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Comparative analysis of antifungal activity of different plant extracts

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Abstract
The increasing incidence of drug-resistant pathogens and toxicity of existing antifungal compounds has drawn awareness towards the antifungal activity of natural products. Hence, in the current study we aimed to determine the antifungal activity of ethanolic extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* against *Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium oxysporum*. Extraction of active principles of plant from the dried powdered samples of leaf and stem of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* was done with 50% ethanol. The antifungal screening was done on *Sorghum bicolor* and *Zea mays* seeds and three plant pathogens associated with these seeds were studied *viz*. *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum.* In agar well diffusion assay itraconazole was used as positive control and ethanol (50%) was used as negative control. *Aspergillus Niger* was markedly affected by leaf and stem extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* and *Fusarium oxysporum* was affected by ethanolic extracts of *Coriandrum sativum* but it was not affected by ethanolic extracts of *Mentha piperita* and *Trigonella foenum*. *Aspergillus fumigatus* was not affected by ethanolic extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum.* The results obtained from this study would help in establishing the use of ethanolic extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* to control plant diseases associated with *Aspergillus niger* and *Fusarium oxysporum* and also as a safe substitute to chemical fungicides.

Keywords: *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, ethanolic extracts, Itraconazole

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, diseases caused by these seed borne fungi reduce the vigour and yield of plants. These infected seeds represent a primary source of infection in the field [1, 2]. In addition, mycotoxins are produced by these seed borne fungi which cause diseases to humans or animals when they consume those seeds [3]. Seeds are commonly treated with synthetic fungicides to manage the seed borne fungi. But the use of synthetic chemical fungicides is associated with problems such as pollution, phytotoxicity and development of resistant pathogenic strains [4]. Post harvest treatment of stored seeds with chemical fungicides is also not preferable as it will affect the quality of seeds and cause serious health hazards for its consumers [5]. Therefore considerable attention has been paid in the recent years for using more consumer and nature-friendly protectants in the seed treatment [6].

Aspergillus fumigatus is a mold species in the genus *Aspergillus*. It is one of the most common *Aspergillus* species which cause ailment in individuals with an immune deficiency. *Aspergillus fumigatus* is a saprobe which is widespread in nature and is commonly found in soil and decaying organic matter such as compost heaps where it plays an essential role in carbon and nitrogen recycling. This fungus is capable of growth at 37 °C and can withstand 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. "Black mold" is a common disease caused by *Aspergillus Niger* on certain fruits and vegetables such as grapes, onions, apricots and peanuts and it is a common contaminant of food. The fungus is omnipresent, commonly found in indoor environment. The US Food and Drug Administration classified *Aspergillus Niger* as safe (GRAS) for use in food production, although the microbe produces toxins that affect human health. *Aspergillus Niger* is capable of withstanding very high acidic conditions which makes it especially important for the industrial production of citric acid [22].

Fusarium oxysporum is a large genus of filamentous fungi and is a part of a group often referred to as hyphomycetes (Fungi imperfecti) which is extensively distributed in soil and linked with plants. Most of the fungi of this genus are harmless decomposers and are relatively abundant members of the soil microbial community. Some species of *Fusarium oxysporum* produce mycotoxins in cereal crops that can affect animal health if they enter the food chain. *Fusarium oxysporum* species also produce the toxins such as fumonisins and trichothecenes. Most species are apparently being harmless, some *Fusarium oxysporum* species and sub specific groups are among the most dominant fungal pathogens of plants and animals [21].

There are many studies which have documented the antifungal properties of plant extracts against pathogenic fungi [10, 18]. Similarly in the present study we aimed to determine the antifungal potential of ethanolic extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* on *Aspergillus fumigatus*, *Aspergillus Niger* and *Fusarium oxysporum* by agar well diffusion assay as described by Perez [19].

Materials and Methods

Collection of sample

Zea mays and *Sorghum bicolor* seeds were collected from Monsoon seed company, Bengaluru and matured *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* plants were collected from local market, Bengaluru, Karnataka, India.

Preparation of sample

The fresh leaves and stem of *Coriandrum sativum*, *Mentha piperita* and *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 leaf sample in 100 mL of ethanol individually. Magnetic stirrer was used for mixing the plant powder in a solvent for 4 hours and the solution was then filtered using Whatman No. 1 filter paper. The extract was then allowed for evaporation of ethanol 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 leaf and stem samples of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* in 50% of ethanol individually.

Standard antifungal preparation

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

Organisms used

The pure cultures of the fungi were obtained by standard blotter method. Screening of fungi 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 oxysporum* from *Zea mays.*

Preparation of media

Potato dextrose agar media was prepared according to instructions as given on product label. 39 grams of potato dextrose agar media was measured using electronic balance in a conical flask and 1000 ml distilled water was added using measuring cylinder. Amoxicillin – a commercial antibacterial agent, was added to prevent the growth of bacteria. The media was mixed properly with sterile glass rod and sterilized by autoclaving for 15 minutes at 760 mmHg.

Antifungal Activity

Antifungal activity of plant extracts was studied using agar well diffusion technique as chronicled by Perez [12]. Well grown colonies of *Aspergillus fumigatus* from *Sorghum bicolor* and *Aspergillus Niger* and *Fusarium oxysporum* from *Zea mays* were inoculated on potato dextrose agar media and pure culture of *Aspergillus fumigatus*, *Aspergillus Niger* and *Fusarium oxysporum* were obtained by sub culturing 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 x 106 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 µL (2 mg) of plant extracts individually. 20 µL (20µg) of itraconazole was used as positive control and 20 µL of ethanol (50%) was used as negative control. Experiments were performed in triplicates and the results produced are the mean of triplicates. The treated plates with plant extracts and controls 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

Most of the *Sorghum bicolor* and *Zea* mays seeds inoculated on to petri plates gave rise to different fungal colonies. *Rhizopus stolonifer* was most predominant on *Zea mays* followed by *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum* sp., *Penicillium* sp., *Mucor* sp., and *Aspergillus fumigatus* was predominant on *Sorghum bicolor seeds* followed by *Alternaria* sp., *Aspergillus Niger*, *Aspergillus flavus* and *Fusarium oxysporum* sp.,.

Effect of Plant Extracts on *Aspergillus fumigatus*, *Aspergillus Niger* and *Fusarium oxysporum*

This study revealed the antifungal activity of ethanolic extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* against *Aspergillus niger*. *Fusarium oxysporum* was affected by *Coriandrum sativum* but it was not affected by *Mentha piperita* and *Trigonella foenum.* This study also revealed that the ethanolic extracts of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* were not effective in inhibiting the mycelial growth of *Aspergillus fumigatus.* Out of three plants extracts, leaf extracts of *Trigonella foenum* showed better antifungal activity with 8mm zone of inhibition against *Aspergillus niger* followed by leaf and stem extracts of *Coriandrum sativum* which inhibited both *Aspergillus niger* (with 6mm zone of inhibition) and *Fusarium oxysporum* (with leaf extracts showing 3mm zone of inhibition and stem extracts showing 5mm zone of inhibition) followed by stem extracts of *Trigonella foenum* which showed the zone of inhibition of 5mm against *Aspergillus niger* followed by leaf extracts of *Mentha piperita* which showed the maximum inhibition of 3 mm against *Aspergillus niger.*

Moghtader *et al* had studied the antifungal activity of *Mentha piperita* against *Aspergillus Niger*. Results revealed that *Mentha piperita* was effective in inhibiting the mycelial growth of *Aspergillus Niger* [10]. This result was in accordance to our result where ethanolic extracts of leaves of *Mentha piperita* were effective in inhibiting the mycelial growth of *Aspergillus niger*. B. Wojcik-Stopczynska, P. Jakowienko, G. Wysocka *et al* had studied the effect of *Mentha crispa* essential oil and hydrosol on growth of the photypathogenic fungi such as *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Botrytis cinerea*, *Cladosporium herbarum*, *Fusarium oxysporum* and *Penicillium cyclopium*. Results revealed that essential oil of *Mentha crispa* was least effective in inhibiting the mycelial growth of *Fusarium oxysporum* and hydrosol of *Mentha crispa* showed maximum inhibition against *Aspergillus fumigatus* [18]. These results partially supported our result where ethanolic extracts of *Mentha piperita* was not effective in inhibiting the mycelial growth of *Fusarium oxysporum* and at the same time those results were in contrast to our result were ethanolic extracts of leaves of *Mentha piperita* was not effective against *Aspergillus fumigatus.* Krutika Patel and Mita Vakilwala *et al.* had studied the antifungal activity of *Coriandrum sativum* against *Aspergillus niger*. Results revealed that *Coriandrum sativum* was effective in inhibiting the mycelial growth of *Aspergillus niger* [13]. This result was in accordance to our result where ethanolic extracts of leaf and stem of *Coriandrum sativum* were effective in inhibiting the mycelial growth of *Aspergillus niger*. Mohamed, H.A., M. Abdelaziz and R. Yakoub *et al.* had studied the effect of *Coriandrum sativum* extracts on growth of the photypathogenic fungi such as *Fusarium oxysporum*, *Aspergillus* sp., and *Penicillium* sp.,. Results revealed that extracts of *Coriandrum sativum* were effective in inhibiting the mycelial growth of *Fusarium oxysporum* and *Aspergillus* [14]. These results were in accordance to our result where ethanolic extracts of *Coriandrum sativum* were effective in inhibiting the mycelial growth of *Fusarium oxysporum* and *Aspergillus niger*. Sudan, Puneet Goswami, Manish Singh, Jitender *et al*., had studied the efficacy of *Trigonella foenum* against *Microsporum gypseum*. Results revealed that *Trigonella foenum* was effective in inhibiting the growth of *Microsporum gypseum* [16]. 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 oxysporum*. Results revealed that *Trigonella foenum* inhibited the mycelial growth of *Fusarium oxysporum* [17]. This result was in contrast to our result where *Trigonella foenum* was not effective in inhibiting the mycelial growth of *Fusarium oxysporum*. Ali A. S. Al-Mayah *et al* had studied the antifungal activity of *Trigonella foenum* against *Aspergillus fumigatus* [18]. 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 oxysporum* 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 oxysporum*.

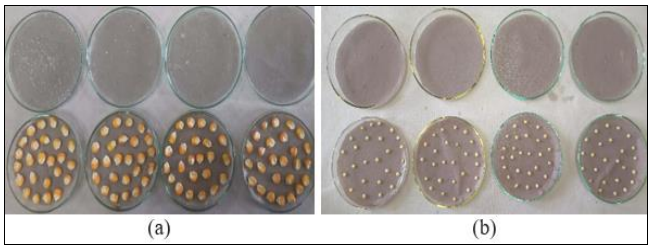

Fig 1: Screening of fungi on *Zea mays* (a) and *Sorghum bicolor* (b)

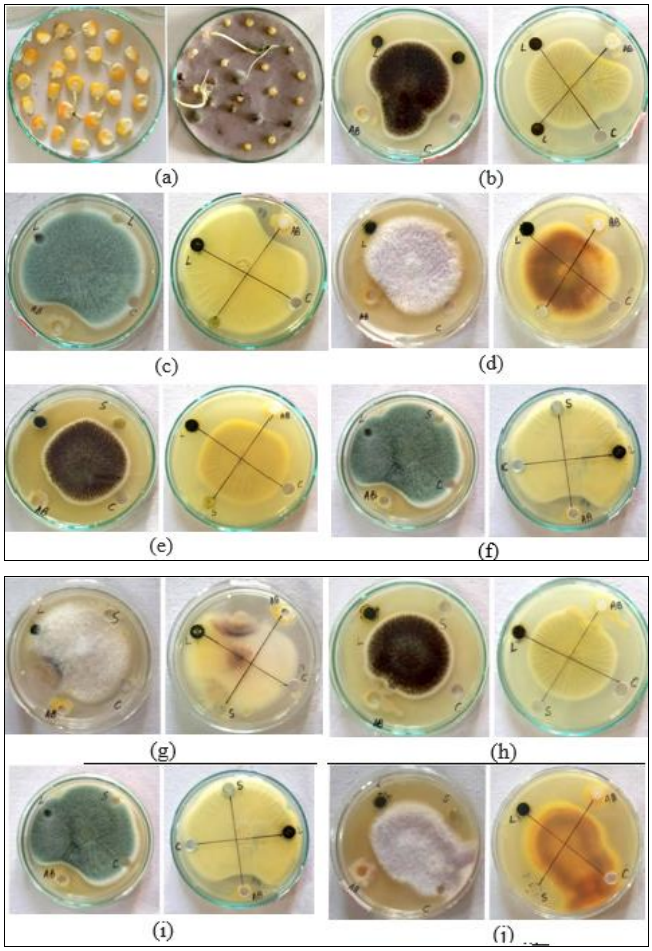

Fig 2: (a) Fungi expressed on *Zea mays* seeds and *Sorghum bicolor* seeds. (b) Inhibitory activity of *Mentha piperita* against *Aspergillus niger*. (c) Inhibitory activity of *Mentha piperita* against *Aspergillus fumigatus.* (d) Inhibitory activity of *Mentha piperita* against *Fusarium oxysporum*. (e) Inhibitory activity of *Trigonella foenum* against *Aspergillus niger*. (f) Inhibitory activity of *Trigonella foenum* against *Aspergillus fumigatus.* (g) Inhibitory activity of *Trigonella foenum* against *Fusarium oxysporum*. (h) Inhibitory activity of *Coriandrum sativum* against *Aspergillus niger*. (i) Inhibitory activity of *Coriandrum sativum* against *Aspergillus fumigatus.* (j) Inhibitory activity of *Coriandrum sativum* against *Fusarium oxysporum*.

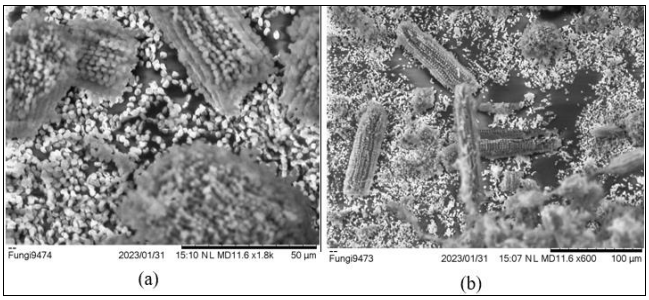

Fig 3: Spores of *Aspergillus fumigatus* under Scanning Electron Microscope (a) magnification 1800x (b) magnification 600x

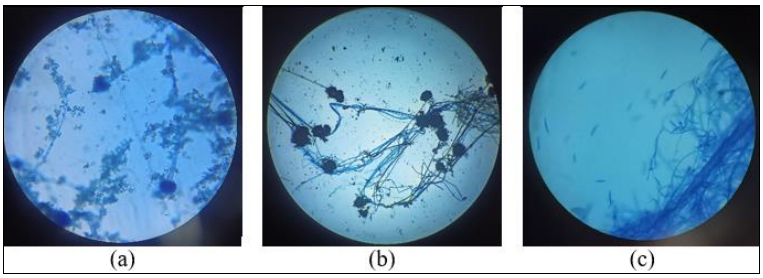

Fig 4: (a) *Aspergillus fumigatus* under binocular microscope (400x) (b) *Aspergillus niger* under binocular microscope (400x) (c) Macroconidia of *Fusarium oxysporum* under binocular microscope (400x)

Table 1: Inhibitory activity of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenm* against *Aspergillus niger* and *Fusarium oxysporum* in milimetre (mm)

Labels on plate	Meaning	Inhibition of *Aspergillus niger* by *C. sativum*	Inhibition of *Aspergillus niger* by *M. piperita*	Inhibition of *Aspergillus niger* by *T. foenum*	Inhibition of Fusarium oxysporum by *C. sativum*
L	Leaf extract	6 mm	2 mm	8 mm	3 mm
S	Stem extract	6 mm	-	3 mm	5 mm
C	50% ethanol	5 mm	5 mm	1 mm	0 mm
AB	Itraconazole	10 mm	10 mm	10 mm	5 mm

Table 2: Inhibitory activity of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenm* against *Aspergillus fumigatus* and *Fusarium oxysporum*

Fungi	Plant extracts	Inhibitory activity
Aspergillus fumigatus	*Coriandrum sativum*, *Mentha piperita* and *Trigonella foenm*	Negative
Fusarium oxysporum	*Mentha piperita* and *Trigonella foenm*	Negative

Conclusion

This study determined the antifungal activity of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* against plant pathogens *Aspergillus niger* and *Fusarium oxysporum.* This study also revealed the ineffectiveness of *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenm* against *Aspergillus fumigatus. Fusarium oxysporum* was inhibited by *Coriandrum sativum* but it was not inhibited by *Mentha piperita* and *Trigonella foenum.* The phytochemicals present in *Coriandrum sativum*, *Mentha piperita* and *Trigonella foenum* exhibited antifungal property against *Aspergillus Niger* presenting it as a potent plant in treatment of plant fungal diseases associated with *Aspergillus niger*. These plant extracts having resistance mechanisms against *Aspergillus Niger* may be useful to control plant diseases such as black mold associated with *Aspergillus niger*. Similarly *Coriandrum sativum* can be used to treat diseases associated with *Fusarium oxysporum.* On the basis of the results obtained during the experiment and reports of success of these plants extracts in controlling plant pathogenic fungi such as *Aspergillus niger* and *Fusarium oxysporum*, the tested plant extracts hold promise for the organic and ecofriendly management of plant diseases associated with *Aspergillus niger* and *Fusarium oxysporum*. 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* and *Fusarium oxysporum*.

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