Evaluation of antifungal efficacy of *Coriandrum sativum*

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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 leaf and stem of *Coriandrum sativum*. Ethanol (50%) was used for extraction of active principles from the dried powdered leaves and stem of *Coriandrum sativum.* The antifungal screening was done with three plant pathogens viz. *Aspergillus fumigatus* associatedwith *Sorghum bicolor, Aspergillus niger*and *Fusarium sp.,* associatedwith*Zea mays.* In agar well diffusion assay *Aspergillus niger*and *Fusarium* was markedly affected by leaf and stem extracts of *Coriander sativum* but it was not effective in inhibiting the growth of *Aspergillus fumigatus.* The results obtained from this study would help in establishing the use of ethanolic extracts of leaf and stem parts of *Coriandrum sativum*to control plant diseases associated with *Aspergillus niger*and *Fusarium* as a safe alternative option to chemical fungicides.

Keywords- Aspergillus fumigatus, Aspergillus niger, Fusarium sp., Sorghum bicolor, Coriandrum sativum, 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]

Aspergillusfumigatus 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. *Aspergillusfumigatus* 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]
Aspergillusniger is a fungus classified within the Nigri section of the *Aspergillus* genus. *AspergillusNiger* 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. *AspergillusNiger* 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. *AspergillusNiger* 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 fumonisinsand trichothecenes. Inspite of most species apparently being harmless, some *Fusarium* species and subspecific groups are among the most important fungal pathogens of plants and animals.[9]

Coriandrum sativum is a member of Apiaceae family. Coriander is native to Southern Europe, Northern Africa, Southwestern Asia. It is a soft plant which grows upto50 cm. The shape of leaves vary from broadly lobed at the base of the plant to slender and feathery higher on the flowering stems.[10] It is widely recognized for its comestible uses and as traditional medicine.[11] Due to the presence of a huge number of bioactives, a broad range of pharmacological activities have been assigned to different parts of this herb, which include anti-microbial, anti-oxidant, anti-diabetic, anxiolytic, anti-epileptic, anti-depressant, anti-mutagenic, anti-inflammatory, anti-dyslipidemic, anti-hypertensive, neuro-protective and diuretic.[12] The different parts of this plant contain borneol, citronellol, monoterpenes, limpnene, α-pinene, γ-terpinene, p-cymene, camphor, coriandrin, geraniol, dihydrocoriandrin,flavonoids and essential oils.[13] There are many studies which have documented the antifungal properties of *Coriandrum sativum* against pathogenic fungi.[15,16] Similarly in the present study we aimed to determine the antifungal

potential of ethanolic extracts of *Coriandrum sativum*on *Aspergillusfumigatus*, *Aspergillusniger* and *Fusarium* sp., by agar well diffusion assay as described by Perez[14]

MATERIALS AND METHODS

Collection of sample

Matured *Coriandrum sativum* plantswere collected from local market, Bengaluru, Karnataka, India.

Preparation of sample

The fresh leaves and stem of *Coriandrum sativum* 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 *Sorghumbicolor* and *Zeamays* seeds followed by isolation of *Aspergillus fumigatus* from *Sorghum bicolor, Aspergillus niger*and *Fusarium* sp., from *Zeamays.*

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.[14] Well grown colonies of *Aspergillusfumigatus* from *Sorghumbicolor* and *Aspergillusniger* and *Fusarium* sp., from *Zeamays* were inoculated on potato dextrose agar and pure culture of *Aspergillusfumigatus*, *Aspergillusniger* 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 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 leaf and stem extracts individually. 20 µL (20µg) of itraconazole a commercial antifungal compound was used as reference standard and 20 µL 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. *Rhizopusstolonifer* was most predominant on *Zea mays* followed by *Aspergillusniger*, *Aspergillusfumigatus*, *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*

This study revealed the antifungal activity of leaf and stem extracts of *Coriandrum sativum* against *Aspergillus niger*and *Fusarium.* It also revealed that ethanol extracts of *Coriandrumsativum* was not effective against *Aspergillus fumigatus.* Leaf extracts of *Coriandrum sativum* showed the maximum inhibition of 6 mm against *Aspergillusniger*and 3 mm against *Fusarium* followed by stem extracts which showed the maximum inhibition of 6 mm against *Aspergillusniger* and 5 mm against *Fusarium.* Itraconazole showed the inhibition of 12 mm against *Aspergillusfumigatus*, 10 mm against *Aspergillusniger* and 5 mm against *Fusarium* while control didn'tinhibited *Aspergillus fumigatus* and *Fusarium* but inhibited *Aspergillus niger*(5mm). Phytochemicals present in *Coriandrum sativum* were effective against the growth of *Aspergillusniger* and *Fusarium* but *Aspergillus fumigatus* has become resistant to those phytochemicals as a result *Coriandrum sativum* was not effective in

inhibiting the mycelial growth of *Aspergillusfumigatus*.Krutika Patel and MitaVakilwala 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*.[15] This result was in accordance to our result were 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 *Coriandrumsativum* extracts on growth of the photypathogenic fungi such as *Fusariumoxysporum*, *Aspergillus* sp., and *Penicillium* sp.,. Results revealed that extracts of *Coriandrumsativum* were effective in inhibiting the mycelial growth of *Fusarium* and *Aspergillus*.[16] These results were in accordance to our result were ethanolic extracts of *Coriandrum sativum* were effective in inhibiting the mycelial growth of *Fusarium* and *Aspergillus niger*.

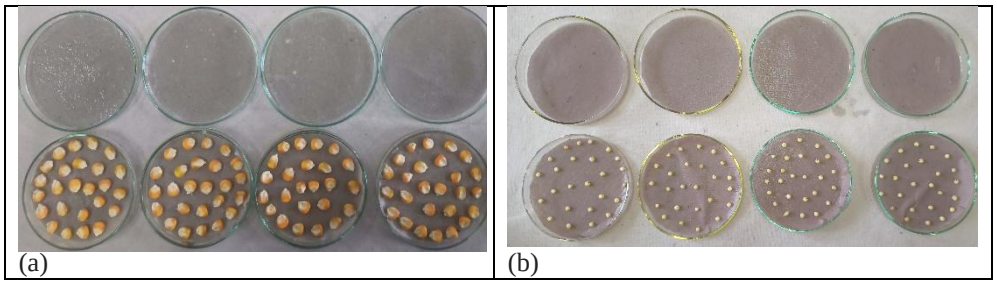

Fig1: Screening of fungi on *Zeamays* (a) and *Sorghumbicolor*(b)

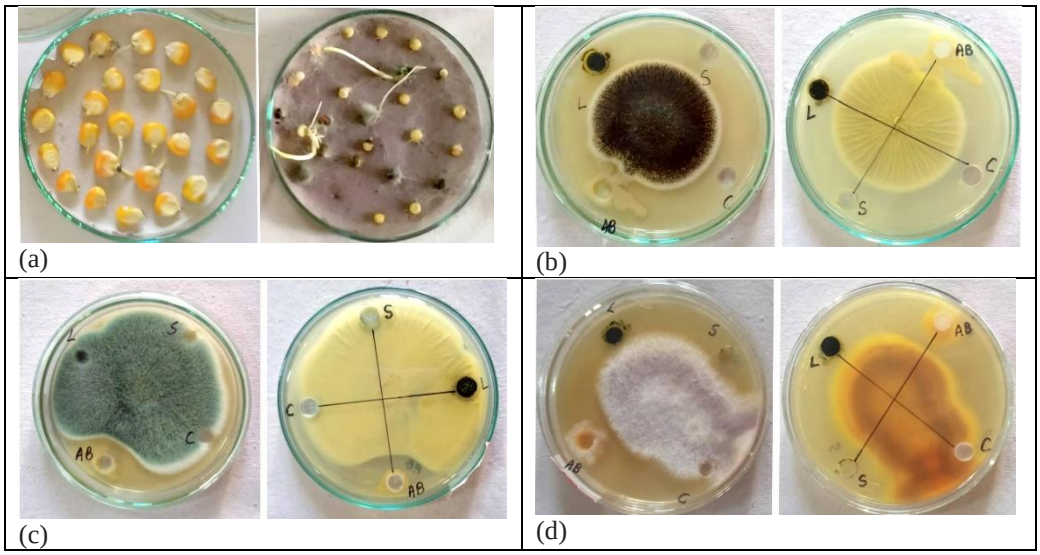

Fig2: (a) Fungi expressed on *Zeamays* seeds and *Sorghumbicolor* seeds.
(b) Inhibitory activity of *Coriandrum sativum* against *Aspergillusniger*.
(c) Inhibitory activity of *Coriandrum sativum*against *Aspergillusfumigatus*.
(d) Inhibitory activity of *Coriandrum sativum* against *Fusarium*.

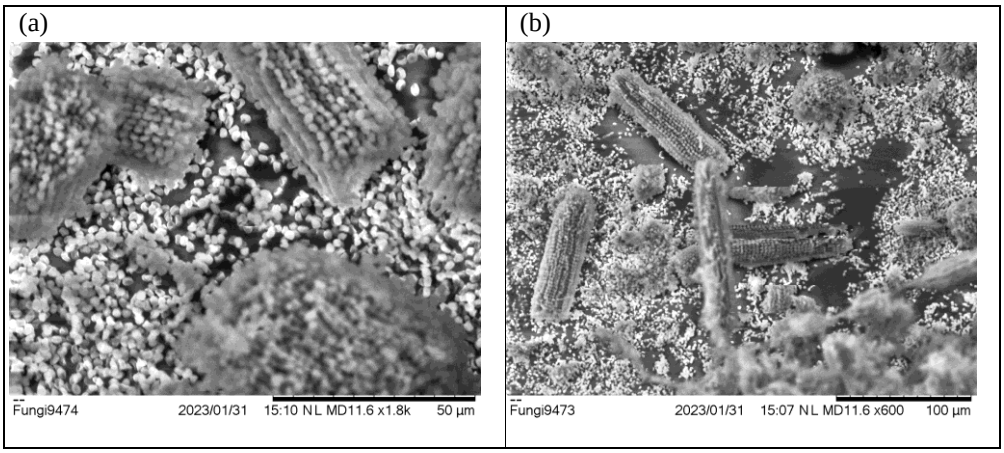

Fig 3: Spores of *Aspergillus fumigatus* under Scanning Electron Microscope (a) magnification 1800x (b) magnification 600x

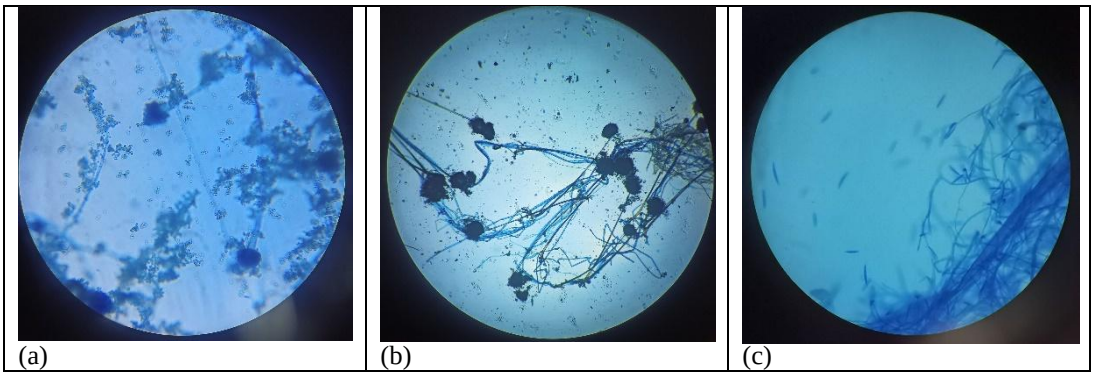

Fig 4: (a)*Aspergillusfumigatus* under binocular microscope (400x)
(b) *Aspergillusniger* under binocular microscope (400x)
(c) Macroconidia of *Fusarium* under binocular microscope (400x)

Table1: Inhibitory activity of *Coriandrum sativum* against *Aspergillusniger* and *Fusarium*

Labels on plate	**Meaning**	**Inhibition of *Aspergillus niger* (mm)**	**Inhibition of *Fusarium* (mm)**
L	Leaf extract	6 mm	3 mm
S	Stem extract	6 mm	5 mm
C	50% ethanol	5 mm	0 mm
AB	Itraconazole	10 mm	5 mm

Table2: Inhibitory activity of *Coriandrum sativum* against *Aspergillusfumigatus*.

Aspergillusfumigatus	Negative inhibition

CONCLUSION

This study determined the antifungal activity of *Coriandrum sativum* against plant pathogen *Aspergillus niger*and *Fusarium.* This study also revealed the ineffectiveness of *Coriandrum sativum* against *Aspergillus fumigatus.* The phytochemicals present in leaf and stem of *Coriandrum sativum* exhibited antifungal property presenting it as a potent plant in treatment of plant fungal diseases associated with *Aspergillus niger*and *Fusarium. Coriandrum sativum* plant extracts having resistance mechanisms against *Aspergillus niger*and *Fusarium* 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 *Coriandrum sativum* extracts in controlling plant pathogenic fungi such as *Aspergillus niger*and *Fusarium*, 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* and *Fusarium.*

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