ANTIFUNGAL ACITUITY OF THIRTY ONE DIFFERENT ESSENTIAL OILS AGAINST PLANT PATHOGENIC FUNGI

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Abstract: In the present study, in vitro antifungal activities of thirty one essential oils were screened against four different fungi. The antifungal activity was performed by disc diffusion assay. The result showed that out of the thirty one essential oils twenty seven oils have antifungal activity to at least one out of four fungal species. Whetgerm, walnut, apricot and almond essential oil did not show any inhibition to any one of the fungal species. The maximum zone of inhibition was observed to Ginger oil showing 57mm of zone of inhibition to *Aspergillus niger*. Different concentration of Peppermint oil showed inhibition of the hyphal growth and reduction in the number of spores. The maximum zone of inhibition showed by thyme, cinnamon, citronella and palmarosa oils. The results indicated that essential oils have shown strong antifungal activity to four fungal species which could be potential candidates for preparation of a suitable formulation used for the control of fugal pathogens.

Keywords: Antifungal activity, essential oil, plant pathogen.

Introduction: Fungi, nematodes, bacteria, and viruses can damages or cause disease in plants (Montesinos, 2003). Fungi are the main pathogen that causes diseases in plant. Several economically important crops yield is reduced due to the pathogenic fungi (Fletcher et al., 2006). *Fusarium* species found in most of grains in maize field and the soil is inoculums for the species Hussein et al. (2002). *Rhizoctonia solani* were regularly cause damping off which observed in several woody perennial plants (Chang, 1997). *Alternaria brassicae* is a common pathogen to cabbage, Chinese mustard, cauliflower and radish (*Raphanus sativus*).

Plant materials are having antifungal activity even many plant comprise of essential oils have been reported to have antifungal activities (Moreira et al., 2010). These essential oils affect the hyphal morphological structure was earlier study by Soylu in 2006. Generally essential oils analyzed by Gas Chromatography. GC gives information of individual components and relative amount of an essential oil. But using thin layer chromatography identification of essential oil is fast and easy (Albuquerque et al., 2015)

In the present research study we want to find antifungal activities of thirty one essential oils against four widely spread pathogenic fungal strains that cause diseases in plant.

Material and methods:

Collection of the essential oil: Total thirty one commercially available essential oils were obtained from Kelkar Foods and Fragrances, Shivajinagar, Pune. All the essential oils were purchased and stored in the tightly sealed bottles in the dark condition kept in refrigerator.

Collection of the fungal strains: Fungal strains used in the study were purchased from National Bureau of Agriculturally important microorganisms. The list of cultures as followes *Alternaria brassicae* NAIMCC-F-00084, *Fusarium solani* NAIMCC-F-01014, *Rhizoctonia solani* NAIMCC-F-01969, *Aspergillus niger* All the culture were observed for purity by wet mounting then maintained on potato's dextrose agar for further use.

Antifungal activity of essential oils using disc diffusion assay: Inhibition effect of the essential oils was observed on potato's dextrose agar. The inoculums of the fungal spores were adjusted 1-5 X 10^5/ml by performing the count of fungal spore in Neubaur chambers. 0.1ml of the fungal spores was spread on the potatos agar plate. 10 µl of essential oil were impregnated on 6mm Whatmans filter paper no 1 disc. These discs and one positive control and one negative control were kept on agar surface.

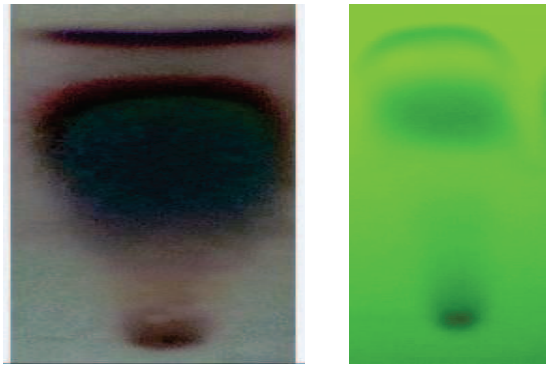
Agar plates were kept for incubated for 48 h at 28°C. Inhibition of zone were determined after 48 h in mm. Fluconazole as standard antifungal antibiotic.

Analysis of essential oil components by TLC: Planar chromatographic separation was performed on 10X10 cm silica gel 60 GF254 (Merkmillipore). Essential oil samples were 3 µl were applied to the TLC plate with capillary pipettes. The plate was developed in n-Hexan: ethyl acetate (90:10). The plates were observed after staining with Vanillin, sulphuric acid and heating the plate at 110°C for 5min.

Effects of the essential oils on fungal morphology: The experiment was performed by the slide culture technique to observe the changes in the morphology of fungal cell. The individual culture was inoculated in liquid broth containing different concentration of essential oil described previously (Espinel-Ingroff et al., 1995). After incubation of 24

hours the slide culture was observed under light microscope.

Results: Thirty one essential oil exhibited different antifungal activity against four fungal pathogenc cultures and the rest were interpreted in Table 1 and fig 3. Out of four fungal species, different oils exhibited different antifungal activities. Maximum activity as zone of inhibition was found to Ginger essential oil calculated as 57 mm, Citronella 56mm, Lemongrass 51mm other oils have range from 50 to 8mm of zone around the 6mm disc with the essential oil. All the four fungal species show inhibition, but *Fusarium solani* inhibited by twenty three oils out of the thirty one. *Rhizoctonia solani* show inhibition by eleven essential oil out of thirty one when compared with rest of three fungal species. Four essential ois Tea tree, Palmarosa, Rosemary and Nutmeg inhibited all the four fungal species with different intensity of zone of inhibition.

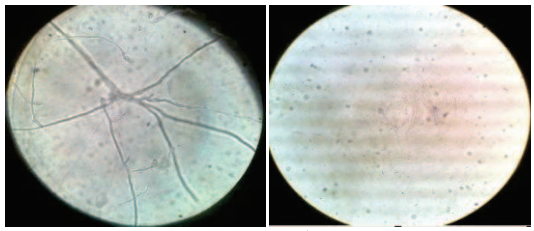


a) b)

Fig 1. TLC of Peppermint oil showing pink and blue colour spots after developed with a)Vanillin, sulphuric acid b) uv at 254 nm spray reagent followed by heating to 110 °C.

Thin layer chromatography analysis indicate of the peppermint oil on silica gel 60 GF254 show the presence of β- caryophyllene and methyl acetate as pink and blue spot present on plate. The spots were visualized under uv at 254 show dark black spots.

Effects of the peppermint essential oils on hyphal morphologies of *Fusarium soloni:* Hyphal morphology of *Fusarium soloni* exposed to 5, 15, 20 µl/ml of peppermint oil showed changes in the morphological changes compared with control plate (Fig 2). Spore count and the degeneration of hyphal structure Simple microscopic observation of *Fusarium soloni* hyphae in the presence of different concentration of essential oil



a) b)

Fig 2. Wet mount of *Fusarium solani* observed under light microscope. a) control b) with essential oil concentration 5 µl/ml

Summery Conclusion: In summary, this study shows that out of thirty one essential oils four oils did not show any activity to the pathogenic fungi.

These screening results specify that essential oils with suitable formulation could be used for the control these fungal pathogen on the crop on field.

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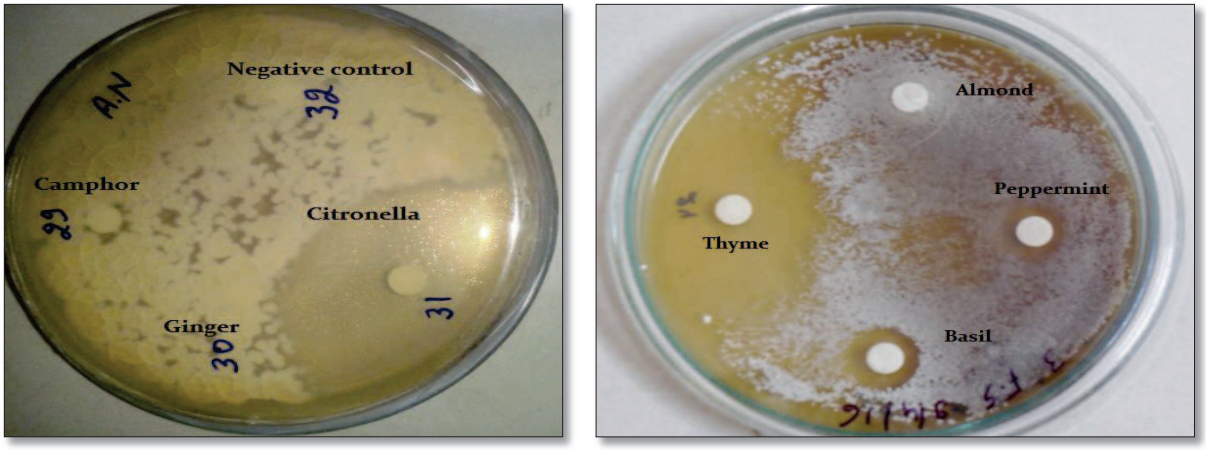
Essential oil	Plant pathogenic Fungal culture			
	Aspergillus niger	*Fusarium solani*	*Rhizoctonia solani*	*Alternari brassicae*
Cinnamon	50	40	NG	46
Lime	50	30	NG	7
Lemongrass	51	10	NG	12
Turmeric	36	20	NG	14
Tea tree	10	20	41	8
Wheatgerm	NI	NI	NI	NI
Frankinsence	NI	25	10	NI
Jatamansi	NI	NI	14	NI
Myrrah	NI	NI	14	10
Palmarosa	30	30	38	26
Vetiver	40	NI	18	20
Rosemary	16	20	18	24
Black pepper	NI	16	NI	9
Cedar wood	NI	NI	9	10
Lemon	NI	18	NI	8
Eucalyptus	NI	10	NI	NI
Walnut	NI	NI	NI	NI
Lavender	NI	NI	NI	10
Nutmeg	20	40	20	18
Orange	NI	20	NI	8
Anise	NG	50	NG	26
Apricot	NG	NI	NI	NI
Basil	NG	15	NI	NI
Thyme	NG	40	NG	33
Almond	NG	10	NI	NI
Peppermint	49	41	NI	33
Bay	46	47	NI	46
Clove	50	45	NI	NI
Camphor	NI	18	NG	7
Ginger	NI	14	57	NI
Citronella	50	24	56	NI
Negative	NI	NI	NI	NI
Positive	NI	55	NI	20

NI= No inhibition, NG=No growth

Table 1.Zone of inhibition in mm against four different plant pathogens



a) (b)

Fig 3. Antifungal activity of essential oil a) Zone of inhibition around Citronella against *Aspergillus niger* b) Zone of inhibition Thyme, Peppermint and Basil against *Fusarium solani*

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