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Antifungal activity of plant products against *Aspergillus niger*: A potential application in the control of a spoilage fungus

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Abstract : Eight commonly used spices *Syzygium aromaticum*, *Cinnamonum zeylanicum*, *Zingiber officinale*, *Murraya koenigii*, *Piper nigrum*, *Trachyspermum ammi*, *Allium sativum* and *Allium cepa* were tested for *in vitro* antifungal activity on *Aspergillus niger*, a causative agent of different destructive disease. Out of eight plant materials used, five showed significant antifungal activity against the test pathogen by poisoned food technique. *Syzygium aromaticum* and *Allium sativum* showed 100% inhibition of mycelial growth at 20% concentration. Results of the present investigation indicates that spices possess antifungal activity and can be exploited as natural fungitoxicant to control the growth of storage or spoilage fungi, *A. niger* and thus reduce the dependence on the synthetic fungicides.

Keywords : *A. niger*, Antifungal activity, aqueous extracts, spices

INTRODUCTION

Fungal contamination of stored commodities is a very serious problem in tropical warm regions of the world. Contamination by storage fungi and their mycotoxins is of great concern in herbal drug and food industry. Fungi, especially the species of *Aspergillus* and *Penicillium* are among the major reported genera having the ability to produce mycotoxins during storage (Gautam and Bhadauria, 2008; 2009). These fungi producing related mycotoxins reduce the quality of food products and the medicinal potential of herbal drugs. In previous reports, *A. niger* (commonly known as black *Aspergilli*), was recorded as a most dominating fungal species to be associated with herbal drugs during storage (Bugno *et. al.*, 2006, Gautam and Bhadauria, 2008; 2009). *A. niger* is a saprophytic and filamentous fungus found in soil, forage, organic debris and food product, causing black mould of onion, Shallot; stem rot of *Dracaena*; root stalk rot of Sansevieria; and boll rot of Cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune (Bobbarala *et. al*., 2009).

The use of chemical pesticides is a very popular practice to control various plant diseases management as compare to natural one which are prepared from plants or plant parts. But, consumer now demands less use of synthetic fungicides due to the non-biodegradability, pollutive nature and residual toxicities of chemical pesticides. Several studies have revealed the plant extracts as source of natural pesticides that make excellent efforts for new pesticide development (Arokiyaraj *et. al*., 2008; Gangadevi *et. al*., 2008; Brindha *et. al*., 2009).

Since, many spices and herbs have been used for centuries as preservatives for foods and medicinal purposes, some of them possess antimicrobial potential in combination and is considered as alternatives to conventional antimicrobial agents especially in this era of antimicrobial drug resistance (Nwaopara *et. al.*, 2009).

The preservative actions of herbs and spices have received much attention in the literature where studies have been reported and showed that mycotoxin-producing molds may be inhibited by some herbs and spices. They generally produce many secondary metabolites such as alkaloid, flavonoids, tannins and phenolic compounds which are the important sources of microbicides, pesticides and many pharmaceutical drugs (Mahesh and Satish, 2008). Therefore, the main objective of this study was to evaluate the antifungal efficacy of commonly used spices against the growth of *A. niger*.

MATERIAL AND METHODS

A. Plant material

In the present study, eight commonly used spices of different families have been selected to analyze their effectiveness on the growth of *A. niger* Table 1.

Table 1 : List of plant species tested for antifungal activity.

S.No.	Plant Name	Part used	Family
1.	*Syzygium aromaticum* (L.) Merrill & Perry	Flower bud	Myrtaceae
2.	*Cinnamomum zeylanicum* Blume	Bark	Lauraceae
3.	*Zingiber officinale* Rosaceae	Rhizome	Zingiberaceae
4.	*Murraya koenigii* Spreng.	Leaf	Rutaceae
5.	*Piper nigrum* (L.)	Seed	Piperaceae
6.	*Trachyspermum ammi.* (L.)Spragne	Seed	Umbelliferae
7.	*Allium sativum* (L.)	Bulb	Liliaceae
8.	*Allium cepa* (L.)	Bulb	Liliaceae

B. Test Organism

The test organism *i.e.*, *A. niger* (#7414.09, confirmed at IARI, New Delhi), was obtained from mycology and plant pathology laboratory, School of Studies in Botany, Jiwaji University Gwalior, Madhya Pradesh, India.

C. Screening of spices for their antifungal activity

For the preparation of aqueous extracts, 10g of each dried sample was grinded into a fine powder with 100 ml sterile distilled water and left for overnight (24 hours) at room temperature (30 ± 2°C). The content of the flask was then filtered through filter paper to obtain clear infusion in laminar air flow (Chaudhary and Tariq, 2006). Poisoned food technique was used for the evaluation of antifungal potential (New, 1971). Different concentrations *i.e.*, 10%, 15% and 20% of the aqueous extracts were added in Czapek Dox Agar (CDA) media and media without plant extract served as control. Five replicates of each concentration were maintained. Five mm disc of 3-4 days old culture of the test fungi (*A. niger*) was placed at the centre of each petriplates. The plates were incubated upside down at 37°C for five days and diameter of colony in each case was measured at 3rd, 4th and 5th day. All the results were analysed statistically.

RESULTS AND DISCUSSION

Eight commonly used spices were screened in the present investigation for their antifungal activities against the growth of *A. niger* using poisoned food method. A significant variability was observed in efficacy of all aqueous extracts against the growth of *A. niger*. The antifungal activity of aqueous extracts of all spices is depicted in Table 2. The growth reduction in percentage was taken into consideration and antifungal effect was evaluated. An enhancement in antifungal activity against *A. niger* was observed, with increase in concentration of aqueous extracts. Maximum percentage inhibition was detected at 20% concentration.

Table 2 : Percent inhibition of mycelial growth of *Aspergillus niger.*

Plant extracts	Concentrations (%)	Percent Inhibition			
		3rd day	4th day	5th day	Mean ± SD
Syzygium aromaticum	10	20	27.8	31.3	26.36 ± 5.78
	15	51.6	62.6	64	59.4 ± 6.79
	20	100	100	100	100 ± 0.0
Cinnamomum zeylanicum	10	15.2	21.4	26.8	21.3 ± 5.80
	15	23.5	28.5	34.2	28.73 ± 5.53
	20	49.4	52.1	55.7	52.4 ± 3.16
Pipper nigrum	10	16.25	23.2	29.1	22.85 ± 6.43
	15	31.25	35.2	40	35.48 ± 4.38
	20	42.5	46.4	49.7	46.2 ± 3.60
Trachyspermum ammi	10	18.1	27.7	37.5	27.76 ± 9.70
	15	36.3	42.1	50	42.8 ± 6.87
	20	45.4	47.3	54.1	48.93 ± 4.57
Allium sativum	10	25	36.3	40.6	33.96 ± 8.05
	15	50	54.5	62.5	55.66 ± 6.33
	20	100	100	100	100 ± 0.00
Murraya koenigii	10	NE	NE	NE	NE
	15	NE	NE	NE	NE
	20	NE	NE	NE	NE
Zingiber officinale	10	NE	NE	NE	NE
	15	NE	NE	NE	NE
	20	NE	NE	NE	NE
Allium cepa	10	NE	NE	NE	NE
	15	NE	NE	NE	NE
	20	NE	NE	NE	NE

NE-Not effective

Although eight spices were screened in present investigation for their antifungal activity, aqueous extracts of only five namely, *Syzygium aromaticum*, *Allium sativum*, *Cinnamomum zeylanicum*, *Trachyspermum ammi* and *Piper nigrum* were found inhibitory against the *A. niger*. *Syzygium aromaticum* and *Allium sativum* showed complete inhibition

(100%) at 20% conccentration Fig.1. In *Cinnamomum zeylanicum*, *Trachyspermum ammi* and *Piper nigrum,* a moderate inhibition (52.4%, 48.93%, and 46.2%) was recorded whereas, no inhibition was in other plant products. Eugenol in clove and cimnamelaldehyde in cinnamon is reported as the main active compound, which might be responsible for their antifungal potential against *A. niger* in present study (Bullarmann, *et. al.*, 1977).

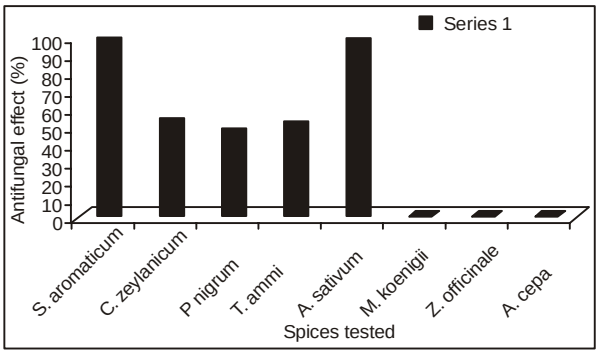

Fig.1. Antifungal effect of spices on mycelial inhibition of Aspergillus niger.

The antifungal effect of *S. aromaticum* and *C. zeylanicum* found on *Aspergillus* spp. and *Penicillium* spp. was also reported earlier (Garg *et. al*., 1992; Tewari and Dixit, 1994; Vazquez *et. al*., 2001). Presence of ajoene and alliicin in *A. sativum* might be the reason for their complete inhibition of *A. niger* (Yoshida *et. al*, 1987; Naganawa *et. al*., 1996). A strong inhibition was observed in case of *T. ammi* (55.7%) and *C. zeylanicum* (54.1%) whereas a moderate inhibition was recorded in black pepper (49.7%). This variability in antifungal potential in plant materials may be firstly due to the difference in the chemical compositions and secondly their solubility in water. This also in agreement with the reports of Qasem and Abu-Blan (1996) and Amadioha (2000). The aqueous extracts of other spices like *Murraya koenigii*, *Zingiber officinale*, and *Allium cepa* were not proved to be effective against the growth of *A. niger*. The ineffectiveness of these spices on *A. niger* might be due to insolubility of their active compounds in water (Qasem *et al*, 1996; Amadioha, 2000).

Because the aqueous extracts of *Syzygium aromaticum* (clove), *Allium sativum* (garlic), *Cinnamomum zeylanicum* (cinnamon), *Pipper nigrum* (black pepper) and *Trachyspermum ammi* (ajwoin) is found effective against the growth of test organism. Therefore, this study suggests that aqueous extracts of these spices would be helpful in treating diseases in plants caused by *A. niger*. In conclusion, the findings of this experiment confirmed that plant extracts can be used as natural fungitoxicant to control the growth of pathogenic fungi (*A. niger*) and thus reduce the dependence on the synthetic fungicides.

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