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In-vitro study on the efficacy of essential oil from *Ocimum* against some plant pathogens

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Abstract
In the present study, essential oil from two species of *Ocimum* was used to inhibit the growth of the phytopathogenic fungi viz. *Bipolaris sorokiniana* and *Colletotrichum henanense*. The Poisoned food technique was used to check the antipathogenic activity of essential oil. The oil was used both as pure (100, 125, 150, 175, 200 and 225 ppm concentration) and diluted with organic solvent (10, 12.5, 15, 17.5, 20 and 22.5 ppm concentration). *Ocimum gratissimum* showed lesser growth of the fungal pathogens and more inhibitory effect over *Ocimum sanctum*. *Bipolaris sorokiniana* recorded lesser fungal growth as compared to *Colletotrichum henanense*. It had been also found that increasing the concentration of oil (both in pure and diluted), increased the fungal growth inhibition. Maximum fungal growth inhibition (94.78% and 79.99%) was recorded in *Bipolaris sorokiniana* treated with the oil of *Ocimum gratissimum* @ 225 ppm and 22.5 ppm as pure and in diluted form respectively. Diluted form of essential oil showed less growth inhibition of fungi than the concentrated oil, but it was much cost-effective because using only 1/10th volume of the essential oil, an inhibition of 79.99% was observed at 22.5 ppm, while pure oil resulted in 94.78% inhibition at 225 ppm. Dilution of the oil was also significant for covering a large mass of infected plant populations. Hence, it can be concluded that the essential oil of *Ocimum* species can be effectively used as a fungistatic agent in controlling the plant fungal diseases. Hence, the essential oil of *Ocimum* species may be effectively utilized in controlling the phytopathogenic fungi.

Keywords: Ocimum, fungi, *Bipolaris sorokiniana*, *Colletotrichum henanense*

Introduction
Ocimum belongs to the family Lamiaceae, is one of the important medicinal plant which is very important for their therapeutic potentials. *Ocimum* have around 30-160 species, found in tropical Asia, Africa, Central and South America. Some of the important species of genus *Ocimum* are *Ocimum sanctum* L., *Ocimum gratissimum* L., *Ocimum canum* Sims, *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum americanum* L. and *Ocimum micranthum*, which grow in different parts of the world and are known to have medicinal properties. The major constituent in *Ocimum* oil include linalool, geraniol, citral, camphor, eugenol, methyl chavicol, safrole, thymol, methyl cinnamate etc. *Ocimum sanctum* is commonly known as holy basil. It is a shrub with hairy stems and simple alternate green leaves with a distinct fragrance. According to studies, *Ocimum sanctum* contain anti-inflammatory, antipyretic, antidiabetics hepatoprotective, analgesic hypolipidemic, and antistress effects. Extracted essential oil have also been shown to include physiologically active components that are insecticidal, nematicidal, and fungistatic (Verma, 2019) [12]. The major volatile constituent found in the essential oil of *O. sanctum* is methyl eugenol (92.4%). The other minor constituents are eugenol (2.4%) and β-caryophyllene (1.3%). *Ocimum gratissimum*, commonly called in India as 'Ram Tulsi', is a tall, heavily branched perennial aromatic shrub that is widely distributed in tropical Africa and Brazil. Eugenol (75.1%) is the primary component of *Ocimum gratissimum* and other minor compounds are terpinolene (14.2%) and germacrene D (3.9%) (Joshi, 2013) [5]. The physiologically active chemical compounds present in essential oil of *Ocimum* species can be used effectively in agriculture for protection of plants from variety of pathogen. Alternative antimicrobial plant protection needs to made from medicinal plants are required for the treatment of infectious disorders. The high expense of typical synthetic chemicals, along with their negative effects on the environment and potential for generating disease resistance, have caused ecological issues. Therefore, the present study was carried out to investigate the antifugal property of *Ocimum sanctum* and *Ocimum gratissimum* on some selected fungus like *Bipolaris*

sorokiniana and *Colletotrichum henanense.*

Materials and Methods

Collection of Planting material

The leaves of *O. sanctum* and *O. gratissimum* was collected from the surroundings of Pundibari, Cooch Behar, West Bengal during December, 2021.

Extraction of essential oil

The leaves (1 kg) of *O. sanctum* and *O. gratissimum* was collected and wash with distilled water to remove any unwanted materials and shade dried for 8 hours. It was then ground and 100 g of powder was taken in a Clevenger apparatus along with 500 ml of distilled water and hydro distillation was done for 3 hours. Oil was collected in vials and kept in refrigerator (Rathnayaka, 2013) [10].

Antifungal activities of the fungi

Fungal pathogens like *Bipolaris sorokiniana* and *Colletotrichum henanense* were obtained and evaluated under the Department of Plant Pathology, Uttar Banga Krishi Vishwavidyalaya, Pundibari, Cooch Behar, West Bengal, India. The antifungal activity of essential oil of *O. sanctum* and *O. gratissimum against* the oil was tested by poison food method. Potato Dextrose Agar was made and autoclave for 20 minutes at 15 PSI at a temperature 121 °C. Further different concentration of essential oil (100, 125, 150, 175,200 and 225 ppm pure oil and 10, 12.5, 15, 17.5, 20 and 22.5 ppm diluted oil) were added aseptically in melted PDA medium. Each essential oil (1ml) was diluted in 10% aqueous Dimethyl sulfoxide (DMSO). 10% 0f DMSO was reported to non-toxic to microorganism (Joshi, 2013) [5]. Then 10 ml media was poured into the sterile petri plate (9 cm) immediately. Small disc of fungus culture (5 mm) was cut from 6-7 days old culture with sterile cork borer and transferred aseptically in the centre of the petri dish containing the medium with desire fungicidal oil concentration. For control, suitable checks were kept where the culture discs were grown under the same condition on PDA without fungi-toxicant. Petri plate was kept in BOD incubator by maintaining the temperature at 26 ±1 °C for ten days. The fungal colony diameter was measured at every 24 hours and record of observation was done when the pathogen growth in the control covered the full petri plates. Each treatment was replicated 3 times and antifungal effect of essential oil was measured by mycelial growth inhibition percentage (MGI%) and pathogen growth was calculated by using following formula:

$$\text{Mycelial growth inhibition (MGI\%)} = (Dc–Dt)/Dc) \times 100$$

Here, Dc is Colony diameter in in control plate (mm) Dt is Colony diameter in treatment plate (mm) (Piyo *et al*., 2009) [9].

Results and Discussion

The fungitoxicity of *Ocimum sanctum* and *Ocimum gratissium* essential oil was evaluated in pure and diluted form for *Bipolaris sorokiniana* and *Colletotrichum henanense*. *Ocimum gratissimum* performed significantly best result as compared to the oil derived from *Ocimum sanctum* in mycelia growth inhibition of the fungus. It showed that maximum growth inhibition (94.78%) was found in *Bipolaris soroikiniana* treated with pure essential oil of *Ocimum gratissimum* @ 225 ppm and lowest inhibition was 78.70% @ 100 ppm while the same oil when diluted with organic solvent and applied @ 22.5 ppm gave maximum inhibition (79.99%) and lowest inhibition was 50.86% @ 100 ppm for the same fungi. In case of another fungi, *Colletotrichum henanense* also showed maximum growth inhibition (87.50% @ 225 ppm and 70.26% @ 22.5 ppm) and minimum growth shown in (68.52% @100 ppm and 40.50% 22.5 ppm) in concentrated and diluted form of *Ocimum gratissimum* oil respectively. On the other hand, *Ocimum sanctum* inhibit maximum mycelial growth of *Bipolaris soroikiniana* was 91.31% @ 225 ppm and minimum at 100 ppm, it inhibits growth upto 78.26%. For *Colletotrichum henanense* maximunm inhibition percentage was 87.06% @ 225 ppm and lowest inhibition was observed in 100 ppm in 68.52% in concentrated form whereas in diluted form 22.5 ppm showed highest inhibition of 73.12% and lowest inhibition was 33.61% in 10 ppm. The oil of *Ocimum sanctum* was found to be less effective as compared to the oil of *Ocimum gratissimum* in respect of mycelium growth inhibition in both of plant pathogens in this study. Hence, in all cases, it had been observed that increasing concentration of essential oil of both *Ocimum gratissimum* and *Ocimum sanctum* increase the growth inhibition percentage of both the fungus viz. *Bipolaris soroikiniana* and *Colletotrichum henanense.*

To control fungal plant diseases, use of essential oils from plants may act as an alternative source to synthetic fungicides due to its environment friendly non-hazardous nature. In the present experiment, it had been shown that essential oils of both *Ocimum* had a significant influence on growth inhibition of *Bipolaris soroikiniana* and *Colletotrichum henanense*. The trends are similar to the work of Piyo *et al*., (2009) [9] where they found that *Ocimum gratissimum* could inhibit mycelium growth of all pathogenic fungi when the concentration applied was over 0.8 %. The present study showed that the fungistatic efficacy against both the fungi increased with an increase in concentration of the botanical extracts. The highest inhibition was observed at 225 ppm in concentrated form and in diluted form it was 22.5 ppm. Yasmin (2016) [13] and Islam *et al*. (2021) [4] also found that mycelial growth inhibition percentages increased with the increase in botanical extracts concentration. Lemos *et al*., (2005) [6] also recorded that the extracts of *O. gratissimum* showed activity in-vitro towards *Cryptococcus neoformans* by using agar dilution method. They found that 100% of *C. neoformans* isolates were inhibited by essential oil at concentrations of 250 µg/ml. Mohr *et al*. (2017) [7] observed that *O. gratissimum* essential oil was a highly potent inhibitor of the evaluated phytopathogenic fungi. Sharma *et al.* (2019) [11] found that *Ocimum sanctum* leaf extract suppressed the multiplication of the test fungus. Abbaszadeh *et al.* (2014) [1] showed that the compounds like thymol, carvacrol, eugenol, and menthol was observed most effective in inhibition against the fungus Aspergillus spp. and *Cladosporium spp*. Philippe *et al.* (2012) [8] recorded that *Ocimum gratissimum* essential oil had the most antifungal activity as a result of its notable content of the phenolic component thymol. Dubey *et al*. (2000) [3] had already established that thymol a constituent of *O. gratissimum* essential oil was highly active against *T. rubrum*, *T. mentagrophytes*, *C. neoformans*, *C. albicans*, and *Malassezia pachydermatis*. From the economic point of view, treatments with essential oil applied at 1% causing the management of pathogen up to 71% were found more profitable compared to treatments with essential oil applied at 10%. (Bahadar *et al*., 2016) [2].

Table 1: Mycelium growth inhibition (%) of the fungus under different concentration of pure essential oil derived from *Ocimum gratissimum* and *Ocimum sanctum*

Pathogenic fungi	Source of oil	Mycelium growth inhibition (%)						
		Control	Oil @ 100 ppm	Oil @ 125 ppm	Oil @ 150 ppm	Oil @ 175 ppm	Oil @ 200 ppm	Oil @ 225 ppm
Bipolaris soroikiniana	*O. gratissimum*	0	78.70	81.30	84.36	86.94	91.30	94.78
	O. sanctum	0	78.26	80.43	83.48	86.53	89.13	91.31
Colletotrichum henanense	*O. gratissimum*	0	68.52	73.29	75.01	82.77	84.92	87.50
	O. sanctum	0	66.36	69.82	73.26	78.43	81.89	87.06

Table 2: Mycelium growth inhibition (%) of the fungus under different concentration of diluted essential oil derived from *Ocimum gratissimum* and *Ocimum sanctum*

Pathogenic fungi	Source of oil	Mycelium growth inhibition (%)						
		Control	Oil @ 10 ppm	Oil @ 12.5ppm	Oil @ 15 ppm	Oil @ 17.5 ppm	Oil @ 20 ppm	Oil @ 22.5 ppm
Bipolaris soroikiniana	*O. gratissimum*	0	50.86	56.08	63.03	67.37	72.60	79.99
	O. sanctum	0	55.21	60.87	64.77	67.39	74.78	76.08
Colletotrichum henanense	*O. gratissimum*	0	40.50	43.52	47.82	51.71	59.46	70.26
	O. sanctum	0	33.61	38.34	48.24	53.42	61.17	73.12

Conclusion

In the present experiment, though it had been found that diluted form of essential oil showed lesser growth inhibition of fungi than the concentrated oil, but it was much cost-effective. Using only $1/10^{th}$ volume of the essential oil, an inhibition of 79.99% was observed at 22.5 ppm, while concentrated oil resulted in 94.78% inhibition. Dilution of the oil will also be effective for covering large area of infected plant populations. Hence, the essential oil of *Ocimum* species may be effectively utilized in controlling the phytopathogenic fungi as a safe mode of fungicide.

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