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In vitro evaluation of *Ocimum sanctum* against ten fungal pathogens

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Abstract
Sacred basil (*Ocimum sanctum*) Syn. *Ocimum tenuiflorus* known as "Tulsi" is a well-known sacred plant of the Hindus, widely used in religious rites since vedic times. It contains active ingredients such as alkaloids, enzymes and other inorganic elements. With a view to assess the fungitoxicity of *Ocimum sanctum* against the fungal pathogens. *Ocimum sanctum* leaves was evaluated in the form of crude (10%), powdered (10%), boiled (10%) and ethanol (1%) extracts against ten fungal pathogens viz., *Rhizoctonia solani, R. bataticola, Phoma sorghina, Colletotrichum gloeosporioides, Fusarium oxysporium* f.sp. *pallidoroseum, F. oxysporium* f.sp. *ciceri, Sclerotium rolfsii, Sclerotinia sclerotiorum, Alternaria solani* and *A. alternate.* The growth of the species of *Phoma sorghina, Colletotrichum gloeosporioides, Fusarium oxysporum f.sp. ciceri, and Fusarium oxysporium f.sp. pallidoroseum* was more effectively inhibited under its crude form than the other four forms, while the powdered form of *Ocimum sanctum* leaf extract was found more suitable for the control of *Sclerotinia sclerotiorum.*

Keywords: *ocimum sanctum,* evaluation, and *rhizoctonia solani, rhizoctonia bataticola, phoma sorghina, colletorichum gloeosprioides, fusarium oxysporium f.sp. pallidoroseum in vitro*

1. Introduction
Ocimum sanctum L. (also known as *Ocimum tenuiflorum*, Tulsi) has been used for thousands of years in Ayurveda for its diverse therapeutic properties. Tulsi, the Queen of herbs, the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. The sacred basil, Tulsi, is renowned (Warrier *et al* 1995) [13] for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East. Tulsi extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria.

Traditionally, *O. sanctum* L. is taken in many forms, as herbal tea, dried power or fresh leaf. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects. It Contains vitamin C and A, and minerals like calcium, zinc and iron, as well as chlorophyll and many other phytonutrients (Anbarasu and Vijayalakshmi 2007) [1]. Also enhances the efficient digestion, absorption and use of nutrients from food and other herbs. The plants of genus Ocimum belonging to family Labiate are very important for their therapeutic potentials. *Ocimum sanctum* L (Tulsi), that grow in different parts of the world and are known to have medicinal properties. (Sen 1993; Chopra *et al* 1956) [8, 2].

Tulsi has anti-bacterial, anti-viral and anti-fungal activity (Mediratta *et al*., 2002) [6] that includes activity against many pathogens responsible for human infections. Tulsi has also been shown to boost defenses against infective threats by enhancing immune responses in nonstressed and stressed animals (Tripathi *et al*., 2008) [10] and healthy humans (Monda *et al*., 2011). In the last few decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungi to different fungicidal used in medicinal practice (Singh *et al*., 2014) [6].

Control of plant diseases by chemicals has some limitations such as development of resistant strains of pathogens, toxicity towards both plants and animals etc. moreover use of chemicals through drenching contaminates the running and sub soil water (Richardson, 1991). Therefore the development of bio-pesticides has been focused as a viable disease control strategy. In previous years plant extract and essential oil show antifungal activity against a wide range of fungi (Wilson *et al.,* 1997 and Abd-Alla *et al.,* 2001). Extracts of several plants, viz., *Allium cepa* L., *Allium sativum* L., *Ocimum sanctum* L. and Mont., *Mentha piperata* L. and *Beta vulgaris* L. were found inhibitory to *Alternaria tenuis* (Shekhawat and Prasada, 1971) [9]. Objective of this study is *in vitro* evaluation of *Ocimum sanctum* against different fungal pathogens.

2. Materials and Methods
Ten different fungal pathogens were evaluated against *Ocimum sanctum* at department of Plant Pathology, College of Agriculture, Gwalior during the year 2011-12. The leaves of Tulsi were collected from the courtyard. Plant materials were washed with distilled water and dried in oven at 60° C for 2 days. The leaves were ground in an electric grinder to produce powder. *Ocimum sanctum* leaves was evaluated in the form of crude (10%), powdered (10%), boiled (10%) and ethanol (1%) extracts against ten fungal pathogens viz., *Rhizoctonia solani, R. bataticola, Phoma sorghina, Colletotrichum gloeosporioides, Fusarium oxysporium* f.sp. *pallidoroseum, F. oxysporium* f.sp. *ciceri, Sclerotium rolfsii, Sclerotinia sclerotiorum, Alternaria solani* and *A. alternate.* Potato Dextrose Agar (PDA) was used for isolation of fungal cultures. Culture medium was used under aseptic condition for bioassay of the test fungus. Three replications were

maintained. The radial growth of the mycelium was measured after 10 days of incubation at 21 ± 1°C.

Plant extracts: The fungi toxicity of *Ocimum sanctum* were evaluated against different fungal pathogens under *in-vitro* condition in the form of following extracts:

a) **Powdered extract:** For preparation of powder extract, the fresh leaves of *Ocimum sanctum* was thoroughly washed in ordinary tap water and dried in oven at 60°C for two consecutive days. After drying the leaves were easily crushed by mixer. These crushed leaves were Ieved (52 mesh) and was stored in the airtight plastic bottles. The powder was used at the concentration of 10 per cent. For preparing 10 per cent concentration 100 gm powder was incorporated into 500 ml of distilled water.
b) **Fresh extracts (Crude):** Crude extracts were prepared by grinding the required quantity of *Ocimum sanctum* leaves. Before grinding equal quantity of water were added (1:1 weight/volume basis). The crushed extracts were used @ 10 per cent by adopting poisoned food technique.
c) **Boiled extract:** The fresh leaves of *Ocimum sanctum* were washed, dried in shadow, weighted and boiled for one hour, thereafter it was filtered and water was incorporated into maintained 1:1 weight/volume basis. The extract was stored and used for bioassay of test fungus at the 10 per cent concentration.
d) **Ethanol extracts:** Hundred gram dried powdered leaf of *Ocimum sanctum* was incorporated into flask containing, 500 ml of ethanol then it was kept open on hot plate at the temperature of 42±2 ^{0}C for the evaporation of ethanol, after then the crust of the extract remained in the flask was scraped, weighted and used for bioassay @ 1% concentration.

For standardization of the concentration of the effective form the crude, boiled and powdered extracts were used @ 5, 10, 15 and 20% concentrations while the ethanol extract was used @ 1 per cent concentrations. The in-vitro evaluation of above extracts against the tested fungi was carried by adopting following method:

Bioassay of leaf extract by poisoned food technique

The leaf extract was mixed aseptically in melted potato dextrose agar medium in appropriate proportions and autoclaved. Twenty ml of the medium is poured in each 10 cm diameter petriplate and solidified. One disc (7 mm) of the fungal culture of the pathogen was cut from the 7 days old culture and was transferred in the centre of the petriplate under aseptic condition. The inoculated plates were incubated at 25oC and growth of the pathogen was measured at the interval of 24 hours. The medium without leaf extract was used as control.

Table 1: Efficacy of different forms of *Ocimum sanctum* leaf extracts against *Rhizoctonia solani*, *Rhizoctonia bataticola*, *Phoma sorghina*, *Colletorichum gloeosprioides*, *Fusarium oxysporium f.sp. pallidoroseum*

S. No.	Treatments	*Rhizoctonia solani*	*Rhizoctonia bataticola*	*Phoma sorghina*	*Colletorichum gloeosprioides*	*Fusarium oxysporium f.sp. pallidoroseum*
1	Powder@ 10%	56.67	55.00	51.67	41.67	50.00
2	Crude @ 10%	41.67	35.83	31.67	30.00	26.67
3	Boiled @ 10%	26.67	22.50	45.83	33.33	31.67
4	Ethanol @ 1%	31.67	65.00	41.67	45.33	51.67
5	Control	75.00	80.00	78.00	77.00	72.00
	SE(m)±	4.377	2.234	3.592	3.269	3.204
	CD at 5%	13.969	7.129	11.466	10.432	10.227

Data are the mean of three replications

Table 2: Efficacy of different forms of *Ocimum sanctum* leaf extracts against *Fusarium oxysporium f.sp. pallidoroseum*, *Sclerotium rolfsii*, *Alternaria solani*, *Alternaria alternata*

S. No.	Treatments	*Fusarium oxysporium f.sp. pallidoroseum*	*Sclerotium rolfsii*	*Alternaria solani*	*Alternaria alternata*
1	Powder@ 10%	59.17	35.83	60.00	51.67
2	Crude @ 10%	45.00	46.67	45.00	47.50
3	Boiled @ 10%	55.00	36.67	25.00	29.17
4	Ethanol @ 1%	61.67	44.17	46.67	52.50
5	Control	74.00	78.00	70.00	72.00
	SE(m)±	3.151	2.039	2.413	2.909
	CD at 5%	10.057	6.507	7.702	9.284

Data are the mean of three replications

3. Result and Dissection

The efficacy of *Ocimum sanctum* extracts against *ten* different fungal pathogens were evaluated. Efficacy against *Rhizoctonia solani* all the four forms of *Ocimum sanctum* leaf extracts significantly inhibited the growth of fungal mycelium but none of the form shown complete inhibition of the growth, however minimum growth was recorded under its boiled form (26.67 mm) while a maximum of 75.00 growth was recorded in control shown in fig.1. In case of *Rhizoctonia bataticola* minimum growth was recorded under its boiled form (22.50 mm), followed by crude (35.83 mm), powdered (55.00 mm) and ethanol extract (65.00 mm), while the maximum of 80.00 mm growth was recorded in control. In *Phoma sorghina*all the four forms of *Ocimum sanctum* leaf extracts significantly inhibited the growth of fungal mycelium but none of them completely inhibited the growth, however minimum growth was recorded under its crude form (31.67 mm) while efficiency against *Colletotrichum gloeosporioides* minimum growth of the fungus was recorded under its crude form (30.00 mm) followed by

boiled extract (33.33 mm) and maximum growth of 77.00 mm was recorded in control. In case of *Fusarium oxysporium f.sp. pallidoroseum* minimum growth was recorded under its crude form (26.67 mm) and in *Fusarium oxysporium* f.sp. *ciceri* minimum growth was recorded under crude form (45.00 mm). In *Sclerotium rolfsii* minimum growth was recorded under its powdered form @ 10% (35.83 mm) followed by boiled extract (36.67 mm). The form of powdered, crude and boiled extract @ 10% and ethanol extract @ 1% significantly inhibited the growth of *Sclerotinia sclerotiorum and in Alternaria solani* minimum fungal growth (25.00 mm) was recorded under its boiled form. Efficacy against *Alternaria alternate* minimum growth of fungal mycelium (29.17 mm) was recorded under its boiled form @ 10%, followed by crude extract @ 10% (47.50 mm), powdered extract @ 10% (51.67 mm) and ethanol extract @ 1% (52.50 mm), while the maximum of 72.00 mm growth was recorded in control.

The results revealed that all the four forms of *Ocimum sanctum* leaf extracts significantly inhibited the growth of all above test organisms but none of the forms could absolutely inhibited the growth of any one of the test fungus. The boiled extract was found significantly superior over other forms for inhibiting the growth of *R. solani*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Alternaria solani* and *A. alternata*. This supports the use of boiled *Ocimum sanctum* leaf extract for the control of these fungi. The growth of the species of *Phoma sorghina*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f.sp. *ciceri*, and *Fusarium oxysporium* f.sp. *pallidoroseum* was more effectively inhibited under its crude form than the other four forms, while the powdered form of *Ocimum sanctum* leaf extract was found more suitable for the control of *Sclerotinia sclerotiorum*. The above finding is supported by the work of Jha *et al.* (2000) [4], Upmanyu and Gupta (2005) [11] and Louis *et al.* (2011) [5]. Upmanu and Gupta (2005) [11] also reported that *O. sanctum* extract completely inhibited the mycelial growth of *Rhizoctonia solani*.

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