

Determination of Antifungal Potential of *Trigonella foenum*

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Abstract-*Trigonella foenum* has been widely used for its various pharmacological properties. Hence, in the current study we aimed to determine the antifungal activity of leaf and stem extracts of *Trigonella foenum*. Ethanol (50%) was used for extraction of active principles from the dried powdered leaves and stem of *Trigonella foenum*. The antifungal screening was done with three plant pathogens viz. *Aspergillus fumigatus* associated with *Sorghum bicolor*, *Aspergillus niger* and *Fusarium* sp., associated with *Zea mays*. In agar well diffusion assay *Aspergillus niger* was markedly affected by leaf and stem extracts of *Trigonella foenum* but it was not effective in inhibiting the growth of *Aspergillus fumigatus* and *Fusarium* sp., Findings of this study demonstrated that the leaf and stem of *Trigonella foenum* possessed antifungal property against *Aspergillus niger* but was not effective against *Aspergillus fumigatus* and *Fusarium* sp., Ethanolic extracts of leaf and stem parts of *Trigonella foenum* could be used to control plant diseases such as black mold associated with *Aspergillus niger* as a safe alternative option to chemical fungicides.

Keywords: *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium* sp., *Sorghum bicolor*, *Trigonella foenum*., *Zea mays*

INTRODUCTION

Plant diseases especially caused by seed borne fungi are among one of the main factors reducing yield and quality of seeds. From seed germination to harvest, soil-borne and seed borne fungi and diseases caused by them reduce the vigour and yield of plants and also infected seeds represent a primary source of infection in the field. [1,2] Furthermore, seed borne fungi produce mycotoxins that cause diseases to humans or animals when they eat those seeds.[3] Seeds are generally treated with synthetic fungicides to manage the seed borne fungi. But the use of synthetic fungicides is associated with problems such as pollution, phytotoxicity and development of resistant pathogenic strains. [4] Post harvest treatment of stored seeds with synthetic fungicides is also not preferable as it influences the quality of

seeds and causes serious health hazards for the consumers. [5] Therefore considerable interest has been developed in the recent years for using more consumer and nature-friendly protectants in the seed treatment.[6]

Aspergillus fumigatus is a species of fungus in the genus *Aspergillus*. It is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency. *Aspergillus fumigatus* is a saprotroph widespread in nature and is typically found in soil and decaying organic matter such as compost heaps where it plays an essential role in carbon and nitrogen recycling. The fungus is capable of growth at 37 °C and can grow at temperatures up to 50 °C with conidia surviving at 70 °C. [7]

Aspergillus niger is a fungus classified within the Nigri section of the *Aspergillus* genus. *Aspergillus Niger* causes a disease known as “black mold” on certain fruits and vegetables such as grapes, onions, apricots and peanuts and it is a common contaminant of food. It is ubiquitous in soil, commonly found in indoor environment. *Aspergillus Niger* is classified as safe (GRAS) by the US Food and Drug Administration for use in food production, although the microbe is capable of producing toxins that affect human health. *Aspergillus Niger* is capable of withstanding extremely acidic conditions which makes it especially important for the industrial production of citric acid. [8]

Fusarium is a large genus of filamentous fungi and is a part of a group often referred to as hyphomycetes which is widely distributed in soil and associated with plants. Most of the species are harmless decomposers and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect animal health if they enter the food chain. *Fusarium* species produce the toxins such as fumonisins and trichothecenes. In spite of most species apparently being harmless, some *Fusarium* species and

subspecific groups are among the most important fungal pathogens of plants and animals. [9]

Trigonella foenum is an annual plant which belongs to family Fabaceae. It has leaves consisting of three small obovate to oblong leaflets and it is cultivated worldwide as a semiarid crop. Its leaves and seeds are common ingredients in dishes from the Indian subcontinent and have been used as a culinary ingredient since old age times. It is used as a food ingredient in small quantities and it is safe to use such small quantities. Fenugreek is used as a spice, herb and vegetable. Sotolon is the chemical which is responsible for the distinctive maple syrup smell of fenugreek. [10] There are many studies which have documented the antifungal properties of *Trigonella foenum* against pathogenic fungi.[11,12,14,15]. Similarly in the present study we aimed to determine the antifungal potential of ethanol extracts of *Trigonella foenum* on *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium* sp., by agar well diffusion assay as described by Perez [13]

MATERIALS AND METHODS

Collection of sample

Matured *Trigonella foenum* plants were collected from local Market, Bengaluru, Karnataka, India.

Preparation of sample

The fresh leaves and stem of *Trigonella foenum* were washed with clean water and sun dried for seven days to remove all moisture. The dried plant samples were grounded in a mortar with a pestle, and then in a blender into powdered form and plant powder was sieved using 0.1 mm sieve.

Extraction of plant materials

Ethanol extraction

The ethanol extracts of the plant were prepared using the powdered sample of the leaf and stem in 100 mL of ethanol individually in a magnetic stirrer for 4 hours. Thereafter filtered using Whatman No. 1 filter paper. The extract was then allowed for evaporation of solvent in a fumigator for 7 hours. The concentrated extract was stored in an air tight container in a refrigerator at 20 °C until it is required for analysis.

Sample preparation

The sample was prepared by dissolving 100 mg/mL powdered sample of the leaf and stem in 50% of ethanol individually.

Standard antifungal preparation

Itraconazole - a commercial antifungal agent was prepared in sterile water (1 mg/mL). 50% Ethanol was used as control.

Organisms used

The pure cultures of the microorganisms were obtained by Standard blotter method. Screening of pathogens was done for all expressed pathogens in the collected *Sorghum bicolor* and *Zea mays* seeds followed by isolation of *Aspergillus fumigatus* from *Sorghum bicolor*, *Aspergillus niger* and *Fusarium* sp., from *Zea mays*.

Preparation of media

Potato dextrose agar media was prepared according to manufacturer's instructions as given on product label. Required quantity of potato dextrose agar media was measured using electronic balance in a conical flask and required distilled water was added using measuring cylinder. Amoxicillin was added to prevent the growth of bacteria. The media was mixed properly and sterilized by autoclaving for 15 minutes at 760 mmHg.

Antifungal Activity

Antifungal activity of plant extracts was studied using agar well diffusion method as described by Perez.[13] Well grown colonies of *Aspergillus fumigatus* from *Sorghum bicolor* and *Aspergillus niger* and *Fusarium* sp., from *Zea mays* were inoculated on potato dextrose agar and pure culture of *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium* sp., were obtained by subculturing the previous culture and incubated at 27 ± 2 °C for 48 h. Fungi were suspended in sterile water and adjusted to a standard inoculum size to $1-2 \times 10^6$ CFU/ml individually. 0.1 ml of fungal suspension was used to inoculate PDA petriplates with a sterile non-toxic cotton swab. Four wells of five millimetres diameter were punched in the agar with the help of a sterile well borer and filled with 20 μ L (2 mg) of leaf and stem extracts individually. 20 μ L (20 μ g) of itraconazole a commercial antifungal compound was used as reference standard and 20 μ L of 50% ethanol was added as control. Experiments were performed in triplicates and the treated plates with plant extracts, reference standard and control were incubated at 27 ± 2 °C for seven days. After incubation the treated plates were observed for zone of inhibition around the wells. Zone of inhibition was measured in millimetres (mm) and recorded.

RESULTS AND DISCUSSION

Fungi were isolated from *Sorghum bicolor* and *Zea mays* seeds and most of the seeds inoculated on to petri plates gave rise to fungal colonies. *Rhizopus stolonifer* was most predominant on *Zea mays* followed by *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium* sp., *Penicillium* sp., *Mucor* sp., and *Aspergillus fumigatus* was predominant on *Sorghum bicolor* seeds followed by *Alternaria* sp., *Aspergillus niger* and *Fusarium* sp..

Effect of Plant Extracts on *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium* sp.,

This study revealed the antifungal activity of leaf and stem extracts of *Trigonella foenum* against *Aspergillus niger* and it also revealed that ethanol extracts of *Trigonella foenum* was not effective against *Aspergillus fumigatus* and *Fusarium* sp.. Leaf extracts of *Trigonella foenum* showed the maximum inhibition of 8 mm against *Aspergillus niger* followed by stem extracts which showed the maximum inhibition of 3 mm against *Aspergillus niger*. Itraconazole showed the inhibition of 12 mm against *Aspergillus fumigatus*, 10 mm against *Aspergillus niger* and 5 mm against *Fusarium*. Sudan, Puneet Goswami, Manish Singh, Jitender et al., had studied the efficacy of *Trigonella foenum* against *Microsporium gypseum*. Results revealed that

Trigonella foenum was effective in inhibiting the growth of *Microsporium gypseum*. [11] Similarly in the present study *Trigonella foenum* was effective against *Aspergillus niger*. Faten Omezzine, Mohamed Bouaziz, Mejda Daami-Remadi, Monique S.J. Simmonds, Rabiaa Haouala et al., had studied the antifungal activity of *Trigonella foenum* against *Fusarium*. Results revealed that *Trigonella foenum* inhibited the mycelial growth of *Fusarium*. [14] This result was in contrast to our result where *Trigonella foenum* was not effective in inhibiting the mycelial growth of *Fusarium*. Ali A. S. Al-Mayah et al had studied the antifungal activity of *Trigonella foenum* against *Aspergillus fumigatus*. [15] Results revealed that *Trigonella foenum* was not effective against *Aspergillus fumigatus*. These results were in accordance to our result where leaf and stem extracts of *Trigonella foenum* were not effective against *Aspergillus fumigatus*. Secondary metabolites present in *Trigonella foenum* were effective against the growth of *Aspergillus niger* but *Aspergillus fumigatus* and *Fusarium* has become resistant to those secondary metabolites as a result *Trigonella foenum* was not effective in inhibiting the mycelial growth of *Aspergillus fumigatus* and *Fusarium* sp..

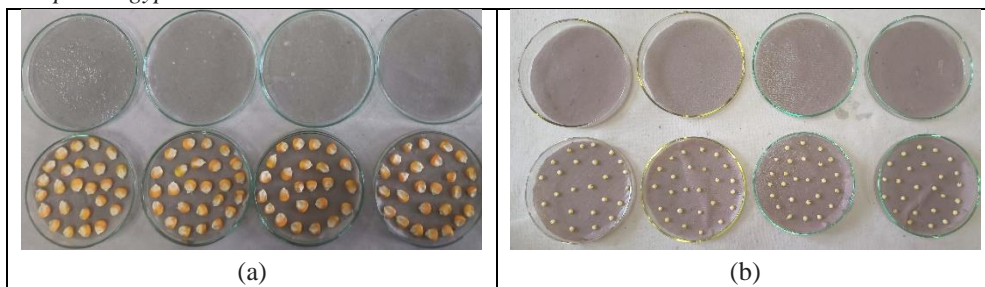


Fig 1: Screening of fungi on *Zea mays* (a) and *Sorghum bicolor* (b)

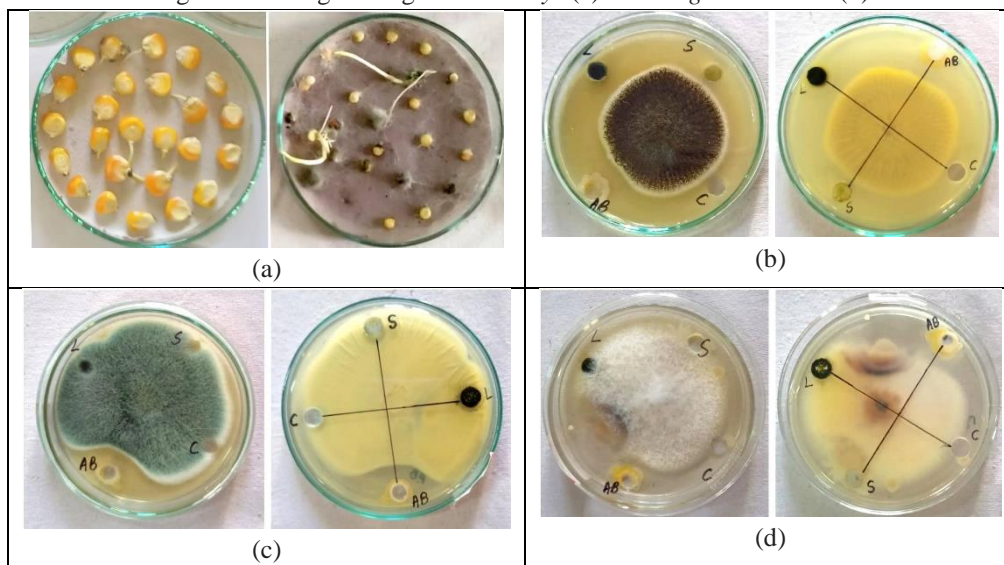


Fig 2: (a) Fungi expressed on *Zea mays* seeds and *Sorghum bicolor* seeds. (b) Inhibitory activity of *Trigonella foenum* against *Aspergillus niger*.

(c) Inhibitory activity of *Trigonella foenum* against *Aspergillus fumigatus*.
 (d) Inhibitory activity of *Trigonella foenum* against *Fusarium*.

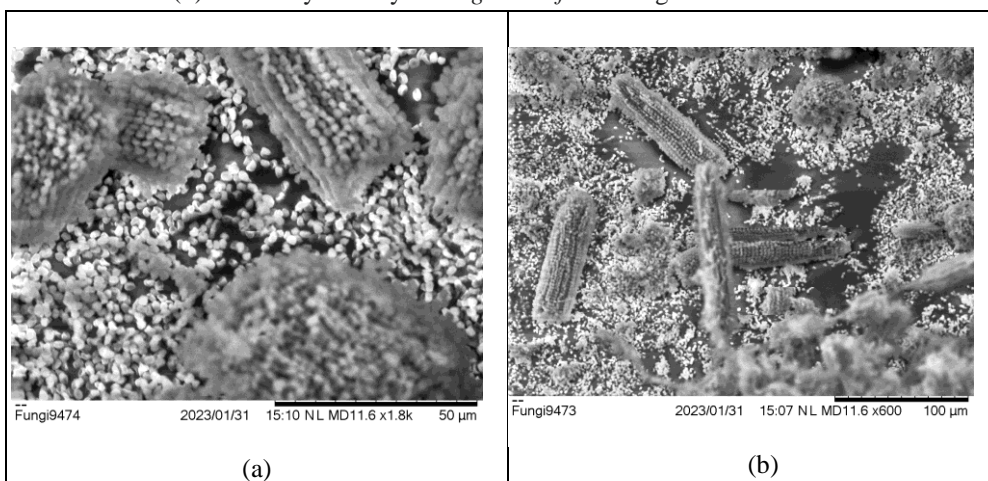


Fig 3: Spores of *Aspergillus fumigatus* under Scanning Electron Microscope (a) magnification 1800x (b) magnification 600x

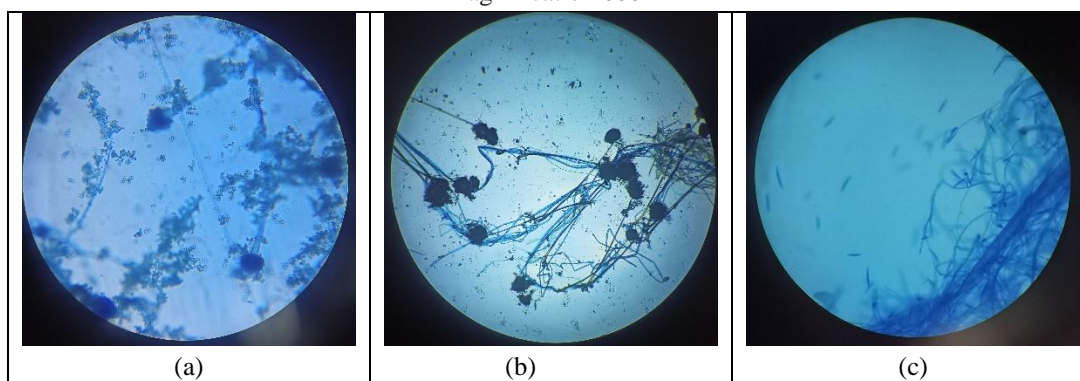


Fig 4: (a) *Aspergillus fumigatus* under binocular microscope (400x)
 (b) *Aspergillus niger* under binocular microscope (400x)
 (c) Macroconidia of *Fusarium* under binocular microscope (400x)

Labels on plate	Meaning	Inhibition of <i>Aspergillus niger</i> (mm)
L	Leaf extract	8 mm
S	Stem extract	3 mm
C	50% ethanol	1 mm
AB	Itraconazole	10 mm

Table 1: Inhibitory activity of *Trigonella foenum* against *Aspergillus niger*

<i>Aspergillus fumigatus</i>	Negative inhibition
<i>Fusarium</i>	Negative inhibition

Table 2: Inhibitory activity of *Trigonella foenum* against *Aspergillus fumigatus* and *Fusarium*

CONCLUSION

This study determined the antifungal activity of *Trigonella foenum* against plant pathogen *Aspergillus niger*. This study also revealed the ineffectiveness of *Trigonella foenum* against *Aspergillus fumigatus* and *Fusarium*. The phytochemicals present in leaf and stem of *Trigonella foenum* exhibited antifungal property

presenting it as a potent plant in treatment of plant fungal diseases associated with *Aspergillus niger*. *Trigonella foenum* plant extracts having resistance mechanisms against *Aspergillus niger* may be useful to control plant diseases associated with that. On the basis of the results obtained during the experiment and reports of success of *Trigonella foenum* extracts in controlling plant pathogenic fungi such as *Aspergillus niger*, the tested plant extracts hold

promise for the organic and ecofriendly management of plant diseases. The findings of these studies may become the foundation for the use of biocontrol agents such as plant extracts as a safe and cost-effective control method against *Aspergillus niger*.

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