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Antifungal properties of extracts of *Ocimum tenuiflorum* and *Datura stramonium* against some vegetable pathogenic fungi

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Abstract
A study was carried out to evaluate the antifungal properties of *Ocimum tenuiflorum* (also known as *Ocimum sanctum*) and *Datura stramonium* extracts on *Fusarium oxysporum* and *Rhizopus stolonifer* using the well in agar method. The *in vitro* studies have been performed by using leaf, stem bark and root bark chloroform, alcoholic and aqueous extracts. All extracts showed antifungal activity. The stem bark alcoholic extract of *D. stramonium* showed maximum activity; while minimum activity was observed with root aqueous extract of *O. tenuiflorum* against the fungi under investigation. Results showed that radial growth in all the test organisms was impaired by the addition of the extracts in the culture medium used. The test organisms differed in their reaction to the different extracts but on the whole, growth inhibition increased with the concentration of each extract. The antifungal activity for both the plants and for both the organisms was found is in increasing order i.e. root bark < leaves < stem bark.

Keywords: Antifungal property, Pathogenic Fungi, Extracts.

INTRODUCTION

Ocimum tenuiflorum also known as *Ocimum sanctum (Tulshi)*. Over the years, control of pathogenic fungi in foods have drawn considerable attention with the use of industrial chemicals such as propionic acid and ammonia in the storage of grains against fungal attack (Frazier and Westhoff, 1998). These chemicals have shown to be effective in preventing fungal growth. However, when they are concentrated on the grains there could induce chemical poisoning, environmental toxicity and development of resistance by fungi to the chemical agent. Some tropical aromatic plants have shown to exert high antimicrobial activities and since they are natural products, mostly consumed by man, there is little or no fear of poisoning even at very high concentrations (Adegoke *et al*., 2002). Some of these plants include *Azadirachta indica* (neem) (Bankole and Adebanjo, 1995) and *Cymbopogon citratus* (Bankole *et al*., 2005).

The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Mohana *et al.,* 2011). Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Balandrin *et al.,* 1985; Hostettmann and Wolfender, 1997). Stem extracts of various plants are known to possess antimicrobial activity. Several workers have observed the antifungal activity of stem and its bark extracts (Boughalleb *et al*, 2005; Vats *et al*, 2009; Upadhyay *et al*, 2010; Sule *et al*, 2011).

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Parekh *et al*., 2006; Buwa and Staden, 2006; Mohana *et al*., 2008). Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promising. In view of these, the author screened some extracts against vegetable pathogenic fungi and the data has been presented in this paper.

MATERIALS AND METHODS

Isolation of disease causing fungal pathogen

Fungal pathogens were isolated on potato dextrose agar (PDA) medium from different stored plant material. Identified fungal cultures were isolated and pure cultures of each fungi made separately on PDA slants. These pure cultures were used for further investigation.

Preparation of plant powder

The fully grown leaf, stems and root of *Ocimum tenuiflorum* (also known as *Ocimum sanctum*) and *Datura stramonium* were collected from S.M.B.S.T. College, Sangamner, Dist. Ahmednagar campus. The collected plant material thoroughly washed with tap water and then rinsed with sterile distilled water. The bark of stem and root removed with help of knife then shed dried and grind in electric mixer. The powder material was kept in air tight glass bottles. This stock powder was used for further extraction.

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Preparation of extracts

Extracts were prepared by using different solvent system as distilled water, alcohol and chloroform. 100 gm of each type of powder was dissolved in 100 ml of solvent system mentioned above. It was filtered through the three layered filter paper. This was saved as stock solution from this stock different concentrations (25%, 50%, 75%, 100%) were prepared. Solvent system used was taken as control. These different concentrations were used as biocide against the fungal pathogen. Bioefficacy of the extract was checked in vitro by well in agar diffusion method (Onkar *et al.*, 1995).

Well in agar method

A loopful of the inoculums suspension test organism was spread uniformly on the solidified sterile culture media in the petridishes for uniform distribution of the organism. Using a sterile cork borer a well of 0.5 cm was made in the media and in each well known quantity of plant extract was filled so as to allow the diffusion of plant extract in the media. The petridishes were incubated at for 24 hours at 30±2°C temperature and the observations were recorded as diameter of inhibitory zone in mm. Well in agar plate filled with sterile distilled water and solvent was used as control in all the experiments. All the experiments were in triplicate and mean has been considered in observation table.

RESULTS AND DISCUSSIONS

Activity of *Ocimum tenuiflorum* extracts against *Fusarium oxysporum* and *Rhizopus stolonifer*

At a concentration of 100%, the leaf alcoholic extract recorded maximum 20.2 mm and 19.5 mm inhibition for *R. stolonifer* and *F. oxysporum* while it is for chloroform extract was 18.9 mm and 18.2 mm and aqueous extras was 15.5 mm and 13.2 mm. The minimum inhibition by leaf extract was recorded 9.2 mm for *F. oxysporum* and 10.4 mm for *R. stolonifer* at 25% aqueous extract.

At a concentration of 100%, the stem bark alcoholic extract recorded maximum 22.6 mm and 21.7 mm inhibition for *R. stolonifer* and *F. oxysporum* while chloroform and aqueous extract exhibited results in decreasing order. The minimum inhibition by stem bark extract was recorded 10.1 mm for *F. oxysporum* and 10.3 mm for *R. stolonifer* at 25% aqueous extract.

The Alcoholic root bark extract at a concentration of 100%, showed maximum 14.1 mm and 12.6 inhibition for *R. stolonifer* and *F. oxysporum* while chloroform and aqueous extract exhibited results in decreasing order. The minimum inhibition by root bark extract was recorded 6.5 mm for *F. oxysporum* and 7.1 mm for *R. stolonifer* at 25% aqueous extract

Table 1. Effect of *Ocimum tenuiflorum* extract on fungal pathogen

Sl. no.	Plant part	Solvent	*Fusarium oxysporum*				*Rhizopus stolonifer*			
			Inhibition* (mm) by extract				Inhibition* (mm) by extract			
			25%	50%	75%	100%	25%	50%	75%	100%
1	Leaf	Alcohol	13.4	16.6	17.1	19.5	13.8	16.5	18.1	20.2
		Chlorf.	10.1	12.1	14.4	18.2	11.2	12.9	15.8	18.9
		D.W.	9.2	11.0	12.6	13.2	10.4	11.6	13.5	15.5
2	Stem bark	Alcohol	14.6	17.5	20.5	21.7	17.5	19.5	21.1	22.6
		Chlorf.	12.2	14.4	17.1	19.4	14.9	17.4	19.5	21.2
		D.W.	10.1	11.3	12.6	14.1	10.3	12.6	14.4	16.5
3	Root bark	Alcohol	9.0	10.1	10.8	12.6	11.0	11.6	12.4	14.1
		Chlorf.	9.4	9.6	10.2	11.1	8.4	8.8	10.3	12.5
		D.W.	6.5	7.8	8.8	9.4	7.1	7.9	8.2	10.6

* - values given are mean values of triplicates; Chlorf- Chloroform; D.W.- Distilled water

Activity of and *Datura stramonium* extracts against *Fusarium oxysporum* and *Rhizopus stolonifer*

The leaf alcoholic extract at a concentration of 100%, exhibited increase of 24.3 mm and 23.5 mm mycelial inhibition while the antifungal efficiency depreciated with Chloroform to aqueous extract. Mycelial growth inhibition finally fell to 9.2 mm and10.4mm at 25% aqueous extract for *F. oxysporum* and *R. stolonifer*.

At a concentration of 100%, the stem bark alcoholic extract produces highest 25.3 mm and 24.4 mm inhibition for *R. stolonifer* and *F. oxysporum* while chloroform and aqueous extract exhibited results in decreasing order. The minimum inhibition by stem bark extract was recorded 11.3 mm for *F. oxysporum* and 12.2 mm for *R. stolonifer* at 25% aqueous extract.

The extract of root bark in alcohol at a concentration of 100%, produced maximum of 15.3 mm 13.5 mm inhibition for *R. stolonifer* and *F. oxysporum* while chloroform and aqueous extract exhibited results as same mentioned above. The minimum inhibition by root bark extract was recorded 7.5 mm for *F. oxysporum* and 8.1 mm for *R. stolonifer* at 25% aqueous extract.

Among the extracts assayed, the stem bark alcoholic extract of *D. stramonium* showed maximum activity; while minimum activity was observed with root aqueous extract of *O. tenuiflorum* against the fungi under investigation. Results showed that radial growth in all the test organisms was impaired by the addition of the extracts in the culture medium used. The test organisms differed in their reaction to the different extracts but on the whole, growth inhibition increased with the concentration of each extract. The antifungal activity for both the plants and for both the organisms was found is in increasing order i.e. root bark < leaves < stem bark.

Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999; Harborne, 1998; Gottlieb *et al.,* 2002). Antifungal activity and preliminary phytochemical analysis of stem bark extracts of *Juglans regia* linn. has been studied by Upadhyay *et al* (2010). Studies on antifungal activity of different extracts of *Cassia fistula* and bioactivity

guided isolation and identification of antifungal agent has been performed by Shilpakala *et al* (2009). Igbinosa *et al* (2009) studied antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas.* Tang *et al* (2010) isolated sphignolipids from the stems of Cucumber (*Cucumis sativus* L.) and observed antimicrobial activity. Priya *et al* (2010) studied antifungal activity of different extracts of *Cassia fistula* and bioactivity guided isolation and identification of antifungal agent. Recently, Londonkar *et al* (2011) reported potential antibacterial and antifungal activity of A*chyranthes aspera*. Antifungal properties and phytochemical screening of extracts of *Ocimum gratissimum* L. (Amadi *et al*).Thus, there is a need to search for alternative approaches to considering these as first step in the present investigation.

Table 1. Effect of *Datura stramonium* extract on fungal pathogen

Sl. no.	Plant part	Solvent	*Fusarium oxysporum*				*Rhizopus stolonifer*			
			Inhibition* (mm) by extract				Inhibition* (mm) by extract			
			25	50	75	100	25	50	75	100
1	Leaf	Alcohol	15.0	17.5	21.3	23.5	16.0	18.3	22.5	24.3
		Chloroform	13.0	14.5	17.5	20.0	14.0	16.5	18.3	22.0
		Dist.Water	10.0	11.0	11.5	12.5	11.0	12.2	14.3	16.0
2	Stem bark	Alcohol	16.5	19.6	22.5	24.4	17.5	19.5	23.9	25.3
		Chloroform	14.3	15.5	18.3	18.5	14.9	17.4	19.4	23.1
		Dist.Water	11.3	12.0	12.5	16.9	12.2	13.5	14.5	18.0
3	Root bark	Alcohol	10.0	11.5	12.1	13.5	11.2	13.0	14.1	15.3
		Chloroform	9.5	9.1	10.4	12.2	10.0	10.5	12.4	14.1
		Dist.Water	7.5	8.2	9.8	10.4	8.1	9.2	10.1	12.0

* - values given are mean values of triplicates; Chlorf- Chloroform; D.W. - Distilled water

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