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EFFECT OF PHYSICAL IRRADIATIONS AND CHEMICAL TREATMENTS ON FUNGAL GROWTH AND DETOXIFICATION OF AFLATOXINS CONTAMINATED ARECA NUTS

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Effects of some physical irradiations like Gamma, UV, IR and X-rays and that of some chemical treatments on fungal growth and detoxification of aflatoxins contaminated areca nuts and its various preparations were investigated. Gamma rays showed 100% detoxification in contaminated areca nuts while UV rays showed 70-75% detoxification, but IR and X-rays were totally ineffective. Chemicals like lime, catechu and tartari (tartaric acid) were also not very effective against various aspergilli. Besides these, some antifungal drugs were also tested against *Aspergillus flavus* and *A. niger*. Hydrogen per oxide and Creosote were found to be most active.

Key Words : Aflatoxins, detoxification, areca nuts, physical irradiations, chemical treatments.

Consumption of aflatoxins contaminated food causes a variety of deleterious effects in human and animal systems. Hence, prevention and control of aflatoxins has been a matter of serious concern. Effect of various fungicides and chemicals (Khatri, 1991; Chaudhary *et al.*, 1994), insecticides (Abdel Kader *et al.*, 1994), antibiotics (Kumar and Singh, 1991), wild and medicinal plant extracts (Singh, 1983; Prasad and Kumar, 1993) on fungal growth and aflatoxin production have been reported. However, little information is available regarding the effects of physical irradiations on fungal growth and aflatoxins production. In the present investigation effects of 4 types of irradiations *i.e.* Ultra Violet (UV), Infra-red (IR), X-rays and Gamma rays have been reported along with some common fungicides and drugs.

MATERIALS AND METHODS

Areca nuts and its various preparations were collected from the local market and on their microbiological examination these were found to be contaminated with various fungi like *Aspergillusflavus, A.fumigatus, A. niger, A. parasiticus, A. nidulans, A. terreus, Penicillium cyclopium, P. purpurogenum* and *Mucor* spp. *etc.* (Chaturvedi and Chaturvedi, 1992) which produced B_1 and G_1 aflatoxins (Chaturvedi and Chaturvedi 1995). The contaminated samples were given physical and chemical treatment and were evaluated for the reduction in the number of fungi and aflatoxins produced.

Physical treatment : Different samples were treated separately by X-rays for 5 seconds; Ultra-Violet (UV) and Infra-red (IR) rays separately for 30 minutes at a distance of 60 cm. The treatment by Gamma rays was for 1 h 40 minutes by 3 Mega red units of Gamma rays. These treatments were selected based on the normal dose and procedure used in medical microbiology.

Evaluation of detoxification effects : The detoxification effects of physical irradiations were evaluated qualitatively by the method of Brown *et al.* (1973) and quantitatively by the method of Ciegler *et al.* (1971) to measure the concentration of B_1 and G_1 aflatoxins.

Chemical treatment : For chemical detoxification, the ingredients like lime, rich in calcium hydroxide and catechu (major component tannic acid) commonly used with areca nuts were selected. Tartaric acid was also included in the study to find out its effects on fungal growth. 5% (w/v) solutions of test substances were used in Sabouraud's Dextrose Agar (SDA, 4% dextrose, 1% peptone and 1.5% agar all w/v) medium and slants were prepared and cultured.

Drug sensitivity test : Some common antibiotics and antifungal agents like Septran, Flagyl, Mycostatin, Griseofulvin, Hydrogen per oxide, Sensoform paint, Borax, Creosote, Catechu (common Hindi name 'kattha'), and Alum generally used in dental and mouth diseases were purchased from the local market and tested using the technique of Grigorin and Grigorin

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Table 1. Effect of physical irradiations on fungal growth.

Type of areca nut	Fungal isolates before exposure (Control)	Fungal isolates after exposure			
		Gamma	UV rays	IR rays	X-rays
Plain cut areca nuts	Afl, Afu, Ani, Alt	ng	Ani	All 4	All 4
Plain cut areca nuts	Afl, Apa, Muc	ng	ng	All 3	All 3
Sugar coated areca nuts (Jhankar supari)	Afl, Afu, Pcy, Muc	ng	Afu	All 4	All 4
Sugar coated areca nuts (suman supari)	Ani, Muc, Alt	ng	Ani	All 3	All 3
Pan masala (Pan parag)	Afu, Ani, Rhi	ng	Rhi	All 3	All 3

Abbreviations : Afl = *Aspergillus flavus;* Afu = *Aspergillus fumigatus*; Ani = *Aspergillus niger*; Apa = *Aspergillus parasiticus*; Alt = *Alternaria alternata*; Pcy = *Penicillium cyclopium*; Rhi = *Rhizopus nigricans*; Muc = *Mucor mucedo*; ng = no growth.

Table 2. Effect of physical irradiations on detoxification of contaminated areca nuts. The figures in parenthesis indicate the % detoxification of the aflatoxin.

Type of areca nut	Amount of aflatoxin before exposure (in µg/kg)	Amount of aflatoxin after exposure (in µg/kg)			
		Gamma rays	UV rays	IR rays	X-rays
Plain cut areca nuts	320 (Aflatoxin G_1)	0 (100%)	96 (70%)	320 (0%)	320 (0%)
Plain cut areca nuts	70 (Aflatoxin B_1)	0 (100%)	17.5 (75%)	70 (0%)	70 (0%)
Sugar coated areca nuts (Jhankar supari)	280 (Aflatoxin G_1)	0 (100%)	70 (75%)	280 (0%)	280 (0%)
Sugar coated areca nuts (Suman supari)	280 (Aflatoxin G_1)	0 (100%)	84 (70%)	280 (0%)	280 (0%)
Pan masala (Pan parag)	93 (Aflatoxin B_1)	0 (100%)	28 (70%)	93 (0%)	93 (0%)

(1970). Drug sensitive filter paper (Whatman No. 1) discs (5 mm diameter) were prepared by dipping 5 discs in each of the test substance in 1 ml of 20% (w/v) solution separately for 2 minutes and dried in hot air oven at 40°C. These discs were placed at equal distance from the centre on the periphery of a Petri plate containing SDA medium. One loopful suspension of spores (10^4 spores/ml) of *Aspergillus flavus* and *A. niger* separately was inoculated in the centre of each test plate. These were incubated at 28°C for 4 days. The zones of inhibition around the paper discs were measured.

RESULTS AND DISCUSSION

Physical Treatment : On comparison of all the 4 radiation methods, it was found that Gamma rays had most significant effect and no fungal species was isolated from areca nuts and its different preparations after exposure to Gamma rays when compared with untreated control (Table 1). On TLC plates, Gamma rays treated samples yielded no spots of aflatoxins indicating 100% detoxification after exposure while UV rays treated samples showed significant reductions in aflatoxins (70-75% detoxification). On the other hand, X-rays and IR treatments caused no changes either in fungal colonization (Table 1) or aflatoxin production (Table 2). Similar type of results were reported by others (Anonymous, 1981; Saxena, 1984) who succeeded in detoxifying toxins to some extent from various food commodities, by exposure through Gamma and UV rays. Bassapa (1983) also reported that exposure of contaminated groundnut oil to IR rays did not destroy aflatoxins which confirms our findings. Aflatoxins are bound to the protein fraction and probably this binding protect the toxin against irradiation (Anonymous, 1981).

Chemical treatment : Lime and catechu which are frequently used with areca nuts and its preparations also demonstrated antimicrobial activity against some fungal taxa (Table 3). It was found that 5% solution of catechu in SDA medium inhibits the growth of *A. parasiticus, A. fumigatus, Penicillium cyclopium* and *Mucor.* Growth of potent aflatoxin producer *A. flavus*

Table 3. Effect of lime, catechu and tartaric acid (Tartari) on survival of different fungal isolates

S.No.	Type of areca nut	Fungal isolates on plain S.D.A. (Control)	Fungal isolates on S.D.A. containing 5% soln. of catechu	Fungal isolates on S.D.A. containing 5% soln. of lime	Fungal isolates on S.D.A. containing 5% soln. of tartaric acid
1.	Plain cut areca nuts	*A. flavus,* *A. nidulans* *Mucor*	*A. flavus* *A. nidulans*	*A. nidulans*	
2.	Plain cut areca nuts	*A. parasiticus*	-	-	-
3.	Half cut areca nuts	*A. flavus,* *A. niger*	-	*A niger*	-
4.	Sugar coated areca nuts	*A. flavus* *A. niger* *Penicillium cyclopium* *Alternaria*	*A. niger* *P. cyclopium*	*A. niger*	*P. cyclopium*
5.	Sugar coated areca nuts	*A. fumigatus* *A. terreus*	-	-	-
6.	Pan masala	*Mucor*	-	-	-
7.	'Kapoori' tobacco	*A. parasiticus* *A. niger*	-	-	*A. niger*

Table 4. Inhibition of growth of *Aspergillus flavus* and *A. niger* by selected drugs.

S. No.	Name of the drug	Growth Inhibition (mm diameter) *A. flavus*	*A. niger*
1.	Septran	Nil	Nil
2.	Creosote	3 mm	2 mm
3.	Hydrogen per oxide	5 mm	3 mm
4.	Mycostatin	Nil	Nil
5.	Flagyl	Nil	Nil
6.	Grisoefulvin	Nil	Nil
7.	Borax	Nil	Nil
8.	Sensoform paint	Nil	Nil
9.	Alum	1 mm	1 mm
10.	Catechu	1 mm	Nil

was also inhibited in few samples but *A. niger* and *A. nidulans* were frequently isolated in treated samples. Similarly, lime demonstrated antifungal activity against *A. flavus, A. fumigatus, A. parasiticus, Mucor* and *Alternaria* spp but had no effect against *A. niger* and *A. nidulans.* Tartaric acid also exhibited antifungal activity against some toxigenic fungal species like *A. flavus, A. fumigatus, A. parasiticus, A. nidulans, Mucor* and *Rhizopus* but no such effect was seen against *A. niger* and *Penicillium cyclopium.* It is clear from these results that these chemicals have some antifungal activity but none of them could completely check the growth of *A. niger.* Saxena (1984) reported that ascorbic and citric acids have the capability to neutralise the aflatoxins.

Drug sensitivity tests : Drug sensitivity test for some common antifungal drugs and chemicals yielded useful informations (Table 4). It was found that best antifungal spectrum was shown by hydrogen per oxide which inhibited the growth of *A. flavus* and *A. niger* both and demonstrated the growth inhibitory zone of 5 mm in the former and 3 mm in the latter. Similarly, Creosote, the buccal cavity antibiotic also showed antifungal property as it inhibited the growth of both *A. flavus* and *A. niger* with growth inhibitory zones of 3 and 2 mm respectively. Alum showed inhibitory zones of 1 mm diameter in both *A. flavus* and *A. niger,* while catechu showed inhibitory zone of 1 mm only against *A. flavus.* Other antifungal drugs and chemicals did not show any growth inhibitory effect against any of the two aspergilli. It may be concluded from this study that hydrogen per oxide and creosote, possess high antifungal properties and may be tested for the treatment of mycoses/mycotoxicoses of buccal cavity and gastrointestinal tract where *A. flavus* and *A. niger* are frequently found as pathogens (Bossche *et al.,* 1988). It is also clear from the present study that drugs and chemicals like Mycostatin, Griseofulvin, Septran, Borax, Sensoform paint *etc.* prescribed for their antifungal properties did not show any effects against natural contaminations by the fungal strains of *A. flavus* and *A. niger.* Therefore, the sensitivity of these drugs need careful evaluation. Verma *et al.* (1988) also reported that organic acids like propionic, benzoic, and sodium

benzoate inhibits the growth of aspergilli and the antibiotics like nystatin (mycostatin) and netamycin showed inhibition against *A. terreus* and other aspergilli. Effective treatment of black rot of onion bulbs caused by *A. niger* by boric acid, copper oxychloride, formaldehyde and captan was also reported by Chaudhary *et al.* (1994). Similarly, Khatri (1991) reported that carbendazim and thiophonate methyl at 0.1% concentration were effective fungicides against fruit rot of date palm caused by *Aspergillus niger, A. flavus, A. fumigatus* and *Nigrospora etc.* which suggest that the various drugs and chemicals act differently against different fungal taxa and more detailed investigations are required to find out the broad spectrum antifungal agents particularly for the detoxification of aflatoxins and/or mycoses/mycotoxicoses.

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