

## *In vitro* antifungal activity of leaf extracts against pathogenic fungi associated on fenugreek

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### Abstract

The *in vitro* antifungal potency of five leaf extracts were evaluated for their botanical fungi toxicants on pathogenic fungi of Fenugreek (*Trigonella foenum-graceum* L.). The antifungal effect of aqueous extracts of leaf namely *Azadirachta indica*, *Ocimum gratissimum*, *Adhatoda vassica*, *Santalum album* and *Aegle mormelos* were selected. The inhibitory effect was tested by food poisoning technique and determined minimum inhibitory concentration (MIC). Due to the presence of bioactive molecules the leaf extracts showed significant inhibition in different concentrations. *Azadirachta indica* leaf extract showed 100% reduction of radial growth of *Alternaria alternata* and *Fusarium oxysporum* at 75% conc. In some extent, *Ocimum gratissimum* also showed significant reduction of *A.alternata* at 100% conc. The inhibitory effect of *Ocimum gratissimum* was also shown in case of *F.oxysporum* at 100% conc.

### INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) of family Fabaceae is an important leafy vegetable and its seeds are used as condiments in India. Fenugreeks are herb that is commonly found growing in the Mediterranean region of the world. The seeds and leaves are primarily used as a culinary spice, it is also used to treat a variety of health problems in Egypt, Greece, Italy and South Asia. (Morcos et al., 1981).

Fenugreek seeds have been found to contain protein, vitamin C, niacin, potassium and diosgenin. Other active constituents in fenugreek are alkaloids, lysine and tryptophan as well as steroidal saponins (Yoshikawa et al., 1997).

In India and China it has also been used to treat arthritis asthma, bronchitis, improve digestion;

maintain a healthy metabolism, cure skin problems, treat sore throat and cure acid reflux. Recent studies have shown that fenugreek helps lower blood glucose and cholesterol levels and may be an effective treatment for both type 1 and 2 diabetes. It is also being studied for its cardiovascular benefits (Patil et al., 1997; Abdel-Barry et al., 2000; Rao, 1996).

Leaf extracts is a stable dispersion of naturally occurring polymer micro particles in an aqueous medium. It is found in 10% of all angiosperms. This complex emulsion consisting of alkaloids, starches, sugars, oils, tannins, resins and gums that coagulates on exposure to air. It is also rich in enzymes like proteases, glucosidases, chitinases and lipases. It has been demonstrated that this substance is a source of natural fungicides (Barkai-Golan, 2001).

Which is regarded as both safe and effective against various diseases of banana, papaya and other fruits. The water-soluble fraction of papaya latex can completely digest the conidia of many fungi, including important postharvest pathogens (Indrakeerthi & Adikaram, 1996). Other latex extracted from several plants showed a strong antifungal activity against *Botryti cinerea*, *Fusarium* sp. and *Trichoderma* sp. (Barkai-Golan, 2001).

Fungicides belong to a group of pesticides which inhibited fungal growth either causing damage to the cells or preventing the fungal development. As pesticides, they offer great economic and social benefits through the protection and preservation of materials, food and the prevention of diseases. Since pesticides are designed specifically to fight harmful or even dangerous life forms and therefore are toxic to them, they may present hazards to the environment by their potential effect upon non-target organisms, including humans, particularly when misused. The need to balance these benefits against the risks presents a challenge to the EPA (Environmental Protection Agency) unlike other chemicals. The aim of this study was to evaluate the antifungal activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by pathogens.

## MATERIALS AND METHODS

### Preparation of Leaf extracts

Fresh leaves of *Azadirachta indica*, *Ocimum gratissimum*, *Adhatoda vassica*, *Santalum album* and *Aegle mormelos* were collected washed and oven dried at 45°C. The oven dried leaves of the above plant sp. were pulverized to obtain dry powder. 100g. powder of each species was taken. Leaf extracts of each plant species was prepared with 100ml water condensed to serve as stock extracts. The toxicity of stock extracts was determined against carbendazim sensitive and resistant isolates of *Alternaria alternata*, *Fusarium oxysporum*, by food poisoning technique (Mishra and Tiwari, 1992) at four (25, 50, 75 and 100%) concentrations.

### Fungal Pathogens

The two fungicide resistant pathogens such as *Alternaria alternata* caused by leaf spot disease and *Fusarium oxysporum* caused by wilt disease were used.

### Determination of antifungal activity

The sensitive and resistant isolates of test fungi were multiplied on CZA medium. Petriplates containing CZA supplemented with different plant

leaf extracts at four concentrations with their three replications were inoculated with fresh 7 days old culture of test fungi (8 mm) cork borer discs and kept upside down. The above plates were incubated in BOD incubator (at 28±2<sup>0</sup> C). Plates without plant extracts were served as control. Radial growth of the fungus on leaf extracts was measured at regular intervals

## RESULTS AND DISCUSSION

### Fenugreek (*Trigonella foneum-graecum*)

Plant leaf extracts was tested against two pathogenic fungi to determine their antifungal activity. Different concentrations of plant latex (25, 50, 75 and 100%) were tested against pathogenic fungi. Minimum Inhibitory Concentration (MIC) was measured to determine the antifungal activity. *Azadirachta indica* leaf extract showed significant reduction of radial growth of *Alternaria alternata* (78.88%) and *Fusarium oxysporum* (68.88%) at 100% conc. respectively. There was no significant reduction of radial growth in case of *Ocimum gratissimum*, *Santalum album* and *Aegle mormelos*.

The result agrees with Takazawa *et al.*, (1982) that there is a need to employ broad range of extractive solvents in the extractions of possible photochemical from medicinal plants. The growth of four test fungi were inhibited by ethanol and chloroform extracts while the aqueous extract was the least effective on the test fungi. The best antifungal activity was recorded in ethanol extract of *C.procera* latex against *Candida albicans* (Kareem *et al.*, 2008). The leaf part of all these plants were used for various medicinal purposes by the local traditional healers. More information on the origin and references of these plants are presented elsewhere (Malabadi *et. al.*, 2007) Leaf extracts, chopped leaves and latex of *C. procera* have shown great promise as a nematicide *in vitro* and *in vivo* (Khirstova and Tissot, 1995). The mycelia growth, percentage spores germination and germ- tube extension in *Fusarium oxysporum* and *Aspergillus carbonaris* decreased when *Calotropis procera* extract concentration increases, where as growth of *Humicola brevis* and *Penicillium lanosum* were not affected (Rizk,2008). The minimum inhibitory concentrations (MIC) were also determined Methanolic fraction had a total inhibition against *Candida albicans* (100%) at a concentration of 500µg/ml and a negative effect against *Cryptococcus neoformans*. *Microsporium canis* was strongly inhibited with methanolic extract (75%) and totally

**Table 1: Antifungal activity of leaf extracts against pathogenic fungi of *Trigonella foneum-graecum*.**

Plant species	Conc (%)	Radial growth of <i>A.alternata</i> (mm)	Inhibition (%)	Radial growth of <i>F.oxysporum</i> (mm)	Inhibition (%)
<i>Azadiracta indica</i>	25	35	61.11	42	53.33
	50	30	66.66	38	57.77
	75	24	73.33	32	64.44
	100	19	78.88*	28	68.88
<i>Ocimum gratissimum</i>	25	40	55.55	30	66.66
	50	36	60.00	28	68.88
	75	31	65.55	25	72.22
	100	26	71.11	21	76.66*
<i>Adhatoda vassica</i>	25	76	15.55	65	27.77
	50	71	21.11	61	32.22
	75	66	26.66	58	35.55
	100	60	33.33	54	40.00
<i>Santalum album</i>	25	70	22.22	71	21.11
	50	64	28.88	66	26.66
	75	60	33.33	61	32.22
	100	57	36.66	55	38.88
<i>Aegle mormelos</i>	25	82	88.88	79	12.22
	50	77	14.44	74	17.77
	75	74	17.77	70	22.22
	100	69	23.33	65	27.77
Control		90	--	90	--
CD (P=0.05)			16.73		14.56

\*Significantly reduced mycelial growth.

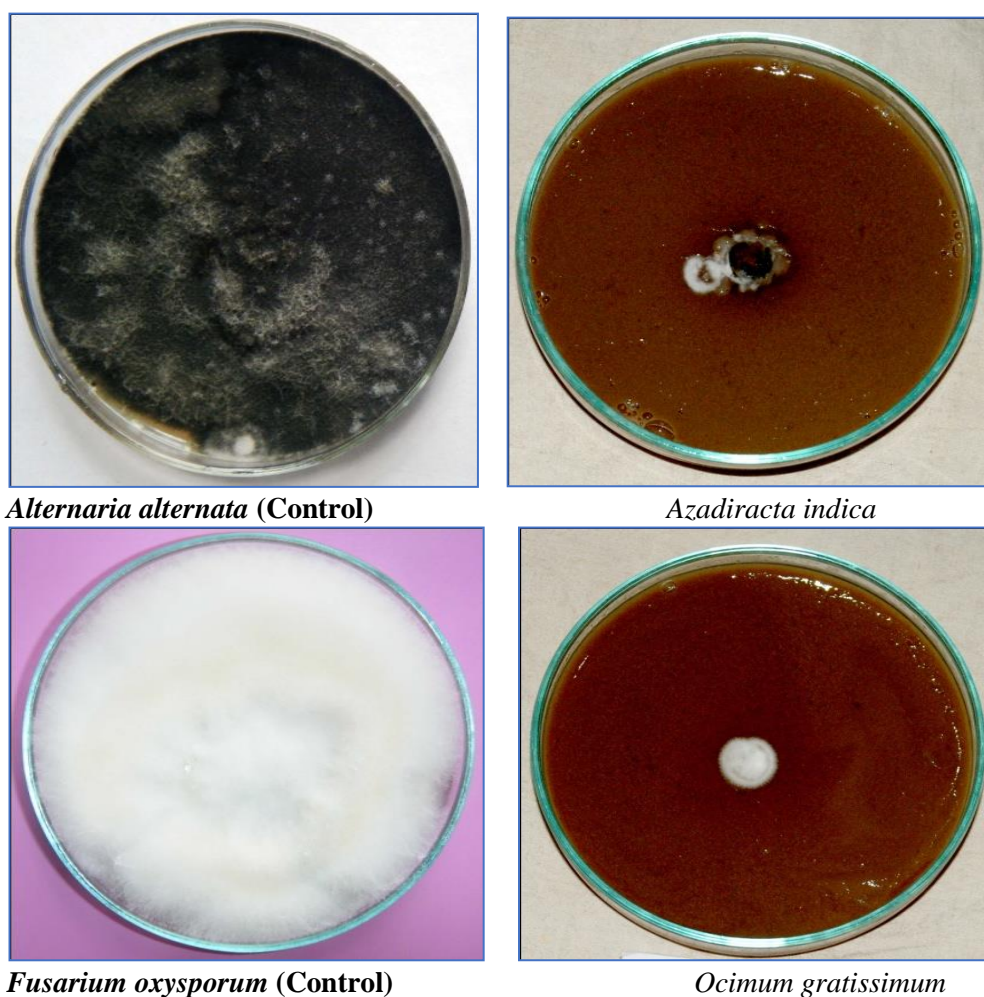
with ethyl acetate extract at a concentration of 750µg/ml (Houda, 2010). The antifungal potency of *C. gigantea* latex extract on the *C. albicans* showed a larger diameter of clearance than that of other fungal strains (Venkatesan and Subramanian, 2010). Raghavendra (2011) reported the latex extract were screened *in vitro* against human pathogenic strains such as Gram positive; *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative; *Salmonella typhi*, *Klebsiella phenonemia* and two fungal strains; *Aspergillus niger* and *Candida albicans*. The inhibitory effect was assessed by agar well diffusion method.

## CONCLUSION

Antifungal activity of leaf extract of *Azadirachta indica*, *Adhathoda vassica*, *Aegle mormelos*, *Santalum album*, *Ocimum gratissimum* were tested against tested fungi. Tested plants, leaf extracts of *Ocimum gratissimum* *Azadirachta indica* were found suitable at 100%. Significant inhibition. Therefore these two plants extracts are suitable for management of infected pathogens.

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**Figure 1: Antifungal activity of leaf extracts against pathogenic fungi of fenugreek.**

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