ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF *CYMBOPOGON CITRATUS* STAPF AGAINST DIFFERENT *FUSARIUM* SPECIES

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Abstract : Experiments were carried out to determine the effect of essential oil of *Cymbopogon citratus* (lemon grass) against seven different species of *Fusarium***.**Antifungal activity assay was carried out by agar well diffusion method. The antifungal activity was evaluated by measuring zone of inhibition of fungal growth surrounding the well with test oil. The experiment was carried out in 3 replicates. The obtained results showed that its essential oil found to be inhibitory to all the *Fusarium* spp tested. The activity of oil was varied significantly depending on a species of Fusarium. The essential oil showed more inhibitory effect against *F.moniliforme* and *F.oxysporum. udum* where as less inhibitory effect recorded against *F. roseum in vitro.* From the results, it can be concluded that the essential oil from the leaves of *C.citratus* may rich in antifungal compounds which possess considerable antifungal properties and a number of biological and medicinal potentials. It is good alternative to the harmful chemical pesticides and can be effectively used to control growth of *Fusarium* spp.

Keywords : *Fusarium* species ,Lemongrass, Essential oil, Antifungal,

INTRODUCTION:

Fungi cause many diseases in plants and yield losses in numerous economically important crops (Fletcher and Bender, 2006).A variety of different chemical and synthetic compounds have been used as antimicrobial agents to inhibit the plant pathogenic fungi. There are problems against the effective use of the fungicides in areas where the fungi have developed resistance **(**Barnard et al., 1997;Isman, 2000). The application of higher concentration of chemicals in an attempt to overcome this problem increases the risk of high level toxic residues in the product, which is particularly serious because fruitand vegetables are consumed in a relatively short time after harvest (Mansoori and Banihashemi, 1982; Maleki et al., 2011). Essential oils are the steam-distillable fraction of plant tissues and are often responsible for a scent or taste. These oils are of rather complex composition, with component compounds generally consisting of low-molecular-weight monoterpenes (10-carbon) and related phenols (Toloza, et al., 2006). The main characteristics of the essential oils are that they areeasily extractable, eco-friendly, biodegradable , possess low toxicity against mammals and are very effective against wide spectra of pests (Isman, 2000;Lucia et al., 2012). Results of several studies have shown that some of essential oils are able to control plant pathogenic pests or at least used as a model for construction of new pesticide compound (Deferera et al., 2003**;** Amini et al., 2012). *Cymbopogon citratus* (DC.)Stapf.(Lemongrass) is a plant in the Poaceae family that contains 1 to 2% essential oil on a dry basis. Lemon grass oil was non-phytotoxic in nature, since it did not exhibit any adverse effects on germination and seedling growth of wheat and rice (Tzortzakis and conomakies, 2007). The specific objectives in the present work were to determine the effectiveness of different concentration of essential oils from *Cymbopogon citrates* on *Fusarium* spp. This aims to identify natural and safer agents for the development of biopesticides to manage *Fusarium* species.

MATERIALS AND METHODS:

Collection of soil samples: The soil samples were collected from six different crop fields in Talukas of Nanded district. The soil samples were collected from different crop fields up to 15 cm depth into a small sterilized polythene bags and brought to laboratory for further studies

Collection of plant materials: Leaves of *Cymbopogon citratus* were collected from N E S Science College,Nanded Medicinal plant garden and air-dried under the shade (25-30^{0}C) .The dried leaves were powdered in grinder, and sieved with a 0.5 mm size mesh.

Isolation of fungi: The fungal strains from the soil samples were isolated by Soil Dilution Plate Method(Waksman,1922) on Martins rose Bengal streptomycin agar. Soil dilutions were made by suspending 1g of soil of each sample in 10ml of sterile distilled water. Dilutions of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Petri dishes, in triplicates of each dilution, containing sterile Martins rose Bengal streptomycin agar. The plates were then incubated at 28+2^{o}C for 8 days. Organisms were easily isolated because they formed surface colonies that were well dispersed particularly at higher dilutions.

The fungal species were identified and characterize based on their morphological characters and microscopic analysis by using taxonomic guides. The identification of fungal taxa was based on illustrated Genera of imperfect fungi (Barnett, 1965),Hyphomycetes (Subramanian,1971), Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes (Ellis, 1971, 1976), Micro fungi on land plants (Ellis and Ellis, 1985) and Manual of soil fungi (Gilman, 1957, 1998).

The Pure cultures of the fungi were maintained. The conidia

suspension was prepared by flooding the surfaces of 10 day old cultures of fungi on plates with 10 ml of 0.05% Tween 80 in sterile distilled water.The conidia suspensions were pelleted by centrifugation at 2000 rpm for 3 min and the pellets were re suspended in 0.05% Tween 80 in sterile distilled water .The concentration of each fungus was adjusted to approximately 10^6 conidia/ml by dilution (Hawksley BS748).

Extraction of essential oil :250grams of the powdered material was placed in a round bottom flask, 1000 ml of distilled water was added and then subjected to hydro distillation in a modified Clevenger apparatus for 8 hours (Bankole, 1997). Theoil recovered was dried over anhydrous sodium sulphate and kept in the refrigerator at 4^0C .

Determination of the antifungal properties : The antifungal properties of the oil was done by colony diameter measurement method Grover and Moore, 1962). Fungitoxic spectrum of the oil was determined at different conc. of essential oil against seven species of *Fusarium* . Fungal toxicity of the essential oil was determined and the experiment was repeated twice, each containing three replicates and the mean value was taken.

RESULTS AND DISCUSSIONS :

Fusarium species are commonly associated with soil particles ,organic matter and plant debris throughout the world (Lim & Varghese,1977;Burgess,1981).Most of the literature on Fusarium species, relates to cultivated soils.It is reported earlier that species of Fusarium are the most active and diverse members of soil- biotic microflora (Burgess & Summerell,1992)

In the present investigations Fusarium species were isolated from the field soil samples collected from seven Talukas of Nanded district were found as *F.moniliforme* ,*F.udum.Fusarium solani, F.oxysporum f. sp.ciceri*, *Fusarium roseum ,F.semitectum and F.equiseti.* Out of these *Fusarium* species only four species were isolated from the soil samples of Degloor while all the seven species of Fusarium were isolated from the soil samples of Ardhapur followed by Nanded (06). The most prevalent species was *F. moniliforme*, which was observed in all sequences and depths from all the Talukas. *Fusarium* species isolated from different soils were facultative parasites and pathogenic. Similarly it is reported that Fusarium species are not only parasitic and pathogenic (Cook & Bruehil,1968) but they can be also involved in the various ecological functions relating to nutrient cycling and plant-soil-microbe interrelationships (Kreutzer,1972; Kommedahl & et.al., 1987).

In the present investigations different concentrations of essential oil of *C. citrates* were tested for their antifungal potential it clear from the results that all the concentrations of essential oil showed strong inhibitory effect against all the seven species of *Fusarium*. The results presented in table 2 clearly indicated that *Cymbopogon citratus* essential oil has the potential for the control of *Fusarium* spp. *in vitro.* On the other hand, 1500 ppm concentration exhibited marked inhibition as compared to 100 ppm and 500 ppm concentrations against all the seven species of *Fusarium*,however no significant difference was observed between control and concentrations of 100 ppm of essential oil of *C. citratus.*The results also showed that a very good effect was obtained when concentrations of essential oils were above 1000 ppm. The essential oil was found to be most effective against *F.moniliforme* followed by *F.solani* ,*F.udum,*and *F.oxysporum ciceri*. The inhibitory effect of essential oil of *C. citratus* against the *Fusarium* spp may be due to the presence of Citral and other phenolic compounds. The GC analysis identified citral as major component with a percentage of 76%. Citral as main components of *C. citratus* an oxygenated terpenoid, which has been identified as a compound exhibiting antifungal properties (Paranagama et al., 2003). This monoterpene has proved effective in controlling mycelial growth and conidial germination of *C. gloeosporioides* (Palhano et al., 2003).Similarly it is reported that the anti-fungal activity of lemon grass oil may be due to the presence of its aldehyde containing the active constituent citral (Gupta et al.,2011).Paranagama, (2003) reported that *C. citratusis* of West Indian origin and yields an essential oil with high content of citral (>70%).In support of antifungal activity, Kumar et al. (2009)reported that *C. citrates* essential oils exhibited broad fungitoxic activity against *Aspergillus flavus*. The essential oils obtained from *Cymbopogon martini* (Roxb.) Wats. and*C. citratus* was studied against some fungi by Singatwadia and Katewa (2001). They found that *C. citratus* oil was found to be effective against *Cladosporium* sp., *Aspergillus niger,* and *Mucor* at lower concentrations, where as that of *C. martini* was more effective against *Candida* sp., *Aspergillus fumigatus* and *Trichophyton rubrum* compared with the oil of *C. citrates* . Results obtained by Tzortzakis and Economakis (2007) revealed that *C. citratus* oil inhibits the fungal spore production of *Colletotrichum coccodes, Botrytis cinerea ,Cladosporium herbarum , and Rhizopus stolonifer* . Gupta et al. (2011) adopted the disc diffusion method to test the anti-fungal activity of the *C. citratus* oil against *Fusarium oxysporum* and found its strong inhibition measured in terms of the average inhibition zone diameter. The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components (Lee, 2007; Kim and Park, 2012).Finally, it is concluded from the results that *C. citratus* oil is a good alternative to the harmful chemical pesticides and can be effectively used as an efficient fungicide against *Fusarium* spp..

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Table 1 Isolation of *Fusarium* spp. from the soil of different Takukas in Nanded

S No	Soil samples	Isolation of *Fusarium*spp						
		Nanded	Loha	*Kandhar*	Ardhapur	Bhokar	Degloor	*Dharmabad*
1	*F. oxysporum* f. sp. *ciceri*	+	+	+	+	+	-	+
2	*Fusarium udum*	+	-	+	+	-	+	+
3	*F.moniliforme*	+	+	+	+	+	+	+
4	*F.roseum*	+	+	+	+	+	+	-
5	*F. solani*	+	-	+	+	-	+	+
6	*F semitectum*	+	-	-	+	+	-	-
7	*F. equiseti*	-	-	+	+	+	-	+

Table 2 Antifungal activity of essential oils against different Fusarium spp.

S No	Concentration of Essential oil (ppm)	Diameter of mycelial growth (cm)						
		F.oxy.ciceri	*F. udum*	*F.moniliforme*	*F.roseum*	*F.solani*	F. semitectum	F. equiseti
1	Control	8.84	8.90	8.86	7.80	7.78	8.53	7.88
2	100	8.15	5.42	3.50	7.45	4.36	8.25	7.50
3	500	6.23	3.59	1.74	6.68	2.86	7.85	6.78
4	800	3.14	2.30	0.50	5.23	1.30	6.40	4.63
5	1200	0.50	0.50	0.50	3.76	0.50	4.56	3.76
6	1500	0.50	0.50	0.50	2.18	0.50	3.37	1.68