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Antifungal Potential of *Ocimum sanctum* Linn. Essential Oil against Fungal Pathogens Causing Economic Losses in *Cymbopogon flexuosus* Nees Ex Steud. (Lemongrass)

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ABSTRACT

Cymbopogon flexuosus Nees Ex Steud. (Commonly known as lemongrass, family Poaceae) is one of the commercially important aromatic plants having medicinal properties. India is one of the largest producers and exporters of lemongrass essential oil in the world; in the year 2021-22, India exported about 610 tons of lemongrass oil, contributing more than 4 lakh US \$ million in Gross Domestic Productivity (GDP). However, despite a good national and international market, this crop suffers quantitative and qualitative losses due to the leaf spot and leaf blight diseases caused by the fungi *Curvularia trifolii* and

Rhizoctonia solani, respectively; therefore, an attempt has been made to manage these fungal pathogens. The essential oil of basil (*Ocimum sanctum* Linn.) was extracted by hydro-distillation method using *Clevenger apparatus*. The oil thus extracted was used for *in vitro* antifungal efficacy against both the test fungi *Curvularia trifolii* and *Rhizoctonia solani*, using the poison food technique. The results show that the minimum inhibitory concentration (MIC) of the basil oil against *C. trifolii* 2000 ppm and *R. solani* was 1600 and however, it was cidal in nature at 3200 and 2400 ppm against both fungi, respectively. The current study's findings show that *O. sanctum* oil could be an alternative to synthetic fungicides after detailed investigations.

Keywords Essential oil, Cymbopogon flexuosus, Ocimum sanctum, Curvularia trifolii, Rhizoctonia solani, Antifungal activity.

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INTRODUCTION

Cymbopogon flexuosus Nees ex Steud. popularly known as lemongrass, Cochin or Malabar grass, is an important aromatic and medicinal herb commonly known for its lemony odour due to citral (a cyclic monoterpene), usually grown and distributed in temperate and tropical/subtropical parts of the world for its essential oil. Citral is used as a basic raw material for synthesis of β -ionone, which is used for synthe-

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sis of a number of useful aromatic compounds and Vitamin A. *C. flexuosus* is an erect herb with a stem that grows up to 1.0-1.5 m long, leaves are linear and lanceolate, and flowers freely. The inflorescence is very large spikes bearing spikelets in pairs (sessile and pedicellate). The sessile spikelet is an awned bisexual floret, whereas the pedicellate is a staminate floret Joy *et al.* (2006), Yogendra *et al.* (2021).

India is one of the leading producers and exporters of lemongrass oil and its products in the world trade. In India, Kerala, Karnataka, Tamil Nadu, Jharkhand, Chhattisgarh, Madhya Pradesh, Assam, Uttar Pradesh, Odisha, Rajasthan, Meghalaya, Nagaland, Tripura, Manipur, Arunachal Pradesh, West Bengal, Uttarakhand and are the major producing states of C. flexuosus essential oil; C. flexuosus is abundantly cultivated in Hardoi, Banda, Sitapur, Kanpur, Sonbhadra, Mirzapur, Raebareli, Unnao, Lucknow, Jalaun, Jhansi, Hamirpur and Lakhimpur Kheri District of the Uttar Pradesh (Joy et al. 2006), Sharma et al. 2022). The presence of important phytochemical constituents, including Myrcene, Linalool, Neral, geranial, contributes to its pharmacological activities, which include anti-microbial, anti-ulcer, radioprotective, cardiovascular, antioxidant, anti-allergic, and antidiabetic activities (Naik et al. 2010, Singh et al. 2011, Mirghani et al. 2012, Olorunnisola et al. 2014, Adukwu et al. 2016, Gao et al. 2020, Mukarram et al. 2022). C. flexuosus is the perennial cash crop as it lies up to 5-7 years from plantation. However, besides this, the crop is affected by various pests and diseases, which cause heavy economic losses to the crop (Yogendra et al. 2021, Sharma et al. 2022).

Literature reveals that fungal pathogen *Rhizoctonia solani* J.G. Kuhn Synonyms: *Moniliopsis solani* (J.G. Kuhn) R.T. Moore causes leaf blight disease in *Cymbopogon* spp., usually starts from the leaf edge, was irregular in shape, The symptoms which developed on leaves and sheaths were characteristic concentric spots that covered large areas of sheaths and leaves brown to dark brown in color and caused more than half of the leaf, or whole leaf die Singh *et al.* (1997), Baiswar *et al.* (2012), Daghir and Madhi (2020) while leaf spot disease of *C. flexuosus* was caused by phytopathogenic fungi *Curvularia trifolii* (Kauffman) Boedijin (Synonyms: *Brachysporium trifolii* Kauffman) having initial symptoms like, lesions appeared as interveinal chlorosis areas that later turned brown. In severe conditions, whole plants had a "burnt" appearance. Damage was particularly severe on younger leaves and when temperature (28 to 32°C) and humidity increased after the rainy season. Both the fungal pathogens cause severe economic loss by

drying and defoliating the lemongrass plant, Singh *et al.* (1997), Zhang *et al.* (2020). Infected slips (planting material) are the primary source of the fungal disease transmission, followed by soil and weather. This infection causes extensive, usually widespread, defoliation after the rain and reduces the herbage, essential oil recovery.

There are many chemical-based fungicides for the prevention and management of fungal pests available in the market, but they are harmful to soil and water health, cattle, and human beings as well as to the agriculturally important microorganisms. After 1990, the consumption of synthetics increased too much; thus, the residue in the soil directly or indirectly affects not only the quality of the food, vegetables and fruit crops but also the medicinal and aromatic crops.

On the other hand, at the same time, some eco-friendly management practices (using viz. micro-organisms, botanicals, and animals) are also available, which are good and safe for the environment and human health. Some researchers have also reported that plant secondary metabolite (s)/ extract (s)/ essential oil (s) have anti-fungal, anti-bacterial and anti-viral properties at different concentrations. Essential oil (s) are obtained from the various aromatic and medicinal plant (s) by the distillation process; for extraction of the essential oil, flower, leaf, stem or root are used. Thus, the distilled essential oil has broad spectrum anti-microbial, anti-nematocidal, anti-repellent, anti-malarial and anti-larvicidal property. Moreover, In the current investigation leaf of the Ocimum sanctum was used for the extraction of essential oil(s). different researchers have proved that essential oil has good antifungal activity under In-vitro and In-vivo analysis.

Cymbopogon flexuosus Nees ex Steud. (Family-Poaceae), commonly known as lemongrass is an essential oil-yielding aromatic grass; cultivated mainly in India, Sri Lanka, Pakistan, Maldives, Brazil, China, Taiwan, Guatemala, Argentina, USA, Uganda, Rwanda and other African countries (Joy *et al.* 2006, Sharma *et al.* 2022). *C. flexuosus* essential oil(s) are rich in phytochemical constituents viz., geranial, neral (geranial + neral = citral), geranyl acetate, linalool, caryophyllene, camphene, geraniol (Nath *et al.* 2002, Sarma and Sarma 2005, Yogendra *et al.* 2021).

Moreover, the Cymbopogon spp. essential oil shows different biological properties, including antifungal (Singh et al. 2011, Gao et al. 2020, Yadav et al. 2021, Mukarram et al. 2022), anti-viral (Mukarram et al. 2022), antibacterial (Naik et al. 2010, Singh et al. 2011, Olorunnisola et al. 2014, Adukwu et al. 2016, Gao et al. 2020, Yadav et al. 2021, Mukarram et al. 2022), anti-inflammatory (Olorunnisola et al. 2014, Gogoi et al. 2020, Yadav et al. 2022), antioxidant (Mirghani et al. 2012, Mukarram et al. 2022), insecticidal (Pinheiro et al. 2013), analgesic (Almeida 2001), anti-diabetic (Mirghani et al. 2012), anticancer (Mukarram et al. 2022), Insecticidal (Verma et al. 2020), antiseptic (Simic et al. 2008). The industrial and therapeutic applications of C. flexuosus essential oil are well documented.

The present study aims to determine the *in vitro* antifungal activity of essential oil of *Ocimum sanctum* L. against two major deteriorating fungal pathogens of lemongrass.

MATERIALS AND METHODS

During the frequent field visit of some districts of Lucknow division, it was found that some of the farmers were suffering with less essential oil recovery. Thus, a survey was conducted among different districts of Lucknow division and concluded that oil recovery was reduced due to the leaf spot and leaf blight diseases. Leaf spot disease of *C. flexuosus* is caused by *Curvularia trifolii* (Kauffman) Boedijn, while leaf blight caused by *Rhizoctonia solani* J. G. Kuhn. This disease generally occurs end of the monsoon season. Disease affects the quantity as well as the quality of the herbage and essential oil.

Extraction of essential oil (S)

For the extraction of essential oil 12 kg fresh herbage

of Ocimum sanctum L. was collected from the experimental farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India (27°15'53" North latitude, 81°04'22" East longitude, and 87m altitude). The collected plant material was gently washed in tap water to remove dust and foreign particles, followed by shade-dry and chopping in to small pieces. Further, shade-dried herbage of O. sanctum was distilled (3 kg/batch in 4 batches) using hydro-distillation method at 100°C for 3-4 hrs in Clevenger apparatus (capacity 5 kg) (Clevenger 1928). A clear light yellow colored oil layer obtained on the top of the aqueous distillate was collected in a glass container, impurities and water contents were removed with the help of anhydrous sodium sulphate (Himedia). The distilled essential oil thus filtered was stored in refrigerator for further experiment. Similarly, the C. flexuosus essential oil was also distilled by hydro-distillation using the Clevenger apparatus to estimate the quantitative losses. Mature C. flexuosus herbage was harvested from the field and taken in to laboratory; followed by washed the sample under tap water. After shed drying, herbage was chopped into small pieces and filled in the Clevenger in a manner so that water and herbage was not more than 50% of its (5L, Clevenger) total capacity (Clevenger 1928). Thus, 3-4 hrs. of distillation process, distilled essential oil was collected in a glass vial and mixed with anhydrous sodium sulphate (Himedia) to remove the contaminants and store in a cool, dark place for analysis (Nath et al. 2002, Joy et al. 2006, Yogendra et al. 2021, Srivastava et al. 2021, Maurya et al. 2024).

Fungal pathogens

Two major deteriorating plant fungal pathogens of *C. flexuosus, C. trifolii* and *R. solani* are used in the current study. Which were isolated from the diseased plant samples of lemongrass on potato dextrose agar medium; later on, purified and identified morphologically as well as molecular level from CSIR -National Chemical Laboratory, Pune Maharashtra. Thus, the isolated fungal pathogens were re-cultured for detail *In-vitro* analysis on Potato Dextrose Agar medium and preserved at 4°C for detailed investigation (Sung *et al.* 2016, Misawa *et al.* 2017, Ajayi-Oyetunde and Bradley 2018, Ji *et al.* 2019, Coelho *et al.* 2020).

Antifungal activity

Poison food technique

The antifungal activity of O. sanctum essential oil was evaluated by poison food technique devised by Grover and Moore (1962) with slight modification of Shukla and Dikshit (2016). The Potato Dextrose Agar (PDA) (Hi-media) medium along with different concentrations of O. sanctum oil diluted in 2% of acetone of the required amount of the medium (400, 800, 1200, 1600, 2000, 2400, 2800, 3200 ppm) and poured on sterilized petri plates (90 mm diameter) to get the uniform thickness. Petri plate containing potato Dextrose agar (PDA) medium with an appropriate amount of 2% acetone served as control. Thereafter, mycelial discs (6 mm) of the 5-7-day-old fungal cultures were inoculated inside center of petri plates and sealed with the help of parafilm. All experiments were performed in triplicate and the inoculated petri plates were incubated at 25± 2°C for 7 days. The colony diameter of the treated and control petri plates was recorded at a regular interval of 24 hrs (Grover and Moore 1962, Singh et al. 1997, Philippe et al. 2012, Shukla and Dikshit 2016, Gakuubi et al. 2017, Maurya et al. 2024). The mycelial growth inhibition (MGI %) was calculated using the formula:

Mycelial Growth Inhibition% (MGI) = $[(D_c - D_t) / D_a]x 100$

 D_c indicates Mean colony diameter in control set D_c indicates Mean colony diameter in treatment set

Nature of toxicity of the essential Oil

The nature of toxicity of *O. sanctum* essential oil was recorded as fungistatic/ fungicidal at their respective minimum inhibitory concentration (MIC) against the both test pathogenic fungi *C. trifolii* and *R. solani*. This was determined following the method of Shukla *et al.* (1999), Van *et al.* (2006), Batish *et al.* (2008), Maurya *et al.* (2024). For defining antifungal activity, the fungal discs from MIC experimental set were re-inoculated upside down on plain fresh PDA medium. Inoculated petri plates were incubated in BOD at $25 \pm 2^{\circ}$ C for seven day and noted the readings. Presence of fungal growth on the 7th day indicates

 Table 1. Antifungal activity of essential oil of Ocimum sanctum against test pathogens.

Concentrations (ppm)	Mycelial growth inhibition (MGI) (%)	
		Rhizoctonia solani
T, 400	15.68	19.54
T ₂ 800	33.56	41.72
T ₂ 1200	76.04	67.81
T 1600	82.17	100
T ₅ 2000	100	100
T ₆ 2400	100	100
T ₇ 2800	100	100
T ₈ 3200	100	100

fungistatic nature of the essential oil, while absence of fungal growth denoted fungicidal nature of the essential oil. All the experiments were designed in triplicates. The minimum concentration of the essential oil having no fungal growth after 7-day incubation was taken as Minimum Cidal Concentration (MCC) (Maurya *et al.* 2024).

Statistical analyses

All the above experiments were done in triplicates so the data were expressed as mean \pm standard deviation. Analysis of variance was performed by ANOVA using IBM SPSS software (Statistics 20) followed by Tukey's Post Hoc test with p \leq 0.05 to determine the significant differences between the results.

RESULTS AND DISCUSSION

Antifungal Activity

The *in-vitro* antifungal activity of the *O. sanctum* oil was investigated against the plant fungal pathogens *C. trifolii* and *R. solani*, using poison food technique. The result shows that the minimum inhibitory con-

 Table 2. Nature of toxicity of the essential oil of O. sanctum against test pathogens.

Pathogen	MIC (ppm)	MCC (ppm)
Curvularia trifolii	2000	3200
Rhizoctonia solani	1600	2400

*MIC - Minimum Inhibitory Concentration. *MCC - Minimum Cidal Concentration.

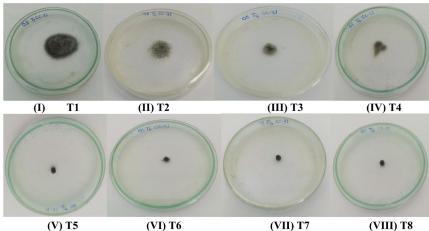


Fig 1 A: Mycelial growth of *Curvularia trifolii* at different concentrations against *O. sanctum*

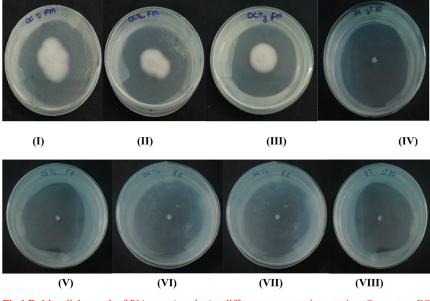


Fig 1 B: Mycelial growth of Rhizoctonia solani at different concentrations against O. sanctum EO

Fig. 1 (A and B). Growth inhibition of fungal pathogens (A) *Curvularia trifolii* (B) *Rhizoctonia solani* by using essential oil of *O. sanctum* at dose of (i) T_1 (400 ppm), (ii) T_2 (800 ppm), (iii) T_3 (1200 ppm), (iv) T_4 (1600 ppm), (V) T_5 (2000 ppm), (VI) T_6 (2400 ppm), (VII) T_7 (2800 ppm) and (VIII) T_8 (3200 ppm).

centration (MIC) of the *O. sanctum* oil was recorded 2000 ppm, and 1600 ppm against the test pathogens *C. trifolii* and *R. solani* respectively. Further, the minimum fungicidal concentration (MCC) of the oil was recorded 3200 ppm against *C. trifolii*, however, in case of *R. solani*, it was 2000 ppm (Figs. 1-2 and Tables 1-2).

Further literature shows that Siva *et al.* (2016) reported the aqueous and acetone extract of *O. sanctum* L. were found to be sensitive against many plant fungi such as *Alternaria tenuis, Helminthosporium* sp. and *Curvularia penniseli*. Kahkonen *et al.* (1999) concluded that essential oil of tulsi was effective against the plant fungal pathogens such

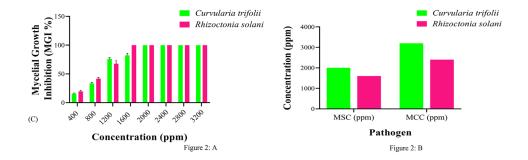


Fig. 2. Effects of different concentration (s) of *Ocimum sanctum* EOs on *C. trifolii* and *R. solani* (A) Antifungal activity of EO at different concentrations showing percent inhibition of mycelia growth, (B) Minimum inhibitory concentration (MIC) and minimum cidal concentration (MCC) of EO against both the test pathogens.

as Alternaria solani, Candida guillermondii, Colletotricum capsici, Curvularia sp. Fusarium solani, and Helminthosporium oryzae. Essential oil of tulsi along with Eugenol check the growth of Aspergillus flavus. Singh et al. (2010) reported that Tulsi leaves shows strong antifungal activity against Aspergillus spp. while Eseential oil of O. gratissimum L. shows strong efficacy against Candida spp. Comparing with the results of present investigations, the literature reveals antifungal efficacy of O. sanctum against several fungal pathogens. However, majority of the studies were focused upto in-vitro evaluation. The present investigation showing the fungicidal activity of O. sanctum oil against R. solani at 2400 ppm and C. trifolii at 3200 ppm concentration(s) by poison food technique prompts us go to the pot and field trials, and the related work are under progress. Constituents of the natural essential oil(s) varying in both ways qualitatively as well as quantitatively depends upon the environment, soil and cultivar, the technology to manage fungal phytopathogens.

CONCLUSION

The findings of the present investigation reveal that MIC of *O. sanctum* oil against the test fungal pathogen (s) *C. trifolii* was recorded 2000 ppm while its MCC was 3200 ppm. Similarly, the MIC and MCC of the *O. sanctum* oil against *R. solani* were recorded as 1600 ppm and 2400 ppm, respectively. Hence, the present findings of antifungal activities of basil essential oil could be used for detailed *in-vitro* investigations; further the results of *in-vitro* findings can be

utilized at pot and field levels and if found effective, could be an alternative to synthetic fungicides.

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