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Antifungal Activity Of Some Spice Extracts On Indoor Dematiaceous Fungi Of Kanpur

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Abstract

Piper nigrum Linn. (Black pepper) *Syzygium aromaticum* Linn. (Clove) spices have been used from mostly countries to aromas and flavor of our food. Spices are effective against some fungi and bacteria that could infect human beings. Aqueous extract of the black pepper and clove was diluted with sterile water to prepared at five different concentrations of 0.25%, 0.5%, 0.75%, and 1.0%. To evaluate the antifungal activities of the extracts were tested on SDA (Sabourauds Dextrose Agar) medium by inoculating isolated dematiaceous fungi *Chaetomium globosum* and *Chrsosporium tropicum* respectively.

The growth of the dematiaceous fungal genera was affected due to the presence of spice extracts. The effectiveness of the extracts increased with increasing the concentration in the SDA medium. A lower concentration of spice extracts showed less antifungal activity.

The aim of this research is to access the study of antifungal activity of *Piper nigrum* (Black pepper) *Syzygium aromaticum* (Clove) extracts on the growth of pathogenic fungi *Chaetomium globosum* and *Chrysosporium tropicum*.

Keywords: antifungal, aqueous, extract, dematiaceous, spice, pathogen.

Introduction

The dematiaceous fungi have a large heterogeneous group of microorganisms that are characterized by a light to dark color pigmentation in their hyphae or conidia.

These fungi are widely distributed among the Ascomycetes, Basidiomycetes, and Zygomycetes. Most strains of these molds are pathogenic and cause the disease to plants, animals, and human beings. The taxa with the largest number of plant pathogens are the class of Deuteromycetes and Ascomycetes of kingdom fungi. Mostly species of dematiaceous fungi cause morbidity and mortality in expanding Immuno-comprised patient population.

Spices have been used in mostly countries to aromas and flavor of our food, According to many researchers, besides these benefits, spices also have antitoxigenic activities, antioxidant, and antifungal effects. As our ancestors have identified the utilization of spices in food preservation and cure of clinical infections and there are many reports on development of antibiotic resistance in various bacterial

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infecions (Mellering and Gold, 1996). This shift in susceptibility of microbes to antibiotics highly affects its ability to successfully cure patient observations. Plant originated products have been used for medicinal objectives since several years. The spices have a unique flavour and aroma which are originated from compounds known as secondary metabolites or phytochemicals (Avato *et al*., 2002).

The secondary metabolites are antimicrobial compounds present in the spices which have capability to attract benefits and repel harmful micro organisms, they also treat as photoprotectants and respond to environmental changes of secondary metabolites including the anthocyanins, isoflavones and flavonids are occurs associated with the spices (Butt. and Chang 1988). According many researchers spices also have Antifungal, antioxidant, medicinal, and antitoxigenic activities (Bullerman, 1974; Bullerman *et al*. 1977; Arun sharma *et al*. 1979; Dewit *et al.* 1979; Hitokoto *et al.* 1980; Azzous and Bullerman, 1982; Faraz *et al.* 1989; Shelef 1983; 1989; Chalfoun *et al*. 2004). The antifungal properties of spices have been reported mainly in relation to their inhibition influence on the infective microorganism.

In vitro studies in this work showed that the spice extracts inhibit fungal growth but this effectiveness varied. The aim of this research was to study the antifungal activity of pepper and clove extract against indoor dematiaceous fungi *Chaetomium globosum* and *Chrysosporium tropicum.*

MATERIAL METHODS:

Plant Collection

Spice *Piper nigrum* (black pepper) and *Syzygium aromaticum* (Clove) was selected for the study. The fresh spices samples are were bought from local market. The spices were cleaned and washed in tap water and air dried at room temperature and powdered by using the grinder.

Spice Extract Preparation

The collected black pepper and Clove were firstly washed and then sterilized with distilled water. 10 gram of powdered spices were weighed and mixed with 100 ml distilled water in conical flasks and kept in rotatory shaker at 160 rpm for 24 hours. After 24 hours it was filtered

with Whattman (No.1) filter paper. The filtrates were evaporated in a hot air oven at 40^{o}C until dry. Each extract was filtered with two layer of muslin cloth and then finally filter with Whattman filter paper (No.1). The clear extract was collected and considered as 100 percentage basic stock. The inhibitory effect of powdered spices in the concentration of 0.25%, 0.5%, 0.75%, and 1.0% was used and mix in Sabouraud's dextrose agar medium and sterilized.

Sterilization of Materials

All glassware used in this test was washed and sterilized in microwave one at 100-120℃ for 3 hours. The SDA medium was sterilized by autoclaving at a temperature of 121℃ for 20 min. at 15 lbs pressure. The cork borer was sterilized by dipping them into 90% Alcohol.

Inoculation

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The sterilized SDA medium was placed in petridishes. Petridish were inoculated by a disc of (6mm) obtained from the periphery of 8-10 days old colony of the isolated both fungus that previously grown on Sabouraud's dextrose agar medium and then incubated at 28±2°C temperature for 8 days. The antifungal effect of pepper and clove extracts and growth of *Chaetomium globosum* and *Chrysosporium tropicum* were observed regularly for each fungus after 10 days of the incubation period. All the experiments were performed in triplicate and values expressed as mean ± standard deviation.

The Sabouraud's dextrose agar medium without any extracts served as control and percent inhibition was calculated by the following formula:

$$\%\ inhibition = \frac{control - treated}{control} \times 100$$

RESULT AND DISCUSSION:

In this experiment both spice extracts were more or less inhibitory to mycelial growth of the isolated fungus. Data are presented in Table (1), and fig (1),(2). Presence of spice extracts affected the normal growth of test fungus.

The results of the inhibition of fungal mycelial growth of *Chaetomium globosum* and *Chrysosporium tropicum* by powdered spices are showed in Table (23), Fig (27, 28). Occurrence of spices affected the normal mycelial growth of isolated fungus. The effectiveness of the spices increased with an increase in concentration and maximum inhibition was observed at (1.0 %). Lower concentration of Black pepper (0.25%) caused 76.20 % inhibition in *C. tropicum* and 85.81 % in *C. globosum* however higher concentration of Black pepper (1.0%) caused 100 % inhibition in *C. tropicum* and *C. globosum*. Inhibition of mycelial growth progressively increased with increase in concentration of the spices at the end of incubation period. It was observed that Clove promoted total inhibition 100% of colony growth of *C. globosum* and *C. tropicum* in all concentrations (0.25%, 0.5%, 0.75%, and 1.0%) tested.

Table 1: Effect of spice extracts on the growth of dematiaceous fungi

Spices	Conc. (%)	Growth of dematiaceous fungi (mm) in different concentration of spices			
		Chrysosporium tropicum		*Chaetomium globosum*	
		mean± $s.d$	% inhibition	mean ±$s.d$	% inhibition
Black pepper	0.25	11.33±0.83	78.62	7.33±2.16	87.98
	0.50	10.33±1.52	80.51	4.00±1.00	93.44
	0.75	8.00±1.00	84.91	0.00±0.00	100.00
	1.00	0.00±0.00	100.00	0.00±0.00	100.00
Clove	0.25	0.00±0.00	100.00	3.00±0.60	95.08
	0.50	0.00±0.00	100.00	0.00±0.00	100.00
	0.75	0.00±0.00	100.00	0.00±0.00	100.00
	1.00	0.00±0.00	100.00	0.00±0.00	100.00
Control		53.00±0.00	0.00	61.00±0.00	0.00

S.d - Standard Deviation of means

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Effect of spices on the growth of dematiaeous fungi

Fig: 1

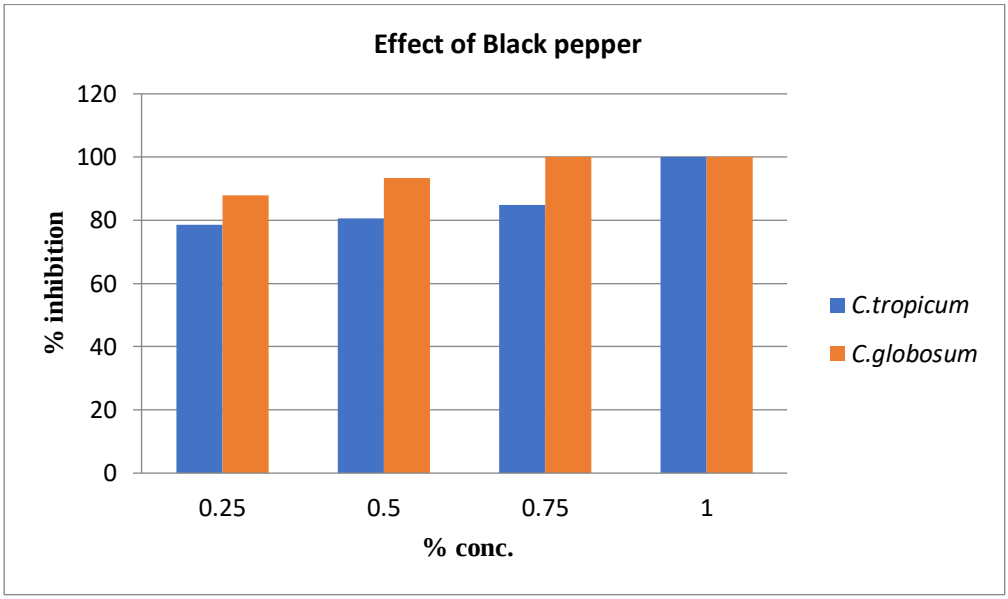
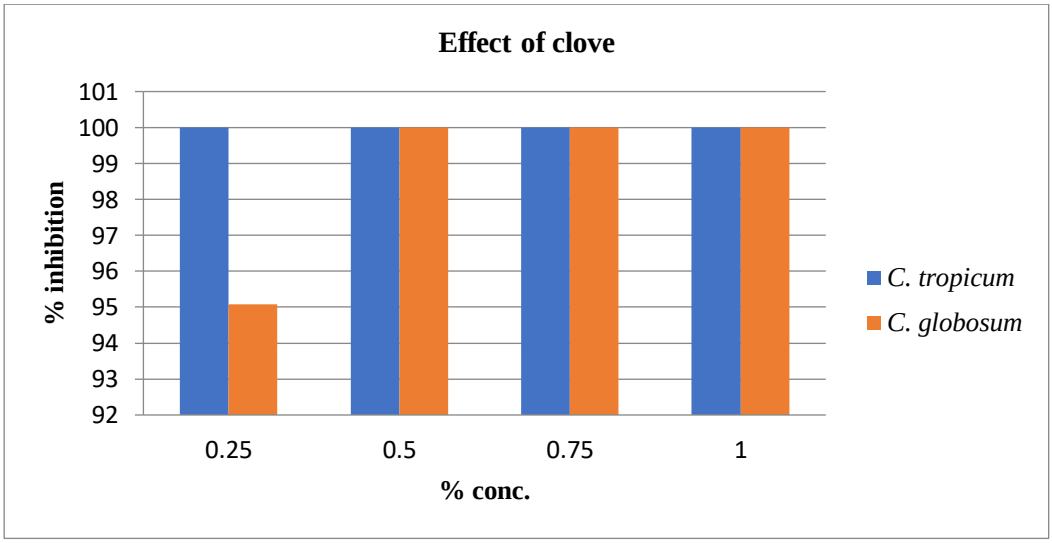


Fig: 2



CONCLUSION:

The selected spices in this study consist of many useful phyto-compounds having important biological properties. The result of this study would lead to find out inhibitory effect of spice extract.

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