.15
40%	0.2	0.4	0.3



Fusariumoxysporum inoculated on Richard's Agar plate with *Xanthium stumarium* ethanolic extract

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*

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

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 liqiud 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% fof 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.

REFERENCES

[1]. Noura S. Dosoky et.al. (2016).Composition and Biological Activities of *Murraya paniculata* (L.) Jack Essential Oil from Nepal. Medicines (Basel) Published online 2016 Feb 26. doi: 10.3390/medicines3010007.

[2]. Ogechi N. Agbenin, P.S. Marley. (2006). In-vitro Assay of some Plant Extracts against *Fusarium oxysporumF. sp. lycopersici* causal agent of tomato wilt.

[3]. Pankaj kumar, Shailja Dhiman, R.P.Bhatt and Lokendra Singh. (2011). In-vitro Antifungal activity of Sapiumsebiferum L. against *Aspergillus niger* and Aflatoxigenic *Aspergillus flavus*. Journal of Applied Pharmaceutical Science, 2011.

[4]. Peyman Mikaili et.al. (2013).Therapeutic Uses and Pharmacological Properties of *Vitex*, Shallot, and Their Biologically Active Compounds Published online 2013 Oct; 16(10): 1031–1048.

[5]. Rawal, P. et. al. (2015). Antifungal activity of *Acorus calamus* against *Fusarium oxysporum f. sp. lycopersici*. International Journal of Current Microbiology and Applied Science. (2015) ISSN: 2319-7706. Vol. 4 (1). pp. 710-715.

[6]. Sahi, I. Y., A. N. Khalid. (2007). *In vitro* biological control of *Fusariumoxysporum* causing wilt in *Capsicumannuum*. Mycopath (2007) 5(2): 85-88.

[7]. ShubhiAvasthi, Ajay K. Gautam and Rekha Bhadauria. (2010). Antifungal activity of plant products against *Aspergillus niger*: A potential application in the control of a spoilage fungus. Biological Forum — An International Journal, 2010