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Research Article

ANTIFUNGAL POTENCY OF *EUCALYPTUS GLOBULES* LABILL ESSENTIAL OIL AGAINST IMPORTANT PLANT PATHOGENIC FUNGI

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ABSTRACT
Extensive use of synthetic chemical fungicides due to their non-degradable nature, involve serious issues to human health and also to the environment. Today there is also an issue arose regarding development of drug resistant pathogens against traditionally used synthetic chemical fungicides. To counteract the problem; scientist are trying to find eco-friendly and more specific control measures through using plant extracts and essential oils to control fungal spoilage and thereby enhancing the self-life of fruits and vegetables. In the present study, *Eucalyptus globules* Labill essential oil was screened to find its potential antifungal activity against eleven important crops destroying fungal strains namely *Alternaria alternata, Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* f.sp. *laginariae*, *Fusarium solani* (GUB05), *Fusarium solani* (GUB06), *Rhizoctonia solani*, *Fusarium oxysporum*, *Rhizopus oryzae*, *Sarocladium oryzae* and *Sclerotium hydrophilum.* Normally the *Eucalyptus* essential oil is used for the medicinal purposes, but here an attempt is made to detect the antifungal potency and to use it against the commonly found crop and yield destructing fungal strains. Moreover they can be used as a fumigant during the storage and transportation of fruits and vegetables to prevent the post-harvest fungal decay and spoilage. *In vitro* Antifungal screening experiments in the present study has revealed that screened essential oil possess good antifungal potential. Thus the data generated, about the effective antifungal properties of oil can be further used to increase the self-life of produce and the results can be further used as a base for supplementary investigation to isolate the active principles, elucidate the structures and evaluate them against wider range of drug-resistant fungal strains.

Keywords: *Synthetic Chemical Fungicides, Fungal Pathogens, Essential Oils, Antifungal Principles, Biofungicide*

INTRODUCTION
Fungal crop destruction and related issues: Fungi are microscopic and are heterotrophic in nutrition. Many are obligate parasites. Fungi play an important role in the nutrient cycle as they decompose the organic matter and return the simpler forms back to the soil. But many times they attack the fresh produce and make them inedible to consume. Fungi largely reproduce by the production of minute and huge quantity of asexual spores, which is a major source of fungal infestation and rapid proliferation (Karbin *et al.,* 2009; Murthy *et al.,* 2009). Fruits and vegetables due to their lower range of pH, higher moisture content and rich nutrient compositions, are highly susceptible to the attack of vastly prevalent pathogenic fungi. Thereby resulting in to a destruction and economical loss of crops and harvested produce (Sharma and Tripathi, 2006). Infestation by microorganisms like species of *Aspergillus* causes losses in terms of pre and post harvest bio-deterioration, spoilage, seed quality and nutritional quality of grains, vegetables, fruits and agricultural produce (Satish *et al.,* 2007). Many fungal strains after vegetative hyphal-mass production, secretes some chemicals broadly known as Mycotoxins; which turn the food in to inferior quality and consumption of this contaminated food results in to a food poisoning. In developing countries 12% pre-harvest and 10 - 30% post-harvest yield losses are reported through fungal diseases which is a huge figure (Baiyewu *et al.,* 2007; Fatima *et al.,* 2009).
Synthetic chemical fungicides are the primary means of control due to ready availability. In field conditions these chemicals often exhibits non-target effects and destroys soil's beneficial mycoflora and thereby the organic matter content in the soil. Also due to their persistent nature they impose various

environmental and residual toxicity issues (Taiga *et al.*, 2008; El-Mougy and Alhabeb, 2009). Thus synthetic chemical fungicides are recently come under special scrutiny by World Health Organization (WHO). Development of resistance by fungal pathogen populations against these traditionally used chemicals has also leaded us to find the alternative strategies that are eco-friendly and bio-safe (Prasad *et al.*, 2010; Wang *et al.*, 2010).

Bio-control of Pathogens: To overcome the issue, botanical extracts and essential oils can serve a best alternative to these hazardous synthetic chemicals due to their eco-friendly, bio-degradable nature and a target specific effect. The plant world is a rich storehouse of natural chemicals commonly referred as Plant Secondary Metabolites (PSMs) or phyto-chemicals, which helps the plants to fight against some diseases and insect pathogens (Khan and Nasreen, 2010; Patel and Jasrai, 2013). These bio-active phyto-chemicals can be formulated in to botanical fungicides for pathogen control and disease management (Mohana and Raveesha, 2007). But due to lack of information on the evaluation of diverse plants for their antibacterial activity, many species of higher plants have not been evaluated for presence of biologically active novel sources of antifungal constituents and developed in to value added fungicide product. Also the plants used in traditional medicines should be scientifically investigated as a potential source of novel antimicrobial compounds to counteract undesirable microorganisms (Joseph *et al.*, 2008).

At a cellular level, the antifungal principles can disrupt the membrane, resulting in to a cell leakage and cytoplasmic evacuation and thereby results in the fungal growth inhibition. Many antifungal compounds restrict the spore production by the fungal hyphae and thereby prevents the further spread of the fungi. Antifungal property of many plants has been studied earlier by many researchers in order to control plant diseases in a bio-safe way.

Mostly, fungi gain entrance through natural openings and wounds created during harvesting, transporting, handling and marketing. There are also several research work carried out to find the storage fungi responsible for the yield loss. Amienyo and Ataga (2007) in their study, isolated storage fungi from the rotted sweet potato *Ipomoea batatas* tubers such as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Fusarium oxysporum* and *Rhizopus stolonifer*. They have found an excellent antifungal property of *Zingiber officinale* water extract against mentioned fungi. Klich (1984), has observed that *Aspergillus flavus* introduced into cotton plants through natural openings before anthesis and thus infect the seed. Ray and Majumdar (1976), have reported considerable antifungal activity against pathogenic fungi from Indian plant species like *Carum copticum* seeds, *Lawsonia inermis* leaves, *Pinus longifolia* stem, *Plumbago zeylanica* roots, *Saussurea lappa* roots, *Alpinia officinarum* rhizome and *Tamarindus indica*, *Terminalia belerica* and *Emblica officinalis* fruits.

Many *in vitro* testing methods of antimicrobial activity of extracts are quite lengthy and difficult to perform routinely. An easy and instant *in vitro* method for MIC (Minimum inhibitory concentration) value of test sample determination is suggested Shafi (1975) called Paper disc diffusion method. It is a simple method of incorporating the antimicrobial drug in agar. Paper disc agar diffusion assay method provides qualitative information on the efficacy of test compounds and can be routine laboratory method to evaluate the antifungal activity of extracts (Kishore *et al.*, 2007). In a study done by Hossain *et al.*, 2008 using disc diffusion method showed, that the hydrodistilled essential oils, methanol extract and its fractions of *Orthosiphon stamineus* leaves and stems at 5µl (1000 ppm) concentration, displayed potential antifungal activity against phytopathogenic fungi *Fusarium solani* with MIC 500 µg/ml and *Rhizoctonia solani* with MIC 1000 µg/ml.

MATERIALS AND METHODS

Plant material: In the present investigation, a good quality essential oil of *Eucalyptus globules* Labill, purchased from the local market of Bangalore, India and used to screen the potential antifungal activity against some important crop destroying fungi (Table 1, 5). The tree grows up to several feet height. *Eucalyptus* essential oil obtained from mature leaves is having very pleasant and characteristic smell. The oil is also found to be having highly important medicinal effects and is mostly used as an external application, and oral intake is not recommended.

Paper Disc preparation for the Assay: Whatman paper (no 1) sheet was punched to prepare discs (6.6 mm) and were autoclaved to make them sterile. The discs were impregnated with the known quantity of essential oil. The impregnated discs were stored in sterilized glass vials at low temperature until further use (Parekh and Chanda, 2007). The plant essential oil loaded paper discs were further utilized for screening antifungal activity against eleven phyto-pathogenic fungal strains (Table 5).

Table 1: Eucalyptus tree, Active Phyto-chemicals and Medicinal Importance

Common name: Eucalyptus
Scientific name : *Eucalyptus globules* Labill
Habit: Tree
Useful part: Leaves
Family: Myrtaceae
% Average oil Yield: 1%

Phyto-chemical constituents: 1,8- eucalyptol, α- and β- pinene, α-terpineol, globulol, epiglobulol, alloaromadendrene, limonene, linalool, cymene, phellendrene, terpinene, α-eudesmol, L-pinocarveol, β-sabinene, terpinolene, aromadendrene, citronellal, camphene and fenchene.

Medicinal Properties: Mature leaves and essential oil are aromatic, deodorant, refreshing, inhalant, astringent, anaesthetic, antiseptic, antibronchitic, anticatarrh, CNS-stimulant, expectorant, sedative, anticonvulsants, antidepressants, antispasmodic, febrifuge, antiperiodic, antichloristic, diaphoretic, hypoglycaemic, hemostat, rubefacient, emmenagogues, suppurative and vermifuge.

Medicinal uses: Inhalation of oil vapour relieves cough and cold, blocked nasal passages, sore throats and lung infections, asthma, pulmonary tuberculosis, etc. External application of diluted oil (can be mixed with coconut oil) cures ulcers, angina, wounds, skin infections, herpes, acne, gingivitis, diptheria, dysentery, dyspepsia, grippe, inflammation, laryngitis, leprosy, malaria, miasma, phthisis, rhinitis, vaginitis, muscular aches and pains, rheumatoid arthritis and sprains, etc.

Isolation Fungal Pathogen from Infected Plant Material: For the requisite study, the pathogenic fungi can be isolated under laboratory conditions from the infected material of the host plant, using artificial nutrient media and further identified based on their microscopic characters including spores (Nduagu *et*

al., 2008). In the present study, the infected material first washed and surface sterilized with 0.1% $HgCl_2$ treatment for 1 min. The treated material was rinsed (five times) with sterilized water. The piece of infected material (5 × 5 mm) was cut down with sterilized blade and inoculated on PDA media (Table 2) slants under an aseptic condition (Dube, 1990). The slants were incubated (27 ± 1°C) for 4- 6 days. For the identification, the fungal growth on slant was observed under microscope. Precisely, small portion of the hyphal mass was taken under aseptic conditions, mounted on a slide and stained with Cotton blue reagent (Table 3). The prepared slides were observed under a microscope for characteristics of mycelium and sporulation. The recorded data was compared with the characters based on the fungal identification keys (Vashistha, 2000). Following the mentioned procedure, in the present study some highly prevalent fungal strains were isolated from the diseased fruits and vegetables obtained from local market, Gujarat area, India on a SDA and PDA medium and were further identified by staining with Cotton blue stain and observed under microscope. The isolates were used for fungal growth inhibition assays using plant essential oil.

Table 2: Composition of PDA (Potato Dextrose Agar) medium

Ingredients	Quantity (g/l)
Potato	200
Dextrose	20
Agar	20
pH value	5.5

Following the procedure, important seven fungi were isolated from the infected plant material (collected from the local markets of Gujarat region) on PDA (Potato Dextrose Agar) media (Table 2) following the standardized protocols (Dube, 1990) for the study. Fungi namely, *Alternaria alternata* (GUB01) isolated from infected apple fruit, *Aspergillus flavus* (GUB02) from peanuts, *Aspergillus niger* (GUB03) from lemon, *Fusarium oxysporum* f.sp. *laginariae* (GUB04) from bottle gourd, *Fusarium solani* (GUB05) from potato tuber, *Fusarium solani* (GUB06) from tomato fruit and *Rhizoctonia solani* (GUB07) from potato tuber. Fungal cultures were further grown and maintained on SDA (Sabouraud Dextrose Agar) media at 28 ± 2°C.

Table 3: Composition of Cotton Blue stain

Ingredients	Quantity (ml)
Lactic acid	20
Phenol	20
Glycerol	40
Water	20
Aniline Blue (1%)	2

MTCC Lyophilized Fungal strains Activation: For the study, four important plant pathogenic fungi *Fusarium oxysporum* (MTCC No. 284), *Rhizopus oryzae* (MTCC No. 3690), *Sarocladium oryzae* (MTCC No. 2046) and *Sclerotium hydrophilum* (MTCC No. 2157) were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India, as lyophilized forms.

Table 4: Composition of SDA (Sabouraud Dextrose Agar) medium

Ingredients	Quantity (g/l)
Dextrose	20
Peptone	10
Agar	20
pH value	6.5

Thus the cultures were activated on suitable nutrient media, before using for the experiments. Under aseptic conditions in Laminar air flow hood (Labfine, India); a small amount of lyophilized culture was taken from the stock vial and transferred to 150 ml conical flask containing 25 ml of Potato Dextrose broth (Broth is the media without agar) (Table 2). The flasks were incubated at room temperature for culture establishment. After fungal growth activation, the cultures were maintained on SDA media slants (Table 4) with regular sub-culturing (after every 4 weeks). The slant cultures were used as an inoculum to prepare a broth culture for the antifungal assays.

Fungal Spore Quantification: The fungal broth culture was established on SDA broth media and after specific incubation period, spore counting was performed through Haemocytometer (Table 5).

Determination of In vitro Antifungal Properties of Essential Oil

(a) *Primary Screening of Antifungal Activity*

In the present study, plant essential oil was assayed against the selected test fungi (Table 5) using Paper disc diffusion assay following the standardized protocol. For this, sterilized whatman paper (no 1) discs (6.5mm) were loaded with 10 mg (10,000 ppm) of oil, under aseptic conditions for the primary screening experiment to check their fungal pathogen inhibiting potency. The result was segregated based on the positive and negative antifungal response exhibited in the assay (Prasad *et al.*, 2010) and the data was compiled as √ (Positive) and – (Negative).

Table 5: Test-fungi, Incubation Period and Haemocytometer Spore-count

Test Fungi	Stock code	Incubation period (Days) in broth medium*	Fungal Spore count ($\times 10^6$)
Alternaria alternata	GUB01	6	**0.72**
Aspergillus flavus	GUB02	5	**35.64**
Aspergillus niger	GUB03	5	**16.69**
Fusarium oxysporum	MTCC 284	3	**7.86**
Fusarium oxysporum* f.sp. *laginariae	GUB04	3	**4.62**
Fusarium solani	GUB05	3	**1.33**
Fusarium solani	GUB06	3	**1.31**
Rhizopus oryzae	MTCC 3690	2	**5.78**
Rhizoctonia solani	GUB07	5	**No sporulation**
Sarocladium oryzae	MTCC 2046	5	**2.39**
Sclerotium hydrophilum	MTCC 2157	10	**8.40**

Paper disc diffusion assay: Homogenized fungal broth culture (0.1 ml) (Table 5) was poured on solidified SDA medium (20 ml) in each petriplate using micropipette. The 0.1 ml homogenized culture was uniformly streaked with sterilized cotton swab under aseptic conditions. During primary screening, the test-oil impregnated paper discs (10 mg/disc) were placed on the medium. The plates were incubated in upside down position for 72 hr at 28 ±1°C (Erturk, 2006; Patel and Jasrai, 2010). The experiment was performed in triplicates. The antifungal effect was observed as formation of clear zone around the essential oil loaded paper disc after incubation, as ZI (Zone of inhibition). This indicates a presence of potential antifungal property in tested oil, and marked as (√) signs. The extract without any ZI was marked as (–) sign (Table 6).

(b) ***Secondary Screening of Antifungal Activity to find MIC value and Dose Optimization***

In the present study, oil indicating potential antifungal activity (Table 6) against the selected fungal strains during the primary screening; were further pulled for the secondary screening and dose optimization following the standard protocols. The *Eucalyptus* oil was screened from its lower to higher concentrations, using the same Disc diffusion assay to find the minimum effective concentration or the minimal inhibitory concentration (MIC) which is effective to inhibit the fungal growth on seeded SDA medium plate, after the incubation period (Huang *et al.*, 2010; Ogbebor and Adekunle, 2008). In other

terms for the secondary screening, oil was screened at various concentrations against the selected fungal strains. The process of dose optimization is necessary to reduce the wastage of extract/oil while preparing the formulation/product.

The paper discs loaded with different concentration of oils (0.5, 1, 2.5, 5, 8 and 10 mg/disc) were placed on fungal seeded plates under aseptic conditions. These discs loaded with mentioned oil concentration range were placed at an equal distance from each other in an anticlockwise manner (Figure 2). Each plate was replicated thrice. The plates were then incubated upside down for 72 hr and the ZI was recorded by Antibiotic Zone Reader (Labfine, India) (Dawar *et al.*, 2008). In case, if the fungal growth is not in a regular circle, then the mean diameter (average of the longest and shortest diameter of the same colony) was calculated.

RESULTS AND DISCUSSION

A comparative analysis of plant essential oils and various solvent extracts and their use as an antimicrobial agent is been evaluated by various researchers using various techniques. In various parts of world, several researchers have reported the potential antifungal activity from broad range of plants and different solvent extracts; using various *in vitro* screening methods/techniques and different artificial nutrient medias. The studies thus supports that botanicals by their varying and unique mode of action can be implemented as a great alternative against a harmful synthetic chemical fungicides (Patel and Jasrai, 2009; 2010).

Primary Antifungal Screening Results with Disc Diffusion Assay: The primary screening using *Eucalyptus* essential oil for the presence of antifungal activity revealed excellent results and thus signifies the existence of antifungal potential amongst the selected plants. The screening was performed using Paper disc diffusion assay on SDA media. The screening clearly demonstrated the effectiveness of essential oil as potential antifungal agents as shown in the Table 6. The primary screening data signifies the potential use of selected plant as source of novel botanical fungicide due to its broad range of antifungal effect.

Table 6: Result of Primary screening for Antifungal potential of Eucalyptus oil (10 mg/disc)

Test Fungi	Antifungal effect of essential oil
Alternaria alternata	-
Aspergillus flavus	√
Aspergillus niger	√
Fusarium oxysporum	√
Fusarium oxysporum* f.sp. *laginariae	-
Fusarium solani (GUB05)	√
Fusarium solani (GUB06)	√
Rhizopus oryzae	-
Rhizoctonia solani	√
Sarocladium oryzae	√
Sclerotium hydrophilum	√

*[**Note:** (√) = indicates presence of antifungal activity, (-) = indicates absence of antifungal activity]*

In the present study, the essential oil of *Eucalyptus globules* found to inhibit eight fungal stains out of eleven tested. The oil was found ineffective to inhibit the hyphal growth of *Alternaria alternate*, *Fusarium oxysporum* f.sp. *laginariae* and *Rhizopus oryzae* (Table 6).

Secondary Antifungal Screening Results with Disc Diffusion Assay: Based on the positive results obtained with primary screening (Table 6), the plant essential oil was subjected to the secondary screening for dose optimization against fungi. For this, the fungitoxic spectrum or the MIC value (Minimum Inhibitory Concentration) of the essential oil against specific test fungi was determined in terms of ZI (zone of inhibition) demonstrated during the Paper disc diffusion assay.

Table 7: Overview of MIC value obtained with *Eucalyptus* essential oil

Test Fungi	MIC (Minimum Inhibitory Concentration) (mg oil/disc)					
	0.5	**1**	**2.5**	**5**	**8**	**10**
Alternaria alternata	-	-	-	-	-	-
Aspergillus flavus	-	-	-	√	-	-
Aspergillus niger	-	-	-	√	-	-
Fusarium oxysporum	-	-	√	-	-	-
Fusarium oxysporum* f.sp. *laginariae	-	-	-	-	-	-
Fusarium solani (GUB05)	-	-	-	-	√	-
Fusarium solani (GUB06)	-	-	-	-	-	√
Rhizopus oryzae	-	-	-	-	-	-
Rhizoctonia solani	-	-	-	√	-	-
Sarocladium oryzae	√	-	-	-	-	-
Sclerotium hydrophilum	-	-	-	√	-	-

*[**Note: (√)** = MIC reported at respective concentration, **(-)** = indicates absence of antifungal activity]*

As above mentioned the oil was tested in the selected range of concentration from 0.5 to 10 mg/disc during the assay (Table 7). The assay was carried out on SDA medium with three replicates for each concentration of tested oil. ZI (clear zone showing absence of fungal growth) was recorded as the diameter (mm) of complete growth inhibition. SD (Standard deviation) for the obtained readings was calculated (Table 8, Figure 1, 2). In general, microbial inhibition at a lower MIC value indicates very effective inhibitory potential. Therefore prevention of fungal growth at 0.5, 1 and 2.5 mg/disc concentration range is highly important for preparing fungicide formulation with excellent results. Other results showing inhibition from 5 mg/disc to 10 mg/disc concentration range is also significant in terms of presence of antifungal effect.

Table 8: Secondary screening and Dose optimization results obtained against respective fungi

Fungi	MIC value (mg/disc) and respective ZI (mm)					
	0.5	**1**	**2.5**	**5**	**8**	**10**
Aa	-	-	-	-	-	-
Af	-	-	-	6.76 ± 0.03	7.23 ± 0.11	7.68 ± 0.09
An	-	-	-	7.53 ± 0.10	8.67 ± 0.25	10.35 ± 0.45
Fo	-	-	6.85 ± 0.15	9.55 ± 0.19	11.83 ± 0.08	12.63 ± 0.32
Fl	-	-	-	-	-	-
Fp	-	-	-	-	6.74 ± 0.03	7.18 ± 0.16
Ft	-	-	-	-	-	6.65 ± 0.02
Rs	-	-	-	6.85 ± 0.04	8.13 ± 0.32	9.4 ± 0.32
Ro	-	-	-	-	-	-
So	6.66 ± 0.004	6.69 ± 0.02	8.33 ± 0.17	11.17 ± 0.23	11.4 ± 0.15	12.67 ± 0.21
Sh	-	-	-	6.69 ± 0.02	6.78 ± 0.03	6.8 ± 0.02

*[**Note:** (-) = indicates absence of antifungal activity, **Aa** = Alternaria alternata, **Af** = Aspergillus flavus, **An** = A. niger, **Fo** = Fusarium oxysporum, **Fl** = F. oxysporum f.sp. laginariae, **Fp** = F. solani (GUB05), **Ft** = F. solani (GUB06), **Rs** = Rhizoctonia solani, **Ro** = Rhizopus oryzae, **So** = Sarocladium oryzae, **Sh** = Sclerotium hydrophilum]*

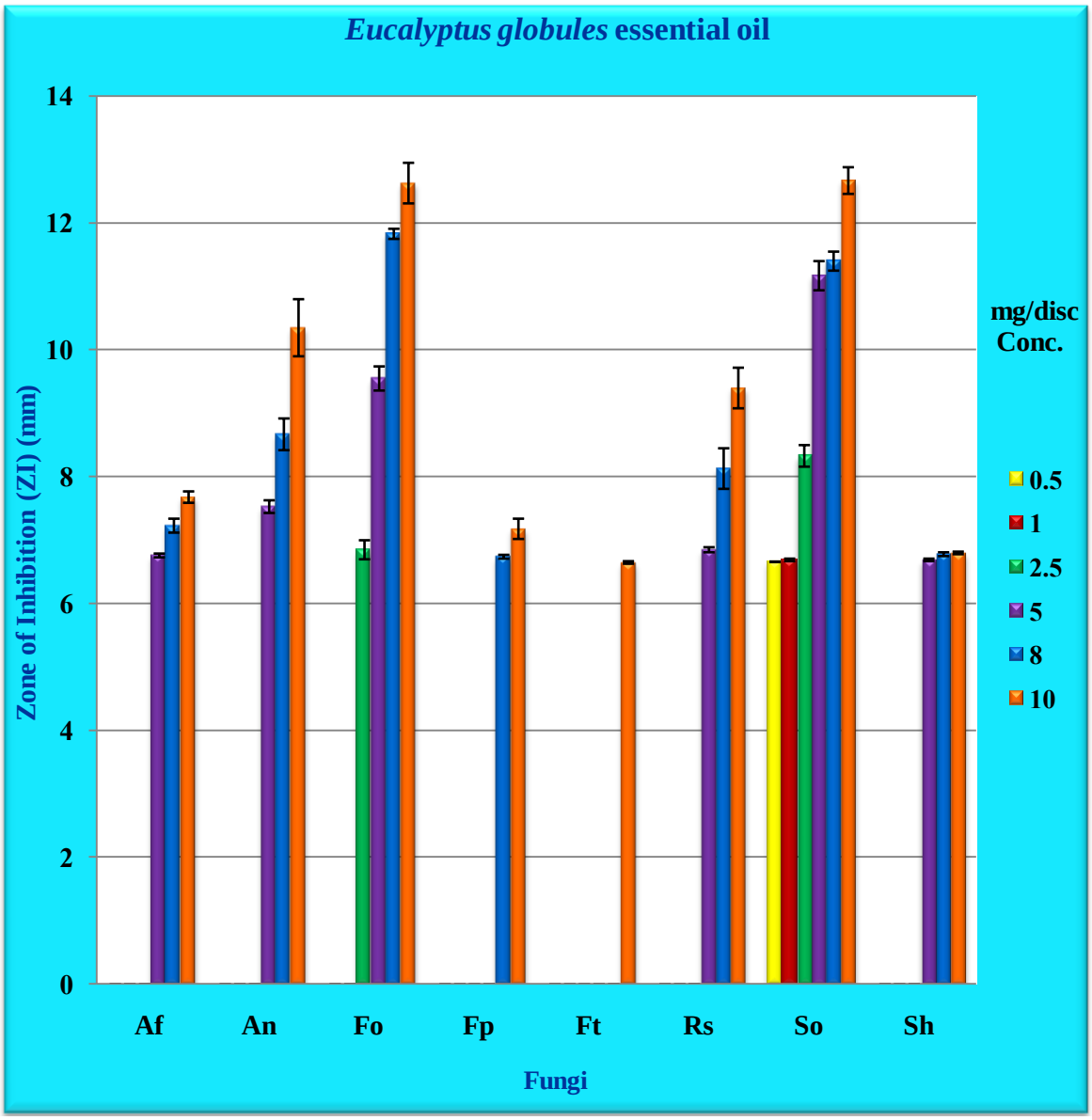
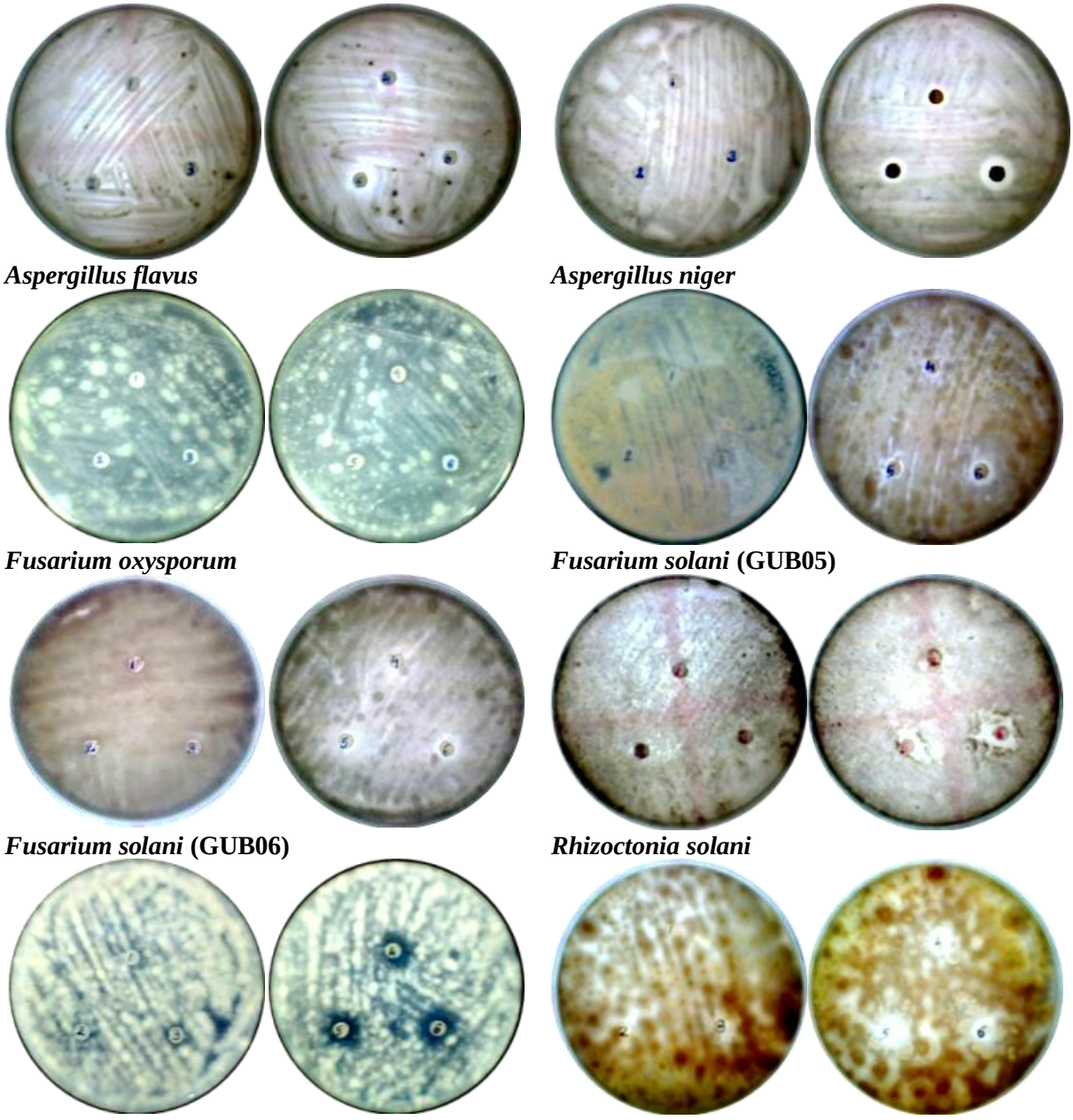


Figure 1: Comparative account of fungal inhibition through essential oil

At MIC 10 mg/disc concentration of *Eucalyptus globules* oil, fungi *Sarocladium oryzae* found to get inhibited with largest ZI (12.67 ± 0.21) mm followed by fungi *Fusarium oxysporum* (12.63 ± 0.32) mm (Table 8). Overall in the study, at a lowest MIC value (0.5 mg/disc) screened, fungi *Sarocladium oryzae* found to get inhibited with ZI 6.66 ± 0.004 mm. This is followed by *Fusarium oxysporum* (6.85 ± 0.15) mm ZI at MIC 2.5 mg/disc concentration. While, four fungi namely *Aspergillus flavus* (6.76 ± 0.03), *Aspergillus niger* (7.53 ± 0.10), *Rhizoctonia solani* (6.85 ± 0.04) and *Sclerotium hydrophilum* (6.69 ± 0.02) mm ZI inhibited at MIC 5 mg/disc concentration *Eucalyptus globules* oil. Other fungi *Fusarium solani* (GUB05) (6.74 ± 0.03) and *Fusarium Solani* (GUB06) (6.65 ± 0.02) mm ZI inhibited at 8 mg/disc and 10 mg/disc oil concentration respectively (Table 8, Figure 1, 2). While, the oil was ineffective to control *Alternaria alternata, Fusarium oxysporum* f sp. *laginariae* and *Rhizopus oryzae* at the tested concentration range in the study. In the present research work, fungus *Sarocladium oryzae* was found most susceptible, while *Alternaria alternata, Fusarium oxysporum* f sp. *laginariae* and *Rhizopus oryzae* were found most resistant fungal strain.



Aspergillus flavus ***Aspergillus niger***

Fusarium oxysporum ***Fusarium solani* (GUB05)**

Fusarium solani* (GUB06)** ***Rhizoctonia solani

Sarocladium oryzae ***Sclerotium hydrophilum***

Figure 2: Antifungal activity exhibited by *Eucalyptus globules* oil against test fungi

*[**Note:** Discs in increasing oil concentration (0.5, 1, 2.5, 5, 8 and 10 mg/disc from left to right side in all figures]*

Conversely, present study is the first report on the control of *Sarocladium oryzae* and *Sclerotium hydrophilum* using botanical extracts or oils. In fact, very little work has been conducted on botanical controls for these selected highly destructive fungal strains. As these fungi are responsible to damage wide variety of important crops and affect the yield; the present findings will contribute to control the fungi in an eco-friendly way.

As per the work of Joseph *et al.*, (2008), *Eucalyptus globules* and *Ocimum sanctum* (20% extract concentration) inhibited *Fusarium solani* f. sp. melongenae growth. Ansari and Shrivastava (1991) had demonstrated that, eucalyptus oil treatment (0.2 ml oil/50 ml) SMKY media concentration inhibited

Aspergillus flavus fungal growth and aflatoxin production. Fungal growth inhibition by essential oils works in various ways such as, involves prevention of hyphal growth and sporulation, interruption in nutrient uptake and metabolism, induction of lysis. They are also responsible in the alternation in fungal physiology by inducing changes in cell wall composition, plasma membrane disruption, mitochondrial structure disorganization and interference with respiratory enzymatic reactions of the mitochondrial membrane (Kishore *et al.,* 2007). In the present study, *Eucalyptus* essential oil revealed excellent and broad-spectrum antifungal activity against the selected pathogens. The fungal growth inhibition activity exhibited at a lower essential oil concentration indicates lower MIC value for the effective fungal growth inhibition, and thus a better affectivity. The inhibition of fungi can be attributed to the complex mixture of secondary metabolites, volatile compounds such as phenylpropanes, terpenoids and their oxygenated derivatives.

Conclusion

Plants are the natural reservoir of biologically active compounds that through their unique mode of action, can affect the metabolic activity of harmful microbial pathogens and this way help to combat the pathogen in an eco-friendly way and without imparting any undesired effects to the environment unlike synthetic chemical fungicides. These compounds are worthy of future investigation to prove their efficacy as potential compounds against phyto-pathogens and this way constitute an important source of commercial microbiocides/ pesticides. The research area is huge, vast and also poorly exploited and there is still further demand for more and defined research on plant fungal pathogens using botanicals. In this context, a broad spectrum antifungal activity was exhibited by the tested *Eucalyptus globules* oil, causing inhibition of eight test-fungi out of eleven selected strains. This has also proved presence of the broad-spectrum antifungal effect among the selected plants for the control of fungal diseases. This has also signified the role of *Eucalyptus globules* oil as potent natural antifungal agent and can be further utilized to develop effective herbal formulation. Thus the present research work is much promising to achieve the above goal to develop bio-safe botanical fungicide and the finding of the present investigation has contributed an important step towards crop protection strategies.

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