ANTIBIOGRAM PROPERTIES OF ALOE VERA EXTRACTS AGAINST FUNGAL ISOLATES FROM AGRICULTURAL LAND

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ABSTRACT

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In soil and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life. In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment. The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies. Currently, there is little evidence on the antimicrobial properties of the medicinal plants under investigation against phytopathogen fungi. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The genus Alternaria Nees is widely distributed in nature and its species are among the most common fungi on the phyllo sphere. It includes both plant-pathogenic and plant-saprophytic species that may damage crops in the field or cause post-harvest decays, causing considerable economics losses for farmers and food industries. Soil samples were characterized for the incidence of fungal strains from contaminated agricultural soils. A total of 5 fungal strains were isolated and 33 fungi were characterized using various isolation and identification methods. Soil samples were also characterized for physiochemical properties. There are limited reports on the antimicrobial effects of isolated Aloe Vera components. Shamir were noted high zone of inhibition with ethanol extracted from Aloe Vera baradenisis against Aspergillums spp, penicillium spp, macro spp, rhizobium spp, Fusarium spp, Candida spp. Antimicrobial susceptibility test showed that both the gel and the leaf inhibited the growth of fungal spp. Experiment has been carried out to define the effect if Aloe vera gels In vitro for the fungi.

Keyword: *Aloe vera, Aspergillus Niger, Candida Albicans*

1.INTRODUCTION

Since antiquity, the plant kingdom has provided a variety of compounds of known therapeutic properties, like analgesics, anti-inflammatory, medicines for asthma, and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world. For example, a large proportion of the South American population use plant extracts obtained from traditional medicinal plants as medicine for many infectious diseases. Plants from the genus Pterocaulon, known as "quitoco", are commonly used in veterinary medicine in southern Brazil to treat animal problems popularly diagnosed as "mycoses". Several works have demonstrated in laboratory trials that different plant tissues, such as roots, leaves, seeds and flowers possess inhibitory properties against bacteria, fungi andinsects.

In addition, the genus produces mycotoxins and phytotoxins, and studies in the last decade have emphasized its toxicogenic properties rather than simply those that cause spoilage. As well, the smallest concentration capable of inhibiting or preventing growth was determined among the species and extracts that demonstrated inhibitoryproperties.

Soil samples were characterized for the incidence of fungal strains from contaminated agricultural soils. A total of 5 fungal strains were isolated and 33 fungi were characterized using various isolation and identification methods. Soil samples were also characterized for physiochemical properties.

The isolated fungal strains were successfully identified belonging to the *phylum Ascomycota, deuteromycota and Zygomycota. Alternaria, Aspergillus, Drechslera* and *Fusarium* were predominant genera. *Curvularia,*

Exserohilum, Humicola, Rhizopus and *Torula* were the most frequently isolated genera. Rests of the strains were not identified owing to the lack of sporulating structures under presently used incubation conditions. Such strains were designated as *Mycelia sterilia*. Further, these species will be used in biodegradation of commonly used pesticides.

Aloe vera belongs to the family Lilaceae, of which there are about 360 species Aloe Vera contain over 75 nutrient and 200 active compounds, including vitamins, enzymes, minerals, sugar, lignin, anthraquinones, sapiens, salicylic acid and amino acids.

It is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities 1. The gel of A. Vera was used to treat stomach ailments, gastrointestinal problems, skin disease, constipation, radiation injury, inflammatory effect, healing wounds and burns, ulcer and diabetes.

Medicinal plants according to the World Health Organization (WHO) defines them as herbal preparations made by introducing plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes, which may be produced as a basis for herbal products or for immediate consumption. *Aloe vera* has modified thick fleshy leaves, it not only has cell wall carbohydrates such as cellulose and hemicellulose but also storage carbohydrates such as acetylated mannans the polysaccharides found in the inner leaves parenchymatous tissue have medicinal importance and also the biological activities are due to presence of large number of compounds. The herb is used internally to combat most digestive problems, including constipation, poor appetite, colitis, irritable bowel syndrome as well as, asthma, diabetes, immune system enhancement, peptic ulcers. *Aloe* is used externally for the treatment of skin irritation, burns, scalds, sunburn wounds, eczema, psoriasis, acne, dermatitis, ulcers, to stimulate cell regeneration. The plant is also used in the treatment of healing properties, effects on skin exposure to UV and gamma radiation, anti-inflammatory, antiviral and antitumor, moisturizing, anti-aging effect, antiseptic, enhance immunesystem.

The study showed that Aloe vera juice has antimicrobial activity against *Candida albicans & Aspergilla's spp, penicilliumspp, macro spp, Fusarium spp, rhizobium spp. Caspian*, found that hydro alcoholic extracts of fresh leaves of Aloe vera have inhibitory effect against the mycelia growth of Fusarium oxysporum, and Penicillium gladioli. Jasso also evaluated antifungal activity of pulp and liquid fraction of Aloe vera on the mycelium development of *Rhizoctonia solani, Fusarium oxysporum &Collectotrichum coccids* and found positiveresults.

2. OBJECTIVE:

- To isolate and identify the fungi spp, from the agricultural soilsample.
- To study about the aloe vera activity against the isolatefungi.
- Optimization of the aloe vera activity under different extract. (gel andpowder)
- To comparison of gel extract and powder extract against isolated fungi.
- To check the MIC of the aloe vera gel and powder against isolated fungi spp, (Well Diffusion Method & Tube testmethod)

3.REVIEW OF LITERATURE:

3.1 SOIL MYCOBIOTA: ROLE AND IMPORTANCE

The saprophytic fungi, typically exemplified by the Phycomycetes, are pioneer colonizers of injured, moribund and dead plant and animal tissues, for which role they are ecologically equipped by an exceptionally high growth rate, and especially by a capacity for rapid germination of resting spores and mycelial cells. (Agrios et al.., 2004)

A rapid 'flare up' in the presence of a suitable substrate, followed by a period of quiescence, appears to be characteristic of such fungi, and it is suggested that the substrate comes to the dormant fungus at least as often as the active fungus contacts fresh substrates by mycelial growth through the soil. (Harris et al..,2001)

Coleman et al…, (2001) discussed on the soil biota, soil systems and major soil processes occurring in those ecosystems. The review describes how soils act as components of ecosystems, their role as organizing centers in ecosystems, various major soil processes and biodiversity in soils. It is apparent that a large proportion of the biota associated with soils are as yet undescribed, with the most extreme cases being the bacteria and fungi. Therefore, it is premature to give even a rough estimate of the total numbers of species that occur in many of these taxa, as such large percentages of the total number of organisms are still unknown. It is incumbent on the rising generation of ecologists and biologists to develop more innovative ways to describe, catalog, and understand the myriad patterns and processes in the biosphere, which are due in large part to the actions of thebiota.

Cowan et al.., (2001) made a review on fungi as life support for ecosystems. Fungi are fundamental to the success and health of almost every ecosystem on the earth, both terrestrial and aquatic, and essential to the sustainability ofbiodiversity.
Fungi are perhaps the most unappreciated, undervalued and unexplained organisms on earth. They perform a crucial role in the transport, storage, release and recycling of nutrients.

Fig 1: Fungal spp, on the soil

Jenkins et al., (2005) describes soil fungi as microscopic plant-like cells that grow in long threadlike structures or hyphae that make a mass called mycelium. The mycelium absorbs nutrients from the roots it has colonized, surface organic matter or the soil. It produces special hyphae that create the reproductive spores. Some fungi are single celled (e.g. Yeast). Fungi have many different structures but they can act in similar ways and thus are not as plant specific in their needs as some soil bacteria such as Rhizobia.
There are three functional groups of fungi: Pathogens, Mutualists and Decomposers that they perform important functions within the soil in relation to nutrient cycling, disease suppression and water dynamics, all of which help plants become healthier and more vigorous. (Demo and olive et al..,2008).

4.SOIL POLLUTION AND ITS IMPACT ON MICROBIOTA AND HUMAN HEALTH:

The antifungal activity and antioxidant activities of the leaf extract was determined by the agar-well diffusion method against plant and human fungal pathogens. Phytopathogenic fungi were *Fusarium oxysporum*, *Pythium sp*. and *Rhizoctonia solani*. The results of the antifungal activity of the Aloe Vera leaf extract were shown to display antifungal activity against all tested fungi (Khaing et al..,2011)
Rabah and Ibrahim et al.., (2010) observed changes in microbial community content as well as physio-chemical properties of soil contaminated with tannery effluents in Sokoto metropolis that were determined using standard procedures. The results showed that the soil sample contained a variety of microorganisms which include *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcensces, Escherichia coli, pyogenes, Klebsiella pneumoniae, A. niger, A. flavus, A. fumigatus, Penicillium notatum, Mucor pusillus and Fusarium sporotrichosis's*. It also revealed high counts of bacteria and fungi in all the sampling sites. The viable count of bacteria was in the range of 8.60±1.80 – 8.70±0.52 ×105 Cfu/g while that of fungi was 1.70±0.30 – 2.0±0.10 × 104 Cfu/g. Similarly, it revealed high levels of Sulphide (0.35-0.44 mg/g), Ammonia (0.40-0.60 mg/g), and Chromium (Cr) (0.20-0.26 mg/g) in all the sampling sites. These levels exceeded the tolerable levels set by the Federal Ministry of Environment. The presence of these microorganisms and chemical substances pose a potential threat to the local inhabitants of these areas.
Despite their central role in ecosystems and their applications in biotechnology, knowledge about fungi remains at a low level. It has been estimated that only 5% of the World's fungi have so far been discovered, and for most of these, little is known about their biology. (cowan et al.., 1999).

5.ISOLATION OF SOIL SAPROPHYTIC FUNGI:

Commercial malt-extract (Bio-Malz), 20 g; K2HPO4, 0.5 g; Fe2+, Mn, Cu, Zn, Mo, B, Co, 1 ppm each (added as soluble salts, not as nitrate); rose Bengal, 1 part in 15,000; agar, 20 g; tap water, 1 liter; pH 6.0 to 6.2. The incorporation of rose Bengal in the medium suppressed and reduced the development of bacterial colonies, which either remained white or were slightly dyed. Comparative results yielding highest counts of actinomycetes and fungi were obtained on von Plotho's glycerol-glycine medium, closely followed by rose Bengal-malt extract- agar with least colonies on oat meal agar.

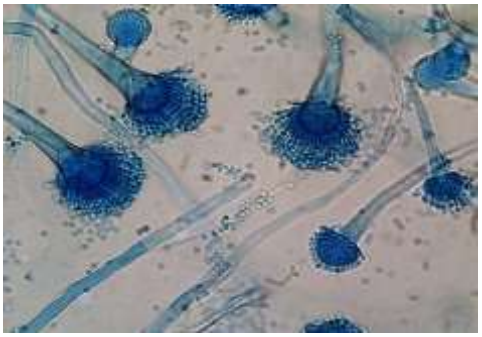


Fig2: *fungal spp,*

Khalil et al.., (2013) analyzed the microbiota composition of the mangrove soil in Egypt by collecting 24 soil samples. Almost all samples showed clay, sandy to sandy loam texture. pH of the soil samples ranged from 7.5 to 8.9 and water content ranged from 8% to 9%. The filamentous fungi of each soil sample were examined using two isolation methods. Fifteen fungal species belonging to nine genera were identified. Results showed that most of the genera detected belonged to the Ascomycotina with fewer proportions belonging to the Deuteromycotina and Zygomycotina. The frequent species were in decreasing order: *Aspergillus* spp, *Cladosporium sp, Alternaria sp, Penicillium sp, Rhizopus sp, Absidia sp, Acremonium sp,* and*Trichodermasp*.One-wayAnalysisofVariance(ANOVA)testshowedsignificant differences of richness and diversity of microflora between sites likewise pH and Na ion of soil analysis. In Egypt as well as in other developing countries, the information on diversity of fungi associated with mangrove soil is limited. Thus, this study was conduct to elucidate the distribution and diversity of fungal species associated with selected mangrove areas.

6. TURBIDOMETRIC GROWTH STUDIES OF FUNGI:

Bajwa et al. (2007) carried out in-vitro studies to evaluate the antifungal activity of Aloe vera shoot extract in aqueous (polar) and organic (non-polar) solvents against few pathogenic species of genus *Alternaria* viz., *A. alternata, A. citri* and *A. tenuissima*. The assessments revealed that Aloe vera contained substantial antimicrobial efficacy. The shoot aqueous extracts caused significant inhibition in growth and biomass production of the three tested fungi.

Taiga et al.., (2011) the efficacy of three plants extracts (*Azadirachta Indica, Nicotiana tabacum* and *Aloe barbadensis*) were tested in-vivo on the fungal pathogens, using four different concentrations of cold and hot aqueous extracts. fungi (*Fusarium oxysporium, Aspergillus niger, Rhizopus stolonifer, Penicillium oxalicum*) pathogens associated with dry rot of yam (*Dioscorea rotundata*) tubers were isolated.

7. *ASPERGILLUS*: DOMINANT SOIL GENERA:

Iram *et al..,* (2011) studied the micro-fungal flora of heavy metals contaminated peri- urban agricultural fields of Pakistan that were investigated in terms of their diversity by soil serial dilution method. A total of 30 micro-fungi were isolated from 6 sampling sites. Of these isolates 24 belong to phylum Ascomycota, 3 to phylum Zygomycota, 2 to phylum Basidiomycotaand1tophylumDeuteromycota.Themostwidespreadgenuswas*Aspergillus*and common species *A. niger*. Frequency percentage showed that Kasur is rich in fungal population as compared to other peri urban areas while Wah Cantt showed maximum fungal Colony Forming Unit (CFU). The aim of present investigation was to see the diversity of fungi in heavy metal contaminated soils of peri-urban agricultural areas and study them in future for heavy metal tolerance and biosorption analysis in reference tobioremediation.

Wahegaonkar *et al..,* (2011) sampled twenty-three soil samples of three ecosystems in and around the city of Aurangabad and were investigated for total number of organisms and the specific composition of hyphomycetous fungi. Total 45 genera distributed in 85 species were isolated, maximum being in agricultural soils. The relationship between the genera of fungi and different ecosystem type was analyzed. No obvious variation was observed in the different soil types.

The dominant genera in all the ecosystem types were also studied. *Aspergillus* was dominant in all the three types of soils followed by *Alternaria, Cladosporium, Trichoderma, Gliocladium* and *Gloeosporium*. Species diversity and diversity indices of these soil types were calculated.

8. CHARACTERISTICS OF ALOE VERA:

Fig 3: Aloevera

SCIENTIFIC CLASSIFICATION

Kingdom	:	plantae
Clade	:	angiosperms
Order	:Asparagales	
Family	:Asphodelaceae	
Subfamily	:Asphodeloideae	
Genus	:Aloe	
Species	:*A. Vera*	

8.1Active components with its properties:

Aloe vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids. (Atherton p etal..,1996)

1. ***Vitamins*:** It contains vitamins A (beta-carotene), C and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline. Antioxidant neutralizes freeradicals.
2. ***Enzymes***: It contains 8 enzymes: aliiase, alkaline phosphatase, amylase, brady kinase, carboxypeptidase, catalase, cellulase, lipase, and peroxidase. Brady kinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars andfats.
3. ***Minerals:*** It provides calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc. They are essential for the proper functioning of various enzyme systems in different metabolic pathways and few areantioxidants.
4. ***Sugars:*** It provides monosaccharides (glucose and fructose) and polysaccharides: (glucomannans/polyominoes). These are derived from the mucilage layer of the plant and are known as mucopolysaccharides. The most prominent monosaccharide is mannose-6- phosphate, and the most common polysaccharides are called glucomannans [beta-(1,4)- acetylated mannan]. Acemannan, a prominent glucomannan has also been found. Recently, a glycoprotein with antiallergic properties, called alprogen and novel anti-inflammatory compound, C-glucosyl chromone, has been isolated from Aloe vera gel. (Hutter JA,Salmon M etal.,)
5. ***Anthraquinones:*** It provides 12 anthraquinones, which are phenolic compounds traditionally known as laxatives. Aloin and emodin act as analgesics, anti-bacterial and antivirals.
6. ***Fatty acids:*** It provides 4 plant steroids; cholesterol, campesterol, β-sisosterol and lupeol. All these have anti-inflammatory action and lupeol also possesses antiseptic and analgesic properties.
7. ***Hormones:*** Auxins and gibberellins that help in wound healing and have anti-inflammatory action.

Others: It provides 20 of the 22-human required *amino acids* and 7 of the 8 essential amino acids. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into the skin. Saponins that are the soapy substances form about 3% of the gel and have cleansing and antiseptic properties

9. MATERIALS AND METHODS:

9.1. SAMPLES COLLECTION:

The soil sample 50 were collected from different agricultural land in around Tirupur, Coimbatore district and transferred aseptically to the lab for further analysis.

9.2. SAMPLEANALYSIS:

9.2.1. Color of soil:

Different color soils were collected from agricultural land.

9.2.2. Temperature ofsoil:

The soil sample collecting areas where be prepared and insert the thermometer into the soil approximately 3-4 deep for 10 -15 mins

9.2.3. Moisture content:

10 g of soil samples collected from agricultural fields were dried at 600C for 72 h in oven and then the moisture content was calculated. Dry weight of the sample was taken till it showed its constant weight.
The percent moisture was expressed as follows:

$$\text{Moisture \%} = \frac{W1 - W2}{100} \times 100$$

Where, W1 = Weight of soil before oven drying W2 = Weight of soil after oven drying

9.2.4.p^H of soilsample:

Soil sample were dried at 60 0C for 72 hrs., and dissolved in distilled water (2.5w/v) and vertexing for 5 minutes at 120 rpm then pH was measured by digital pH meter15.

9.3. ISOLATION OFFUNGUS:

Isolation of fungus spp, was done by spread plate method.

9.3.1. Procedure:

- Collected the soil sample from agriculturalland
- Each of the 50-soil sample ,1g of soil sample was taken and mixed with 9 ml of sterile distilled water which gives10^{-1}
- Take 1ml from this and transferred to 9ml distilled water in a tube which gives 10^{-2}dilution,
- Serial dilution was performed by transferring 1ml of this to the subsequenttubes,
- Poured Potato Dextrose Agar medium into the petri plate, and allowed tosolidify,
- After, Transferred 1ml of the desired soil suspension to sterile petridish,
- To spread the sample by using of "L" rod and rotatingtable,
- After, allowed to incubation at$37^{0,}$
- Observed the development of colonies after 4-7day.

9.4. IDENTIFICATION OF FUNGUS LACTOPHENOL COTTON BLUESTAINING:

The staining technique used for the identification of fungi and to study the morphological characteristics was lactophenol cotton blue staining.
Lactophenol cotton blue stain is recommended for mounting and staining yeast and molds. Itconsists of phenol crystal, lactic acid, glycerin, cotton blue and distilled water. The phenol crystal helps in the penetration of the fungal cell wall and lactic acid helps in fixing the fungal cells to the slides. Cotton blue is an acid dye that stains the chitin present in the cell walls of fungi.

9.4.1. Procedure

- A drop of the fungal culture was taken on a clean glass slide and teased with the aid of sterilizedneedle.
- A drop of lactophenol cotton blue was poured over theculture.
- A cover slip was then placed over it, taking care to prevent the formation of air bubbles.
- The excess stain was removed by means of a blottingpaper.
- The slide was viewed under themicroscope.

9.5. SUBCULTURING:

Most of the plates show the development of a particular fungal colony. The fungal colony was sub cultured to Czapek dox agar (CDA) plates and incubated at 37^0c for 4-7 days and pure cultures were obtained.

9.6. DETERMINTION OF ANTIFUNGALACTIVITY:

9.6.1. Preparation of aqueous extract:

The above dried residue was extracted with 100 ml of distilled water by occasional shaking for 24 hours. The extract was filtered through Whatman No.1 filter paper and then concentrated to one fourth of the original volume by evaporation at roomtemperature**.**

9.6.2. Preparation of brothinoculum:

A part of the fungal colony was then transferred into the Potato Dextrose Broth (PDA) by aseptically punching out 5 mm of the agar plate culture with a cutter. A shake flask culture was carried out in 250 ml flasks containing 50 ml of the medium at 130 rpm and incubated at room temperature over a period of time.

9.6.3. Agar well diffusionmethod:

Antifungal assay was carried out with standard fungal cultures such as *Aspergillus spp, Penicillium spp, Rhizopus spp, Fusarium spp, and Mucor*spp, by well diffusion method.

Czapek Dox Agar	- 49 g
Sucrose	-30.0g
DipotassiumPhosphate	- 1.00g
Magnesium sulphate	- 0.50g
Potassium chloride	- 0.50g
Ferrous sulphate	-0.01g
Agar	-15.00g

The test fungus was grown on CDA plate at 28ºC and maintained with periodic sub culturing and storing at 4ºC. The fungal spore suspension was prepared by flooding the surface of a two-week-old culture of the individual test fungi with 10 mL of sterile phosphate buffered saline, (PBS; 10 mM phosphate buffer, 2.7 mM potassium chloride, 137 mM sodium chloride, pH 7.4). The spore suspensions were stored at 4ºC for furtheruse.

CDA medium was poured in to a Petri dish, allowed for solidification and after that the fungal broth culture was swabbed uniformly with fungal spore suspension of different fungal cultures. The appropriate wells were made on agar plate by using well cutter and loaded with different concentration of powder and gel extracts like 100 µg/ml, 50µg/ml, 25 µg/ml and 12.5 µg/ml and antifungal agent clotrimazole was also loaded in the center well of the agar plate that served as a control. Incubation period of 24-28 hours at 28ºC was maintained for observation of antifungal activity of Aloe Vera aqueous extract and gel extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the aloe vera extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm. (This procedure similar for both powder aqueous extract and gel extract)

9.7.DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC):

9.7.1. Broth dilution method:

Minimum inhibitory concentration (MIC) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganisms after overnight incubation, as minimum fungicidal concentration (MFC) as the lowest concentration of antimicrobial that will prevent the growth of an organisms after subculture on to antibiotic free media.

9.7.2. Procedure

- The plant extract was serially diluted(twofold) in 2ml of potato dextrose broth to obtain a concentration range of 512 mg/ml, 256 mg/ml, 128 mg/ml, 64 mg/ml, 32 mg/ml, 16 mg/ml, 8 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml, and 0.5mg/ml.
- A fungal inoculum, equal to the volume of the broth was added to each test tube. Controls were equally set up by using gentamycin and test organisms withoutextract.
- The test tube was incubated at 37^0c for 24hours.
- The lowest concentration (highest dilutions) of the extract preventing the visible bacterial growth was recorded as theMIC.

(This procedure carried for both gel and powder extract)**.**

10. RESULTS:

10.1. SAMPLES COLLECTION:

Fig5: agricultural land

The soil samples were collected from agricultural land. 50 soil samples were collected in and around Tirupur and Coimbatore area.

10.2. COLOUR, TEMPERATURE,MOISTUR

Fig 6: Soil from sulur**Fig 7:**Different soil sample

TABLE 1: CHARACTERISTICS OF SOIL SAMPLE

SAMPLE	COLOUR	TEMP^{0}C	$_p$H	%MOISTURE
1	Black	30.2	8.26	0.62
2	Gray	30.5	8.56	0.37
3	Black	32.4	8.55	0.51
4	Dark gray	30.3	8.43	0.62
5	Gray	31.0	8.56	0.52
6	Black	32.4	8.37	0.39
7	Brown	30.5	7.96	0.42
8	Brown	32.6	8.57	0.70
9	Gray	31.2	8.55	0.59
10	Black	30.	8.57	0.61
11	Dark gray	30.6	8.57	0.51
12	Gray	31.1	8.57	0.60
13	Gray	30.7	8.43	0.43
14	Brown	30.4	8.18	0.96
15	Brown	31.3	8.25	0.58

16	Black	30.7	8.35	0.36
17	Brown	31.1	8.44	0.62
18	Dark gray	30.5	8.26	0.37
19	Black	31.2	8.56	0.52
20	Gray	33.3	8.56	0.62
21	Black	33.4	8.55	0.51
22	Brown	32.2	8.43	0.69
23	Dark gray	33.1	7.96	0.42
24	Black	32.3	7.63	0.38
25	Brown	33.5	8.55	0.61
26	Dark gray	30.6	8.65	0.51
27	Gray	33.1	8.57	0.63
28	Brown	32.5	8.63	0.36
29	Black	31.1	8.26	0.58
30	Brown	34.0	8.20	0.95
31	Dark gray	30.6	8.26	0.90
32	Gray	31.6	8.82	0.78
33	Black	31.3	8.43	0.35
34	Brown	31.3	0.46	0.69
35	Dark gray	30.5	7.02	0.80
36	Gray	33.1	8.26	0.46
37	Black	30.3	8.56	0.73
38	Brown	29.4	0.04	0.62
39	Gray	30.1	8.55	0.37
40	Brown	32.2	8.43	0.62
41	Gray	32.3	8.76	0.51
42	Black	28.9	8.65	0.70
43	Black	30.5	8.40	0.42
44	Brown	37.2	0.69	0.95
45	Gray	34.0	7.29	0.90
46	Dark gray	31.5	6.00	0.78
47	Black	32.4	8.39	0.35
48	Brown	31.2	8.29	0.69
49	Black	32.3	0.45	0.46
50	Brown	31.0	0.78	0.80

10.3. ISOLATION OFFUNGI:

Isolation of fungus was done by spread plate method. Different fungal colonies were observed on the CDA plate, the colonies that are different colors and they are selected and sub cultured on SDA plates.

10.4. IDENTIFICATION OFFUNGUS

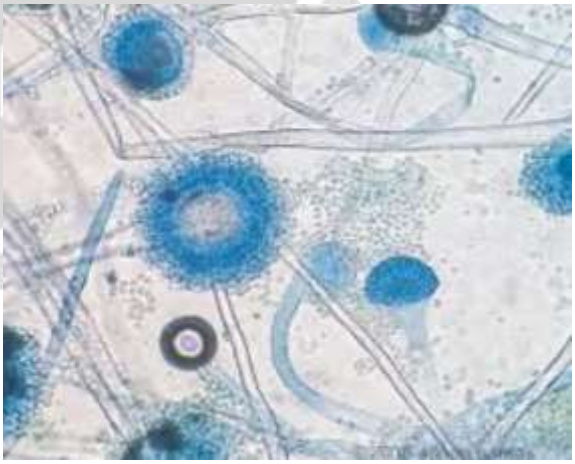
From the collected 50 soil sample, five types of fungi were repeated and isolated. The colonies morphology of the isolated fungal mycelia was observed as Black, Light green, Brown, dark green color fungi respectively.

Table 2: Types Of isolated fungi and their features:

S.NO	ISOLATE	COLONY MORPHOLOGY		NAME OF THE STAIN
		Macroscopic feature	Microscopic feature	
1	Strain 1	Brown-black, downy to powdery. Growth rate varies from slow to rapid.	Septate hyphae, conidiophores are biseriate, flask shaped phialides.	*Aspergillus* spp,
2	Strain 2	Initially white and fluffy colonies, later turns to greenish colour.	Septate hyaline hyphae, simple or branched conidiophores, phialides observed.	*Penicillium*spp,
3	Strain 3	Its fluffy appearance with a height of several cm resembles cotton candy. White initial and become grayish brown in time.	Non-septate or sparsely septate, broad (6-15 µm) hyphae, sporangiophores, sporangia, and spores are visualized.	*Mucor* spp,
4	Strain 4	The color of the colony may be white, cream, tan, salmon, cinnamon, yellow,	Hyaline septate hyphae, conidiophores, phialides,	*Fusarium spp,*
5	Strain 5	The texture is typically cotton candy like. From the front the color of the colony is white initially and turns gray to yellowish brown in time.	Non-septate or sparsely septate broad hyphae sporangiophores, rhizoids sporangia, sporangiospores are visualized.	*Rhizopus spp,*

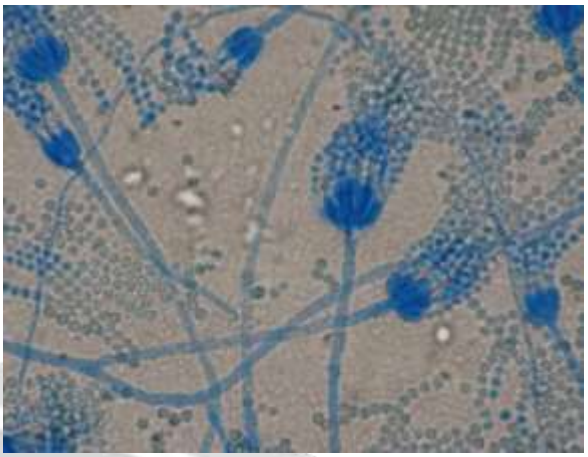
Based on staining and microscopic observations among these five cultures were identified as *Aspergillus spp, Penicillium spp, Mucor spp, Fusarium spp, and Rhizopus spp,*

Strain 1



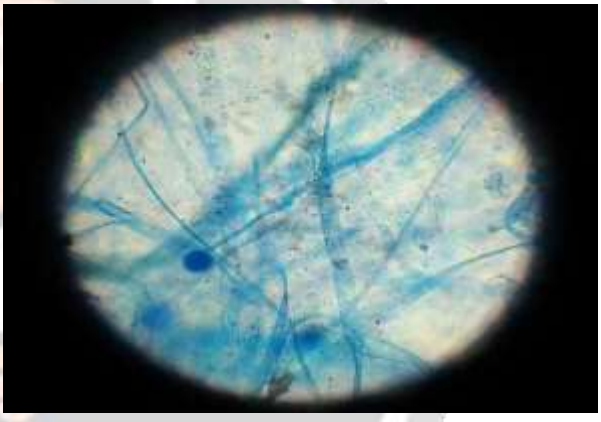
1.subculturing 2.*Aspergillus spp,*

Strain 2

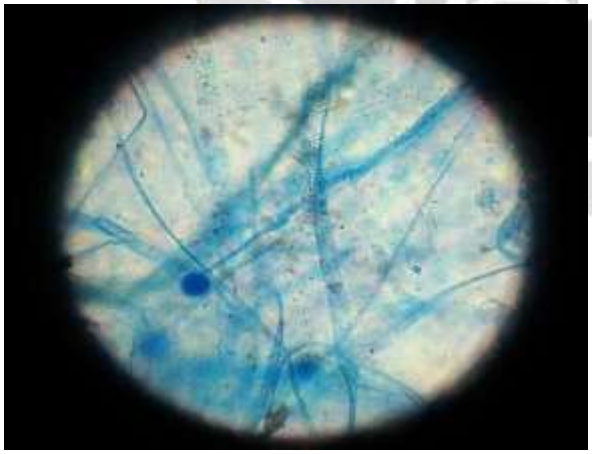
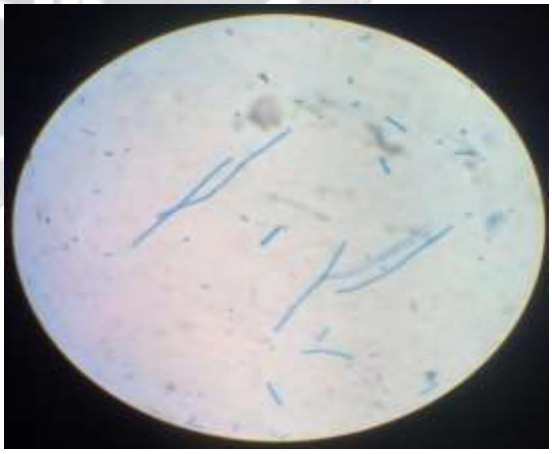
1.sub culturing2.*penicilliumspp*

Strain 3

1.subculturing 2.*Mucor*spp,

Strain 4

1.subculturing 2.*fusarium*spp

Strain 5

1.subculturing 2.*Rhizopus*spp,

TABLE3: ANTIFUNGAL ACTIVITY OF ALOE VERA AGAR WELL DIFFUSION (GEL EXTRACT)METHOD

FUNGAL STRAINS	1.00 g	0.500 g	0.250 g	0.125 g	CONTROL
*Aspergillus*spp,	No zone	0.1	0.5	1.0	No zone
*Fusarium*spp,	No zone	No zone	0.4	0.9	No zone
*Mucor*spp,	No zone	0.1	0.3	0.7	No zone
*Penicilum*spp,	No zone	No zone	0.4	0.9	No zone
*Rhizopus*spp,	No zone	0.2	0.6	1.1	No zone

TABLE 4: ANTIFUNGAL ACTIVITY OF ALOE VERA AGAR WELL DIFFUSION METHOD(Aqueous extract)

FUNGAL STRAINS	1.00 g	0.500 g	0.250 g	0.125 g	CONTROL
*Aspergillus*spp,	No zone	0.1	0.4	0.9	No zone
*Fusarium*spp,	No zone	No zone	0.3	0.7	No zone
*Mucor*spp,	0.1	0.3	0.7	0.9	No zone
*Penicilum*spp,	No zone	No zone	0.4	0.9	No zone
*Rhizopus*spp,	No zone	No zone	0.2	0.7	No zone

TABLE 5: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)
BROTH DILUTION METHOD (GEL EXTRACT)

TABLE 6: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)BROTH DILUTION METHOD (AQUEOUS EXTRACT -POWDER)

FUNGAL STRAINS	512	256	128	64	32	16	8	4	2	1	contro l
*Aspergillus*spp,	-	-	-	-	-	+	+	+	+	+	+
*Fusarium*spp,	-	-	-	-	-	-	+	+	+	+	+
*Mucor*spp,	-	-	-	-	-	-	+	+	+	+	+
*Penicillium*spp,	-	-	-	-	-	-	-	+	+	+	+
*Rhizopus*spp,	-	-	-	-	-	+	+	+	+	+	+

11.DISCUSSION:

Antifungal activity of *Aloe vera* gel was determined against five plant pathogenic fungi viz., *Aspergillus spp*, *Penicillium*spp, *Mucor* spp, *Fusarium* spp, and *Rhizopus* spp,

The *Aloe vera* gel @ 0.125g,0.250g,0.500g & 1.00g concentration tested by agar diffusion plate method caused significantly reduction in the growth of above-mentioned fungi. The rate of growth reduction was directly proportional to the concentration of tested material in the medium.

Result showed that *Aloe vera* gel significantly inhibited the growth of all tested fungi. 0.15% concentration of *Aloe vera* gel posses' remarkable antifungal activity toward all fungi compared to control except *Aspergillus niger*; whereas &*Penicillium spp*, showed moderate antifungal activity at this concentration.

Antifungal activity of *Aloe vera* gel was determined against five plant pathogenic fungi viz., *Aspergillus spp*, *Penicillium* spp, *Mucor* spp, *Fusarium* spp, and *Rhizopus* spp,

The *Aloe vera* gel @ 0.125g,0.250g,.500g, and 1.00g concentration tested by agar diffusion plate method caused significantly reduction in the growth of above-mentioned fungi. The rate of growth reduction was directly proportional to the concentration of tested material in the medium. Result showed that *Aloe vera* gel significantly inhibited the growth of all tested fungi. 0.15% concentration of *Aloe vera* gel posses' remarkable antifungal activity toward all fungi compared to control except *Aspergillus niger*; *Rhizopus* spp, whereas *Penicillium* spp, *Mucor* spp, *Fusarium* spp, showed moderate antifungal activity at this concentration. Only two fungal species viz., *Mucor* spp and *Fusarium* spp, had strong antifungal properties towards *Aloe vera* gel at same concentration. The result in close conformity with the tested antifungal activity of natural *Aloe vera* gel on four plant pathogenic fungi viz.,*Mucor* spp, *Aspergillus niger*; *Rhizopus* spp, the result showed that natural gel suppresses the mycelial growth of *Penicillium* spp, and *Fusarium* spp,

The in vitro antifungal effect of Malaysian *Aloe vera* leaf extracts in alcohol and aqueous solutions on two common pathogenic *penicillium*species, *A. niger* and *C. albicans*, using the zone of inhibition and MIC to determine antimicrobial activity. We found that both alcohol and aqueous extracts demonstrated notable antifungal properties against *A. niger*. The antifungal effect of this study was solvent dependent. The highest concentrations of alcohol and aqueous extracts displayed the maximum zone of inhibition. *Fusarium* spp, showed resistance to both. The no zonesof inhibition of *Aspergillus* spp, for both the gel and aqueous extracts for all five concentrations tested.

The used the agar diffusion method and crude *Aloe vera* leaf extracts in methanol, ethanol, and ethyl acetate and found that all three extracts had no zone of inhibition for the *Fusarium* spp, As for *A. niger*, the zone of inhibition was highest for the methanol extract followed by gel and aqueous extracts. According to Aqueous extract was effective secondary to its constituent of extraction. The resistance of *Rhizopus* spp, towards *Aloe vera* leaf extracts in our study compared to other regions may be due to geographical and climatic conditions, which may affect the phytochemical composition of the plant and its antifungalactivity

12.CONCLUSION:

Fungi have many different structures but they can act in similar ways and thus are not as plant specific in their needs as some soil bacteria such as Rhizobia.
The total hydroalcoholic plant extract obtained from Aloe vera fresh gel and powder extract had antifungal activity against the mycelial growth of *Aspergillus spp, Fusarium*spp,*Penicillium* spp, *Mucor spp, and Rhizopus spp,* compared to the control (Gentamycin).
Results bring new information to the literature data about the antifungal activity of A. vera plant extract against the mycelial growth, on Czapek-agar medium, of phytopathogenic fungi isolated from ornamental plants.
These isolated fungal spp, are sensitive to maximum amount of ALOE VERA extract the zone were observed in 1.00g and 0.500g of concentration of A. Vera.

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