



RESEARCH ARTICLE

BIOCONTROL POTENTIALS OF SOME PLANT AND SPICE EXTRACTS AGAINST FUNGI CONTAMINATING POST HARVESTED STORED GRAINS.

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Abstract

In the present investigation antifungal activity of some plants and spice extracts was studied against fungi contaminating post harvest stored grains. A total of 135 fungal isolates were obtained from contaminated cereal and pulse samples collected from Jalukbari and Maligaon markets of West Guwahati, Assam. The frequently isolated fungal species were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and fungi belonging to genera *Penicillium* and *Mucor*. Antifungal evaluation of plants and spice extracts were carried out against the test fungal strains by Agar Cup Diffusion assay. The result indicated susceptibility of the test fungal species against the extracts in varying degrees. *Aspergillus flavus* was found to be most susceptible against water extract of *Cinnamomum zeylanicum* obtained at 100°C and also against Ethanol extracts of *Streblus asper* and *Litsea chinensis*. Since most of the isolated fungal genera produced aflatoxin which causes toxicity to living organisms including humans, the inhibition of these fungal genera using plants and spice extracts may be a promising eco-friendly approach to control such post harvest contamination.

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Introduction:-

Cereal grains have been the principal component of human diet for thousands of years and are grown in greater quantities than any other type of crops; therefore serve as staple food crops worldwide. They have high energy values, mainly from the starch fraction, but, also from the fat and protein portions along with traces of minerals and vitamins. Along with cereals pulses have also been grown since millennia and have been contributed as a vital ingredient of the human diet in India (Ayachit, 2002). In India pulses are the second source of dietary protein (27%) after cereals (55%). India is the largest producer of pulses in the world with 25% share in the global production (Ali, 2003). These food items must be stored properly after harvest as the point of production is not always the point of consumption and the time of production is not always the time of consumption. However, during storage and transportation, a number of fungal pathogen attacks these food items leading to their qualitative and quantitative loss. On an estimate, around 25% of agricultural food items become unsuitable for consumption annually due to the invasion by different food-borne molds and their toxic metabolites (Pittet, 1998). Due to their chemical composition these food products are particularly susceptible to molds. The condition is more serious in tropical and sub-tropical regions of the world due to the congenial factors like high temperature, relative humidity and moisture content of stored products which favor the development of fungal population. The major fungi found associated with stored

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food items include *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Curvularia* spp., members of Mucorales etc. (Prakash *et al.*, 2015). Most of them are known to produce mycotoxins (Samson *et al.*, 2004 and Pitt *et al.*, 2009). The development of mycotoxin producing fungi leads to contamination with mycotoxins and these toxins diffuse into the grain and can be found in all ground fractions, due to their thermo-resistance properties (Bullerman *et al.*, 2007). Mycotoxins are well known carcinogens, teratogens, tremorogens, mutagens, nephrotoxins and neurotoxins to a wide range of organisms (Refai, 1998) and therefore is a matter of serious health concern. Use of synthetic chemicals to control growth of fungal contaminant could have serious health consequences as most of the chemicals have negative effects to living cells and the environments. Therefore, various natural plant products as such as plant extracts could be used as eco-friendly alternatives to control mycotoxin producing fungi. They have a narrow target range with specific mode of action, therefore are suitable for a specific target and as they degrade rapidly from sunlight, air, and proper moisture, which generally make them less toxic to the environment. Since long herbs and spices have been used as flavoring agents and also as folk medicine and food preservatives (Beuchat, 1994; Nakatani, 1994; Cutler, 1995). They are also found to possess antimicrobial activities (Abu-shanab *et al.*, 2004; Holly and Patel, 2005; Brandi *et al.*, 2006; Agaoglu *et al.*, 2007; Shan *et al.*, 2007; Narayan *et al.*, 2010). Therefore present investigation was carried out to evaluate the efficacy of different plants and spice extracts in controlling fungi contaminating post harvested stored grains.

Materials and methods:-

Collection and isolation of fungi from post harvested stored grains:

Stored food grains of two cereals viz., *Triticum aestivum* and *Oryza sativa* and two pulses viz., *Lens culinaris* and *Arachis hypogaea* were collected from West Guwahati markets, Assam, India. For isolation of associated fungi from the collected samples two methods were used (serial dilution and direct plating). Ten (10) grains of each sample was added to 10ml of sterilized distilled water and shaken for 3 minutes to get a stock solution. 1ml of the stock was pipetted into 9ml of sterilized distilled water in a test tube to make a serial dilution of 10^{-1} , 1ml of 10^{-1} serial dilution was pipetted into 9ml of sterilized distilled water in a test tube gave 10^{-2} serial dilution. Similar method was carried out to give final concentrations of 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} dilution. Each dilution was inoculated into Potato Dextrose Agar (PDA) media. While in the direct isolation, ten (10) contaminated grains of each sample were directly inoculated in Potato Dextrose Agar (PDA) media. Samples were inoculated in the media and incubated at $30 \pm 2^\circ\text{C}$ for 48-72 hours. Fungi growing out of the inoculated samples were carefully isolated as pure culture as stored in 4°C in refrigerator for further study. The isolated fungal species were identified based on their colonial traits and detailed microscopic studies using standard identification manual of Gilman (1971).

Preparation of crude water extracts of different spices:

A total of nine spices namely *Elettaria cardamomum*, *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Trigonella foenum graecum*, *Cuminum cyminum*, *Coriandrum sativum*, *Foeniculum vulgare*, *Acorus calamus* and *Amomum aromaticum* were collected from local vendors of West Guwahati market and were kept in sterilized polybags and brought to the laboratory. Here 100gm of each spice were taken in a conical flask and to it 100ml of sterilized distilled water was added. It was then placed on a heating mantle and temperature was set to 50°C and the solution mixture was heated for 10 minutes. Then it was allowed to cool down and filtered through Whatman filter paper to obtain the spice water extract. Similarly extraction was carried out at 100°C for 10 minutes. The crude water extracts so obtained in two different temperatures were labeled and stored in refrigerator at 4°C for use in further assay.

Preparation of plant extracts of *Litsea chinensis* and *Strobilus asper*:

Three different organic solvents namely ethanol, methanol and petroleum ether and distilled water were used to obtain the crude plant extracts. The extracts were prepared by dissolving 20gm of fine powdered leaf material of both *Litsea chinensis* and *Strobilus asper* separately in 200ml of ethanol, methanol and petroleum ether and distilled water respectively. The contents were air tight and kept in arbitrary shaker for 96 hours. Then the extracts were filtered with the help of whatman filter paper. The filtrates so obtained were heated in water bath at 40°C for 10-20 minutes to evaporate the respective solvents. Crude extracts so obtained were stored in refrigerator at 4°C for use in further assay.

Determination of antifungal activities of the plant based extracts:

The antifungal activity of the plant based extracts against the isolated fungi was performed by Agar cup diffusion method. For this purpose, first the culture of the selected isolates were inoculated in Potato Dextrose Broth and incubated at 30°C for 5-6 days till sporulation. At the meantime Potato Dextrose Agar plates were prepared and 1ml of the broth culture of sporulating test fungi were inoculated on the respective PDA plates and it was evenly

inoculated throughout the PDA plate with the help of sterilized cotton swabs. Now agar cups were prepared by scooping out the medium with the help of sterile cork borer (7mm in diameter). Each cup was then loaded with different plant crude extracts and essential oils separately. The plates were incubated at 30°C for 48-72 hours and the zone of inhibition was measured thereafter.

Results:-

A total of 135 fungal isolates were isolated from contaminated post harvest stored grains. The frequently isolated fungal species were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and fungi belonging to genera *Penicillium* and *Mucor* (Table 1 and Table 2).

Table 1:- Occurrence of fungal species isolated from contaminated cereals

Fungi	No of isolates
<i>Aspergillus niger</i>	20
<i>Aspergillus fumigatus</i>	15
<i>Aspergillus flavus</i>	20
<i>Penicillium nordicum</i>	06
<i>Penicillium italicum</i>	13
<i>Mucor</i> sp.	04
Total	78

Table 2:- Occurrence of fungal species isolated from contaminated pulses

Fungi	No of isolates
<i>Aspergillus niger</i>	16
<i>Aspergillus fumigatus</i>	11
<i>Aspergillus flavus</i>	15
<i>Penicillium nordicum</i>	03
<i>Penicillium italicum</i>	10
<i>Mucor</i> sp.	02
Total	57

Determination of antifungal activity of oil against tested fungal species:

The water extracts of selected spices obtained at 50°C and 100°C showed varying degree of antifungal activity against the selected isolates of fungi obtained from samples of cereals (Table 3). The water extract of *Cinnamomum zeylanicum* obtained at 100°C showed significant zone of inhibition against all the fungal isolates. It showed maximum activity against *Aspergillus flavus* isolated from both *Oryza sativa* and *Triticum aestivum* with 20mm zone of inhibition. It was followed by *Penicillium italicum* isolated from *Oryza sativa* (18mm) and *Triticum aestivum* (17mm) and *Aspergillus niger* isolated from *Oryza sativa* (16mm) and *Triticum aestivum* (15mm). The minimum activity was recorded against *Aspergillus fumigatus* isolated from *Oryza sativa* and *Triticum aestivum* with 15mm and 12mm zone of inhibition respectively. Similarly the water extract of *Syzygium aromaticum* obtained at 100°C also showed considerable activity except on *Penicillium italicum* isolated from *Oryza sativa*. The other spice extracts did not show such considerable antifungal activity.

Table 3:- Antifungal activity of crude water extracts of selected spices against most frequently contaminating fungi isolates isolated from cereals

Zone of inhibition (mm)						
Fungal species	Cu100	Cu50	BE50	So50	CI100	Ci100
<i>Aspergillus niger</i>	*--	--	--	--	11	16
<i>Aspergillus flavus</i>	--	--	--	--	10	20
<i>Penicillium italicum</i>	--	--	--	--	--	18
<i>Aspergillus fumigatus</i>	--	--	--	--	10	15
<i>Aspergillus flavus</i>	--	--	--	--	12	20
<i>Penicillium italicum</i>	--	--	--	--	10	17

*-- indicates antifungal activity; Cu₁₀₀ = Water extract of *Cuminum cyminum* obtained at 50°C; Cu₅₀ = Water extract of *Cuminum cyminum* obtained at 100°C; BE₅₀ = Water extract of *Ammomum aromaticum* obtained at 50°C; So₅₀ = Water extract of *Foeniculum vulgare* obtained at 50°C; Cl₁₀₀ = Water extract of *Syzygium aromaticum* obtained at 100°C; Ci₁₀₀ = Water extract of *Cinnamomum zeylanicum* obtained at 100°C.

Similarly, antifungal activity of the spice extracts were also carried out against fungi isolated from contaminated pulses. The result indicated that water extract of *Cinnamomum zeylanicum* obtained at 100°C showed significant zone of inhibition against all the fungal isolates (Table 4). It showed maximum activity against *Aspergillus flavus* isolated from *Lens culinaris* with 25mm zone of inhibition. It was followed by *Aspergillus flavus* isolated from *Arachis hypogaea* (22mm), *Aspergillus niger* isolated from *Arachis hypogaea* (15mm) and *Aspergillus fumigatus* isolated from *Lens culinaris* (12mm). Further, the water extract of *Syzygium aromaticum* obtained at 100°C also showed considerable activity against *Aspergillus flavus* isolated from *Arachis hypogaea* and *Aspergillus fumigatus* isolated from *Lens culinaris* displaying 11mm zone of inhibition. Water extract of *Foeniculum vulgare* obtained at 50°C also showed significant activity against *Aspergillus flavus* isolated from pulses with 21mm zone of inhibition. Other spice extracts did not show such considerable antifungal activity.

Table 4:- Antifungal activity of crude water extracts of selected spices against most frequently contaminating fungal isolates isolated from pulses.

Zone of inhibition (mm)						
Fungal species	Cu100	Cu50	BE50	So50	Cl100	Ci100
<i>Aspergillus niger</i>	--*	--	--	--	--	--
<i>Aspergillus flavus</i>	--	--	--	--	11	22
<i>Aspergillus fumigatus</i>	--	--	--	--	11	12
<i>Aspergillus flavus</i>	--	--	--	21	--	25

*-- indicates no antifungal activity; Cu100 = Water extract of *Cuminum cyminum* obtained at 50°C; Cu50 = Water extract of *Cuminum cyminum* obtained at 100°C; BE50 = Water extract of *Ammomum aromaticum* obtained at 50°C; So50 = Water extract of *Foeniculum vulgare* obtained at 50°C; Cl100 = Water extract of *Syzygium aromaticum* obtained at 100°C; Ci100 = Water extract of *Cinnamomum zeylanicum* obtained at 100°C.

Antifungal activity of the fungal isolates was also evaluated with crude solvent extracts obtained from two plant species namely *Litsea chinensis* and *Streblus asper*. The result indicated that organic solvent extracts of *Litsea chinensis* and *Streblus asper* showed varying degree of inhibition against fungal isolates isolated from cereals (Table 5). The Ethanol extract of *Litsea chinensis* showed highest degree of activity against *Aspergillus flavus* isolated from *Oryza sativa* with 19mm zone of inhibition. It was followed by *Aspergillus niger* isolated from *Oryza sativa* (18mm), *Aspergillus flavus* isolated from *Triticum aestivum* (18mm), *Penicillium italicum* isolated from *Triticum aestivum* (17mm), *Penicillium italicum* isolated from *Oryza sativa* (13mm), *Aspergillus fumigatus* isolated from *Triticum aestivum* (13mm). Besides this the Ethanol extract of *Streblus asper* showed considerable activity against *Aspergillus niger* (15mm) and *Penicillium italicum* (15mm) isolated from *Oryza sativa* and *Aspergillus fumigatus* (14mm) isolated from *Triticum aestivum*. Other plant extracts did not show any considerable activity against the tested fungal isolates.

Table 5:- Antifungal activity of crude organic solvent and water extracts of *Litsea chinensis* and *Streblus asper* against most frequently contaminating fungal isolates obtained from contaminated cereals

Zone of inhibition (mm)						
Fungal species	S.A. _{P.E.}	S.A. _{W.E.}	S.A. _{E.}	S.A. _{M.}	L.S. _{W.E.}	L.S. _{E.}
<i>Aspergillus niger</i>	--*	--	15	--	--	18
<i>Aspergillus flavus</i>	--	--	--	--	--	19
<i>Penicillium italicum</i>	--	--	15	--	--	13
<i>Aspergillus fumigatus</i>	--	--	14	--	--	13
<i>Aspergillus flavus</i>	--	--	--	--	--	18
<i>Penicillium italicum</i>	--	--	--	--	--	17

* -- indicates no antifungal activity; S.A._{P.E.} = Petroleum ether extract of *Streblus asper*; S.A._{W.E.} = Water extract of *Streblus asper*; S.A._{E.} = Ethanol extract of *Streblus asper*; S.A._{M.} = Methanol extract of *Streblus asper*; L.S._{W.E.} = Water extract of *Litsea chinensis*; L.S._{E.} = Ethanol extract of *Litsea chinensis*.

Similarly, antifungal activity of the organic and water extract of the two plant species was evaluated with fungi isolated from contaminated pulse samples. The result indicated varying degree of antifungal activity (Table 6). The ethanol extract of *Streblus asper* showed maximum inhibition against *Aspergillus niger* isolated from *Arachis hypogaea* with 18mm zone of inhibition. It also showed considerable inhibition against *Aspergillus flavus* isolated from *Arachis hypogaea* (14mm). The extract also showed activity against *Aspergillus fumigatus* and *Aspergillus flavus* isolated from *Lens culinaris* with zone of inhibition of 14mm and 12mm respectively. Ethanol extract of *Litsea chinensis* also showed very good activity against *Aspergillus flavus* which was isolated from *Arachis hypogaea* with 16mm zone of inhibition. This extract also showed considerable inhibition against other fungal isolates isolated from pulses. It showed 14mm zone of inhibition against *Aspergillus flavus* isolated from *Arachis hypogaea*, which was followed by *Aspergillus flavus* (13mm) and *Aspergillus fumigatus* (12mm) isolated from *Lens culinaris*. Methanol extract of *Litsea chinensis* also showed antifungal activity against *Aspergillus niger* isolated from *Arachis hypogaea* and *Aspergillus fumigatus* isolated from *Lens culinaris* with 12mm and 11mm zone of inhibition respectively. It was also found that the methanol extract of *Streblus asper* showed some activity against *Aspergillus flavus* isolated from *Arachis hypogaea* and *Aspergillus fumigatus* isolated from *Lens culinaris* with 11mm zone of inhibition each. Water extract of *Streblus asper* showed some activity against *Aspergillus niger* isolated from *Arachis hypogaea* with 11mm zone of inhibition. It was observed that other extracts did not show any considerable antifungal against the tested fungal contaminants.

Table 6:- Antifungal activity of crude organic solvent and water extracts of *Litsea chinensis* and *Streblus asper* against most frequently contaminating fungal isolates obtained from pulses

Zone of inhibition (mm)						
Fungal species	S.A. _W	S.A. _M	S.A. _E	L.C. _W	L.C. _M	L.C. _E
<i>Aspergillus niger</i>	11	--	18	--	12	14
<i>Aspergillus flavus</i>	--	11	14	--	--	16
<i>Aspergillus fumigatus</i>	--	11	14	--	11	12
<i>Aspergillus flavus</i>	--	--	12	--	--	13

*-- indicates no antifungal activity; S.A._W= Water extract of *Streblus asper*; S.A._M= Methanol extract of *Streblus asper*; S.A._E= Ethanol extract of *Streblus asper*; L.C._W = Water extract of *Streblus asper*; L.C._M= Methanol extract of *Litsea chinensis*; L.C._E = Ethanol extract of *Litsea chinensis*.

Discussion:-

In current scenario, there is wide search for new effective antimicrobial agents from natural sources. Numerous studies have been carried out with the various plants extracts for exploration of their antimicrobial activity against pathogenic microorganisms. Thus the present investigation indicates that the majority of the plants tested are important source of antifungal compounds that may provide renewable sources of useful antifungal drugs against plant and human pathogenic fungi. Several strategies are being used to control fungal growth and mycotoxin biosynthesis in stored grains by chemical treatment with ammonia, acids and bases or with food preservatives by physical methods and by biological methods. These methods require sophisticated equipment and expensive chemicals and reagents. Use of natural plant extracts provides an opportunity to avoid chemical preservatives. Several edible botanical extracts have been reported to have antifungal activity (Reddy *et al.*, 2009; Pradeep *et al.*, 2003). López-Malo *et al.*, (2007) studied the effects of selected combinations of cinnamon extracts and sodium benzoate on the growth response of *Aspergillus flavus* inoculated on potato-dextrose-agar medium and found positive results. Similar results were obtained in our findings where the water extract of *Cinnamomum zeylanicum* obtained at 100°C showed significant zone of inhibition against all the fungal isolates. Growth of fungi in food materials mainly during post harvest periods is the chief reason for the worsening of food products in the sequence of storage and delivery that leads to decrease in the value of food products and also its self life causing great economic damages. Both quantitative and qualitative food losses to the exceptionally unpredictable extent occur at all junctures in the post-harvest method from harvesting, during handling, storage, dispensing and selling to ultimate delivery to the end user. Fungal attack in food items leads to their bio-deterioration through increase in free fatty acid content, change in colour and texture and decrement in nutritional value and germination ability (Dhingra *et al.*, 2001). Thus the present findings proved to be significant as biological control agent for control of fungi contaminating stored post harvested grains.

Conclusion:-

The present finding suggests that plant and spice extracts could be successfully used for control of fungi contaminating post harvested grains. Since most of the chemicals used for controlling such contaminants are harmful and have negative impact on living organisms and the environment the study becomes more significant as most of the plants and spices extracts are used by human as food and do not proved to be harmless.

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