J. Asiat. Soc. Bangladesh, Sci. 40(2): 179-186, December 2014

FUNGI ASSOCIATED WITH COMMON SPICES IN BANGLADESH

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Abstract

Association of fungi in chili (*Capsicum fruticance* L.), coriander (*Coriandrum sativum* L.) and turmeric (*Curcuma longa* L.) was investigated. A total of 19 species of fungi under ten genera and one sterile mycelial fungus was isolated from the three spices. Out of ten genera three belong to Phycomycetes, one genus belongs to Ascomycetes and rest belongs to Deuteromycetes. The most frequent contaminants of the spices were *Aspergillus niger* van Tieghem, *A. flavus* Link, *Fusarium nivale*, *Pestalotia* sp. and *Rhizopus* sp. Dried fruits of the spices showed maximum number of fungal association in comparison with the respective commercial brand powder samples. Out of three plant extracts, *A. sativum* was found to inhibit the growth of all the test isolates at all concentrations.

Key words: Fungi, Spices, Bangladesh

Introduction

Fungal contamination of spices may occur when they are not properly dried or stored in a highly humid environment. The presence of fungi in spices with formation of noxious orders and other adverse effects increases the risks for mycotoxin formation under favourable condition which is harmful for consumers. Many researches has been carried out in abroad about fungal contaminants of spices (Ahene *et al.* 2011, Imandel and Adibenia 2000 and Stakvileviciene 2003). So far no report is available in this respect from Bangladesh.

Plant parts and their constituents of some higher plants have already been reported to be successful as fungitoxicants (Panday *et al.* 1983). Fungitoxicity of *Allium sativum* has been reported by Misra and Dixit (1977) and Ahmed and Sultana (1984). Application of plant extracts which are easily available for controlling plant diseases are non pollutive, cost effective and non hazards. On account of their non phytotoxicity and biodegradability (Fawcett and Spencer 1970) and renewable nature, such substances appear to be the ideal antifungal agents.

Considering the importance of the spices in our daily life and lack of information about the association of fungi with spices in Bangladesh, the present investigation was undertaken to detect the fungi associated with the dried fruits and dry powders of three common spices used in Bangladesh and their *in vitro* management.

Materials and Methods

The materials of this investigation were the powdered spices of three different companies (Pran, Radhuni and Fresh brands) and ungrined whole of chili, coriander and turmeric, respectively. Samples were collected from Tongi market. Forty five samples of three spices were collected during the period of November, 2011 to July, 2012 and stored at room temperature (19-30^{0}C) and humidity was 60-65% before these were used for assay.

The fungi associated with the collected samples were isolated following "Tissue Planting Method"(CAB 1968). In case of intact chili and turmeric, 2^2 mm size of inocula were used. In case of coriander single fruit was used as an inoculum. Inocula were washed with sterilized distilled water separately within the sterilized Petri plates. One g brand powder of each sample was taken in sterilized Petri plate and mixed with one ml sterilized water to make a paste. One loop full of (2mm dia.) inocula was transferred on PDA medium at three points. Inoculated plates were incubated for 5 days at 25-28^0 C. Percent frequency of the occurrence of the fungal isolates were calculated by adopting the formula of Spurr and Welty (1972).

Identification of the fungi was made following the standard literature (Gilman 1967, Subramanian 1971, Barnett and Hunter 1972, Booth 1971, Ellis 1971, Ellis 1976, Thom and Raper 1945, Raper *et al.* 1949 and Sutton 1980).

Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysporum, *Curvularia lunata* and *Rhizopus* sp. isolated from spices were selected as the test fungi. Bulbs of *Allium cepa* and *A. sativum* and tubers of *Zingiber officinale* were selected for screening of antifungal activity against the selected fungi. Different concentrations i.e, 5, 10, 15 and 20% plant extracts were used in this study.

The toxicity of the crude plant extract was determined against the test fungi following poisoned food technique (Bashar and Raj 1991). The per cent inhibition of mycelial growth over control was calculated using the following formula:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment.

Results and Discussion

Three common spices such as coriander, chili and turmeric powders of Fresh, Pran and Rhaduni brand companies were tested for the detection of fungi. The dried spices and powders of the brand companies and the association of fungi with the powders are presented in Plate 1.

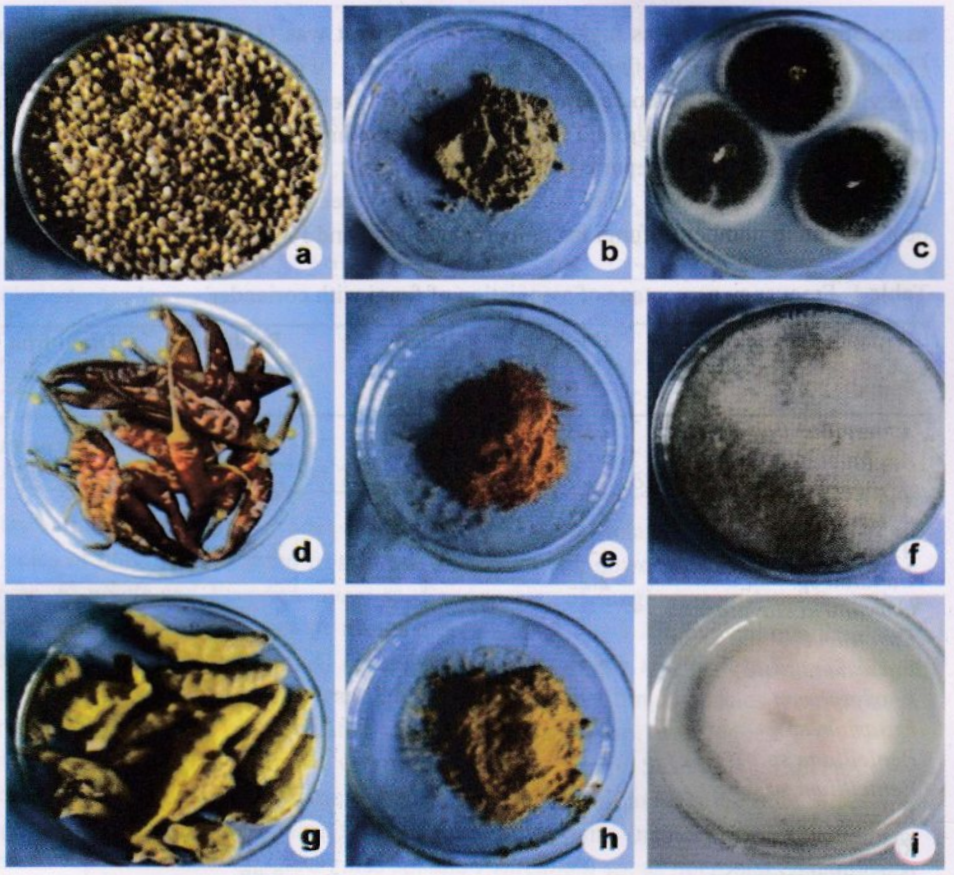

Plate 1. Different spices and fungi associated with them. a-b: Fresh fruits and powders of coriander and c shows the growth of *Aspergillus niger*. d-e: Fresh fruits and powders of chili and f shows the growth of *Rhizopus* sp. g-h: Fresh fruits and powders of turmeric and i shows the growth of *Fusarium nivale*.

A total of 16 species of fungi belonging to eight genera and one sterile mycelium was isolated from coriander (Table 1). Seven species of fungi were associated with Fresh brand coriander powder. Frequency percentage of association of *Aspergillus niger* was the highest (15.55) and the lowest (2.22) was in *Candida* sp., *F. nivale*, *Fusarium* sp. 1 and *Penicillium* sp.1. Three species of fungi were isolated from Pran brand coriander powder. Frequency percentage of association of *A. niger* was highest (86.67) and lowest(8.89) was in *A. flavus*. Radhuni brand power showed eleven species of fungi. In this case *Pestalotia* sp. showed highest frequency percentage (33.34) and *A. versicolor* showed lowest frequency percentage (2.22). Ten species of fungi and one sterile mycelium were associated with fruits of coriander of which frequency percentage of

association of *A. niger* was highest (40.75) and lowest was (1.27) in Sterile mycelium (Table 1). The present findings are in agreement with the previous reports of Stakvileviciene (2003) who reported a total of 23 species of parasitic and saprophytic micro fungi on the above ground parts of coriander. Similar observation was noticed by Ahene *et al.* (2011) who reported presence of *A. flavus*, *Eurotium* , *Fusarium*, *Rhizopus* and *Penicillium* in case of other spices. Out of the three brands Radhuni was found to bear maximum number of fungi in comparison to Pran and Fresh brands.

Table 1. Frequency percentage of association of fungi with coriander.

Name of fungi	Fresh	Brand powder Pran	Radhuni	Dried fruit
Aspergillus flavus	13.33	8.89	2.23	12.35
A. glaucus	-	-	-	3.71
A. niger	15.55	86.67	20.00	40.75
A. terreus	4.44	-	-	-
A. versicolor	-	-	2.22	-
Candida sp.	2.22	-	-	-
Curvularia clavata	-	-	-	3.71
Eurotium sp.	-	-	2.23	-
Fusarium nivale	2.22	13.33	33.33	4.94
F. oxysporum	-	-	-	18.52
Fusarium $sp._1$	2.22	-	6.67	8.65
Penicillium $sp._1$	2.22	-	13.33	8.65
Penicillium $sp._2$	-	-	2.23	-
Pestalotia sp.	-	-	33.34	28.89
Rhizopus sp.			20.00	2.47
Syncephalastrum sp.	-	-	8.88	-
Sterile Mycelia 1				1.27

Eleven species of fungi were isolated from chili of which two species of fungi were recorded from Fresh brand powder. Results presented in Table 2 show that frequency percentage of association was highest in *Rhizopus* sp. (97.77) and lowest was (2.23) in *A. niger*. Six species of fungi were isolated from Pran brand powder. Frequency percentage of association of *A. niger* was highest (53.34) and lowest was (2.23) in *Rhizopus* sp. From Radhuni brand powder two species of fungi were isolated of which frequency percentage of association was highest in *Rhizopus* sp. (62.23) and lowest in *A, niger* (20). Eleven fungal species were isolated from chilli fruits. *Apergillus niger* was the predominating (48.15) fungus while *A. glaucus* and *Rhizopus* sp. were the least (1.24) counted fungi.

Table 2. Percentage frequency of association of fungi with chilli.

Name of fungi	Fresh	Brand powder Pran	Radhuni	Dried fruit
Aspergillus flavus	-	26.68	-	13.58
A. fumigatus	-	-	-	4.94
A. glaucus	-	-	-	1.24
A. niger	2.23	53.34	20.00	48.15
Curvularia clavata	-	-	-	11.12
Fusarium nivale	-	11.12	-	1.24
F. oxysporum	-	-	-	6.18
Fusarium sp. $_1$	-	13.33	-	3.71
Penicillium sp.$_1$	-	8.90	-	3.71
Penicillium sp.$_2$	-	-	-	4.94
Rhizopus sp.	97.77	2.23	62.23	1.24

Eleven fungal species and one sterile mycelium were isolated from turmeric. Three species of fungi were isolated from Fresh brand turmeric powder. Isolated fungi were *A. niger*, *F. nivale* and *Rhaphalomyces* sp. Four species of fungi were isolated from Pran brand turmeric powder. From the results presented in Table 3 it is evident that frequency percentage of association of *A. niger* was highest (73.34) and lowest was (2.22) in *Fusarium* sp.$_1$. From Radhuni brand turmeric powder five fungal species were isolated. Frequency percentage of association of *Syncephalestrum* sp. was highest (19.99) and lowest was (6.66) in *A. flavus, A. versiculor* and *Rhizopus* sp.. Seven fungal species were isolated from rhizome of turmeric. Frequency percentage of association of *A. niger* was highest (45.69) and lowest was (1.24) in sterile mycelium. Imandel *et al.* (2000) from Tehran reported *Mucor* sp., *Apergillus* sp. and *Penicillium* sp. as microbial contaminants of turmeric and black pepper.

Table 3. Percentage frequency of association of fungi with turmeric.

Name of fungi	Fresh	Brand powder Pran	Radhuni	Dried rhizome
Aspergillus flavus	-	11.12	6.66	1.24
A. niger	6.66	73.34	-	45.69
A. terreus	-	-	8.89	-
A. versicolor	-	-	6.66	-
Curvularia clavata				4.94
Fusarium nivale	2.23	33.33	-	1.24
F. oxysporum	-	-	-	11.12
Fusarium sp. $_1$	-	2.22	-	-
Rhizopus sp.	-	-	6.66	37.04
Syncephalestrum sp.	-	-	19.99	-
Rhaphalomyces	2.23	-	-	-
Sterile Mycelium	-	-	-	1.24

Effect of plant extracts on the growth of six test isolates, *viz.*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Curvularia lunata*, *Fusarium oxysporum* and *Rhizopus* sp. is presented in Table 4. Although the plant extracts inhibited the growth of test isolates in most of the cases but it was found to have stimulatory effect on *C. lunata.* The inhibitory effect of the plant extracts increased with their increasing concentrations but in case of *A. niger* with *Allium cepa* and *Fusarium oxysporum* with *Z. officinale* inhibition of growth decreased with the increase of concentrations (Table 4).

The plant extracts of *A. sativum* and *Z. officinale* were found to be most inhibitory to *Rhizopus* sp. Both the extracts completely inhibited the growth of *Rhizopus* sp. The effect of *A. sativum* in all the test isolates was satisfactory followed by *Z. officinale*. The maximum inhibition of *C. lunata* was found with plant extract of *A. sativum* followed by *Z. officinale* . Out of three plant extracts, only *A. sativum* was found to inhibit the growth of all the test isolates at all concentrations (Table 4).

Table 4. Effects of plant extracts on the radial growth of fungi

Name of test fungi	Name of plants (Treatment)	Inhibition of growth at different concentrations (%)			
		5	10	15	20
	Allium cepa	21.62	23.89	25.24	31.08
Aspergillus flavus	*A. sativum*	19.48	53.89	67.53	100
	Zingiber officinale	10.83	18.86	30.86	45.71
A. fumigatus	*Allium cepa*	22.78	30.10	37.42	56.93
	A. sativum	31.71	74.80	100	100
	Zingiber officinale	16.29	18.73	52.05	75.61
A. niger	*Allium cepa*	25.36	24.98	22.01	14.17
	A. sativum	04.94	29.63	88.49	100
	Zingiber officinale	04.31	39.47	53.52	100
Curvularia lunata	*Allium cepa*	+35.0	+23.33	+0.88	10.0
	A. sativum	83.88	100.00	100	100
	Zingiber officinale	32.24	74.20	74.98	100
Fusarium oxysporum	*Allium cepa*	36.10	52.24	56.10	63.34
	A. sativum	94.47	100	100	100
	Zingiber officinale	61.67	59.44	45.57	40.00
Rhizopus sp.	*Allium cepa*	1.08	3.76	4.84	8.60
	A. sativum	100	100	100	100
	Zingiber officinale	100	100	100	100

Effect of plant extracts on radial growth of *Curvularia lunata* presented in Table 4 shows that complete inhibition of the test fungus was at 20% concentration owing to the plant extracts of *Z. officinale* and *A. sativum* at 10 % concentration also completely inhibited the growth of the test fungus. On the other hand *A. cepa* stimulate the growth at 5-15% concentrations whereas slight inhibition was also noticed at 20% concentration (Table 4).

Table 4 represents the effect of plant extracts on the radial growth of *Fusarium oxysporum*. *Allium sativum* inhibited the growth of the test fungus completely at 10-20% concentrations. The trend of inhibition of growth was also observed with *A. cepa* but in case of *Z. officinale* the inhibition decreases with increase of concentration of plant extract in culture media (Table 4).

The foregoing findings indicate that a plant extract which is inhibitory to a number of fungi may have also a stimulatory effect on other fungi. The inhibitory effect of a plant extract is owing to the presence of antifungal agents of various group like antibiotics, phenolic compounds etc (Agrios 1997). Chakraborty *et al.* (2004) reported the efficacy of various cell free extracts of the plants against the growth inhibition of the pathogen. The effectiveness of the extracts varied significantly with dosage, where 100% inhibition of the pathogen was achieved with garlic extract. Antifungal activity of different plant extracts have been reported earlier by several investigators against a number of plant pathogens (Monoharachary and Reddey 1978, Singh and Dwivedi 1987, Asrrafuzzaman *et al.* 1990, Bashar and Rai 1991).Among the three plant extracts *Allium sativum* was found to be most effective followed by *Zingiber officinale.*

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(Received revised manuscript on 30 November 2014)