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Exploration of Ochratoxin A Contamination and its Management in Rice

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Abstract: *Aspergillus ochraceus* contamination was detected in the forty six rice samples collected from areas exposed to rain or flood or stored in godown or mills. These isolates were grown on Pusa basmati rice grains for 7 days at room temperature than extracted the Ochratoxin A (OTA) and identified on TLC plate along with standard in different solvent systems. The OTA was confirmed by changing the colour from greenish blue to blue after spraying 6% NaOH in 20% ethanol on TLC plate. Garlic bulb extract (10%) proved significantly more effective with complete inhibition of *A. ochraceus*. *Pongamia glaberima* kernel extract (20%) showed 71% inhibition on mycelial growth of *A. ochraceus*. Among the culture filtrate of the biocontrol agent, *Trichoderma virens* inhibited completely while the culture filtrate from *T. harzianum* showed a partial inhibition (67%) at 15% concentration. The culture filtrate of *Pseudomonas fluorescens* (DRPf 002) were effectively inhibited (98%) mycelial growth at 15% concentration. Among the fungicides tested carbendazim was effective even at 100 ppm in inhibiting *A. ochraceus*. Propineb completely reduced the growth at 750 ppm. Among the chemicals tested benzoic acid were completely inhibited the mycelial growth at 0.1% concentration.

Key words: Rice, *A. ochraceus*, OTA, plant extracts, biocontrol agents, fungicides and chemicals

INTRODUCTION

Rice (*Oryzae sativa* L.) is the most important staple food crop in India and bulk of rice is grown in *kharif* or wet season. Frequent and heavy rainfall and floods particularly near harvest in coastal areas in eastern, southern and western regions of the country wet the crop and make panicles more prone to invasion by fungi and bacteria. As the harvested sheaves are left in moist fields over strubbles to dry, the grains become infected by *Aspergillus* sp. (Reddy *et al*., 2004). The mycotoxin producing fungi viz., *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. were found in the seed samples rough rice stored in government warehouses (Carlos *et al*., 2000). Species of *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* were the most important mycotoxigenic fungi in rice grains (Shetty *et al*., 1994). Indeed, among more than 100,000 fungal species able to contaminate feeds and foods, some have the ability to synthesize molecules toxic for human and animals. Rice represents a very good substrate for fungal growth and toxinogenesis since it is used as an ideal culture medium to test the toxigenic potential of isolated strains (Bars and Bars, 1992). Some surveys showed that this basic food might be contaminated by mycotoxins such as Aflatoxins and Ochratoxin A (Begum and Samajpati, 2000). The Ochratoxin A contamination was identified in rice purchased from retail shops in the Rabat and Sale area in Morocco (Zinedine *et al*., 2007). That contamination may represent a real threat to human health specially concerning chronic toxicity of these toxins, which are known or suspected to be

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carcinogenic. However, detailed information on OTA contamination in rice is not available. We therefore made an attempt to isolate OTA producing mycoflora in the samples collected from different rice growing areas of India and evaluate plant extracts, biocontrol agents, fungicides and chemicals to limit the growth of *A. ochraceus*.

MATERIALS AND METHODS

This study was carried out from February 2005 to October 2006 at Plant Pathology Laboratory, Directorate of Rice Research, Rajendranagar, Hyderabad, A. P, India. Five hundred and five seed samples were collected from 36 locations in 21 states in the country (Andaman and Nicobar islands, Andhra Pradesh, Assam, Bihar, Chattishgarh, Gujarat, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Orissa, Pondicherry, Punjab, Rajasthan, Tamil Nadu, Tripura, Uttaranchal and West Bengal). Using agar plate method OTA producing mycoflora was isolated from these samples. Seeds were surface sterilized with chlorax (0.1%) and washed twice with distilled water. Two hundred seeds were plated on one-half strength potato dextrose agar medium having Rose Bengal (50 ppm) (Cotty, 1994). The plates were incubated at room temperature and presence of *A. ochraceus* was observed after six days.

For identification of OTA, *A. ochraceus* isolates were grown on pusa basmati rice grains were soaked 2 h in tap water and fermentation was carried out in 500 mL Erlenmeyer flasks containing 50 g of rice inoculated with 1 mL of spore suspension of *A. ochraceus* was originally isolated from rice grains collected from different rice growing areas of India. Three treatments were maintained for each strain. After 7 days of fungal growth on rice grains, than extracted the OTA through the moldy rice (25 g) was blended in waring blender for 5 min with Acetonitrile-4% KCl-6N HCl (88: 10:2) (Eucaris *et al*., 1982). The mixture was filtered through Whatman No. 1 filter paper and concentrated on a rotary evaporator to 1 mL. OTA production was evaluated by standard thin layer silica gel chromatography to confirm the presence or absence of OTA along with standard. The standard was procured from sigma chemicals. Two replicates were analyzed by spotting crude extract of OTA. The TLC plates used were coated with silica gel 60 F_{254} on aluminum sheet, 20×20 cm and the silica gel plates were developed in the following solventsystems (vol/vol): (A) toluene-ethyl acetate-formic acid (6:3:1), (B) benzene-methanol-acetic acid (90:5:5), (C) chloroform-ethyl acetate-formic acid (10:3:1), (D) chloroform-acetone-formic acid (6:2:1), (E) benzene-ethyl acetate-formic acid (3:1:1), (F) benzene-aceticacid (4:1) and (G) chloroform-diethyl ether-acetic acid (17:5:1). The plates were than observed under UV light at 365nm and calculated R_f value. The OTA was confirmed on TLC by changing the colour from greenish blue to blue after spraying 6% NaOH in 20% ethanol (Golinski and Grabarkiew, 1984).

Plant extracts viz., *Allium sativum* bulb extract, *Annona squamosa* kernel extract, *Allium cepa* bulb extract, *Pongamia glabarema* kernel extract, *Azadirachta indica* leaf and kernel extract and *Eucalyptus terticornis* leaf extract, biocontrol agents viz., *Trichoderma viride* (MTCC 800), *T. harzianum* (MTCC 2050), *T. virens* (MTCC 794), *T. reseii* (MTCC 2480) and *T. koningi* (MTCC 796), five strains of *Pseudomonas fluorescens,* fungicides viz., propineb 70 WP, bitertanol 25 WP, carbendazim 50 WP and tricyclazole 75 WP and chemicals viz., acetic acid, benzoic acid, propionic acid, vanillin and sodium chloride were tested for their efficacy on the growth of *A. ochraceus*. The *Trichoderma* cultures were grown in potato dextrose broth for seven days at 28°C and the cultures were filtered through Whatman No. 1 filter paper to get cell-free filtrate. The *Pseudomonas* cultures were grown in King'S B broth for 4 days in 28°C at 120 rpm and the cultures were centrifuged at 10,000 rpm for 10 min to get cell-free filtrate.

Similarly clear extracts from plants (*Pongamia*, onion, custard apple, Neem kernel and garlic @ 25 g/100 mL and *Eucalyptus* and neem leaf @ 3 g/100 mL water) were also obtained. The extracts

of each plant species at 5, 10, 15 and 20% and biocontrol agents at 5, 10 and 15% were tested by poison food technique (Nene and Thapliyal, 1971). Fungicides were tested at 100, 250, 500, 750 and 1000 ppm concentration and chemicals at 0.1, 0.2, 0.3, 0.4% concentration. The medium incorporated with desired treatment was poured into petri dishes, allowed to solidify and a loop full of inoculum of OTA producing culture was transferred. PDA without plant extract, fungicide or chemical served as control and three replications were maintained for each treatment.

RESULTS

The level of infection of *A. ochraceus* varied from 1 to 100% in the 46 rice samples collected from different rice growing regions of India (Table 1). Forty-six isolates of *A. ochraceus* were obtained from these samples. On an average, the infection with *A. ochraceus* was to the extent of 65.3% in the samples collected from Pusa where the seed was stored for one to two years in the godowns and open exposed to flood. A similar level of infection was found in the seed samples that were exposed to rain at Arundhatinagar (Tripura). However, the seed stored for one to two years in godowns showed 46.5% at Titabar (Assam). The seed stored for 2 years in godowns showed 38.5% at Megalaya (Gujarat). The occurrence of *A. ochraceus* in the seed samples open exposed to rain was 35.5% at arundathinagar (Tripura) and 28.5% at Aduthurai (Tamil Nadu). The other samples showed low level of infection from 1.4 to 11% were the seeds stored in godowns, damp condition and the seed collected from standing crop. Fifteen isolates of *A. ochraceus* were purified by single spore isolation and named as DRAo 001 to DRAo 015. Ochratoxin A were isolated from the *A. ochraceus* isolates (DRAo 008 and DRAo 013) and identified on TLC plates by using the different solvent systems. The OTA isolated in this study and standard showed the same R_f values (Table 2) in different solvent systems. The OTA was confirmed on TLC plate by changing the colour from greenish blue to blue after spraying the 6% NaOH in 20% ethanol.

Table 1: Occurrence of *Aspergillus ochraceus* on seed samples

Variety	Storage condition	Percent Infection
Aduthurai (Tamil Nadu)		
ADT 38	Open exposed to rain	10
ADT 39	”	15
ADT 39	”	22
ADT 39	”	38
ADT 39	”	32
CO 43	”	59
CO 43	”	17
CO 43	1 year old seed	73
ADT 44	”	3
Mean		28.5
Arundathinagar (Tripura)		
Kalikasa	Open exposed to rain	1
Kalikasa	”	66
Garomalati	”	28
Pechibadam	”	47
Mean		35.5
Bloomsdale (Andaman andNicobar islands)		
M 55	Damp condition	1
Zengnit	”	1
Quing livan	”	1
MTI 113	”	1
CARI 1	”	3
Mean		1.4
Andhara Pradesh		
BPT 5204	4 years godown	6
Sonamashuri	”	6
Vijaya mashuri	”	11

Table 1: Continued

Variety	Storage condition	Percent Infection
Safari	Standing crop	1
Khitish	Stored in godown	1
NLR 34242	Rice mill	6
NLR 2222	”	3
JJ	1 year old seed	1
Mean		4.3
Mandya (karnataka)		
Mixed seed	3 years old seed	2
Mean		2
Megalaya		
DR 92	Stored in godown	50
Canto 51	”	27
Mean		38.5
Navsari (Gujarat)		
GR 5	4 years old seed	13
Mean		13
Pusa (Bihar)		
Pankaj	2 years old seed	99
Rajshree	”	75
Satyam	”	100
Pankaj	”	28
Kisori	1 year old seed	75
Sugandha	”	42
Prabhat	”	82
Pankaj	Open exposed to flood	24
Pankaj	”	83
Satyam	”	41
Rajshree	”	70
Mean		65.3
Rewa (Madhya Pradesh)		
Jaya	Stored in godown	7
Mean		7
Titabar (Assam)		
Ranjit-sali	2 years old seed	22
Piyolee-sali	”	74
Bahadur-sali	”	89
Mixed seed	1year old seed	1
Mean		46.5
Over all mean		24.1
Number of isolates		46

Table 2: R_f value of ochratoxin A in different solvent systems

Solvent system	R_f value
Toluene-ethyl acetate-formic acid (6:3:1)	0.77
Benzene-methanol-acetic acid (90:5:5)	0.54
Chloroform-ethyl acetate-formic acid (10:3:1)	0.90
Chloroform-acetone-formic acid (6:2:1)	0.95
Benzene-ethyl acetate-formic acid (3:1:1)	0.90
Benzene-aceticacid (4:1)	0.75
Chloroform-diethyl ether-acetic acid (17:5:1)	0.82

Garlic bulb extract (10%) completely inhibited the fungal growth of the *A. ochraceus* while the efficacy of other plant products was only in the range of 61 to 71% even at 20% concentration (Table 3). *Pongamia* kernel extract gave good control (71%) at 20% concentration. But, neem kernel extract (0-69%), neem leaf extract (0-66%), custard apple kernel extract (0-64%) *Eucalyptus* and onion leaf extract (7-61%) were less effective in inhibiting the growth of *A. ochraceus.*

Of the *Trichoderma* isolates evaluated against *A. ochraceus*, culture filtrate of *T. virens* showed a complete inhibition of growth at 15% concentration and was significantly superior over all other *Trichoderma* sp. (Table 4). The order of performance was *T. harzianum*, which showed a moderate inhibition (37-67%) followed by *T. koningi* (8-38%), *T. viride* (10-38%), *T. reseii* (9-32%) and among

Table 3: Effect of plant extracts on the mycelial growth of *A. ochraceus*

Conc.	*Allium cepa*		*Allium sativum*		*Annona squamosa*		*Azadiracta indica Kernel*		*Azadiracta indica leaf*		*Eucalyptus terticornis*		*Pongamia glaberima*	
(%)	RG	INH	RG	INH	RG	INH	RG	INH	RG	INH	RG	INH	RG	INH
5	83	7	31	65	90	0	90	0	90	0	83	7	75	16
10	59	31	0	100	63	30	80	11	73	18	59	31	59	34
15	49	45	0	100	58	38	45	50	53	41	49	45	39	56
20	35	61	0	100	32	64	20	69	30	66	35	61	26	71
Control	90	0	90	0	90	0	90	0	90	0	90	0	90	0
CD (p = 0.5)	2.2	-	2.1	-	3.3	-	4.6	-	2.0	-	6.6	-	3.9	-
CV (%)	1.9	-	2.2	-	2.7	-	3.7	-	1.6	-	5.7	-	3.6	-

RG-Radial growth (mm), INH- % Inhibition over control

Table 4: Effect of *Trichoderma* culture filtrates on mycelial dry weight of *A. ochraceus*

Conc.	*T. viride*		*T. virens*		*T. harzianum*		*T. koningi*		*T. reseii*	
(%)	MDW	INH	MDW	INH	MDW	INH	MDW	INH	MDW	INH
5	4	10	1.3	70	2.8	37	4.1	8	4.0	9
10	3	31	0.7	84	2.0	54	3.1	30	3.3	25
15	2.8	38	0	100	1.4	67	2.7	38	3.0	32
Control	4.4	-	4.4	-	4.4	-	4.4	-	4.4	-
CD (p = 0.5)	0.6	-	0.1	-	0.2	-	0.2	-	0.2	-
CV (%)	8.4	-	3.8	-	4.8	-	3.3	-	3.8	-

MDW- Mycelial dry weight (g), INH-% Inhibition over control

Table 5: Effect of *Pseudomonas fluorescens* culture filtrates on mycelial weight of *A ochraceus*

Conc.	DRPf001		DRPf002		DRPf003		DRPf004		DRPf005	
(%)	MDW	INH	MDW	INH	MDW	INH	MDW	INH	MDW	INH
5	4.1	8	0.9	80	3.3	26	4.1	8	1.0	77
10	3.4	24	0.5	88	2.9	35	3.9	13	0.7	84
15	2.8	37	0.1	98	2.2	51	3.5	22	0.3	93
Control	4.5	0	4.5	0	4.5	0	4.5	0	4.5	0
CD (p = 0.5)	0.14	-	2.1	-	0.15	-	0.1	-	2.5	-
CV (%)	5.5	-	4.2	-	3.8	-	5.2	-	4.1	-

MDW- Mycelial dry weight (g), INH-% Inhibition over control

Table 6: Effect of fungicides on mycelial growth of *A. ochraceus*

Conc.	Propineb		Bitertinol		Carbendazim		Tricyclazole	
(ppm)	RG	INH	RG	INH	RG	INH	RG	INH
100	59	34	78	13	0	100	53	41
250	48	47	68	24	0	100	50	44
500	33	63	53	41	0	100	48	46
750	0	100	49	45	0	100	23	75
1000	0	100	42	53	0	100	0	100
Control	90	0	90	0	90	-	90	0
CD (p = 0.5)	2.0	-	1.8	-	-	-	2.4	-
CV (%)	2.8	-	1.5	-	-	-	3.1	-

RG- Radial growth (mm), INH-% Inhibition over control

the five strains of *Pseudomonas fluorescens* culture filtrates the DRPf 002 were effectively inhibited the growth (98%) at 15% concentration followed by DRPf 005 (93%). The others are less effective in reducing the growth of *A. ochraceus* even at 15% concentration (28-51%) (Table 5).

All the fungicides were significantly effective in reducing the growth of *A. ochraceus* over control (Table 6). Of the test fungicides, carbendazim was significantly effective (100%) even at 100 ppm.

Table 7: Effect of Chemicals on mycelial growth of *A. ochraceus*

Conc.	Acetic acid		Benzoic acid		Propionic acid		Sodium chloride		Vanillin	
(%)	RG	INH	RG	INH	RG	INH	RG	INH	RG	INH
0.1	35	61	0	100	24	73	90	0	82	9
0.2	0	100	0	100	15	83	90	0	71	21
0.3	0	100	0	100	0	100	90	0	65	28
0.4	0	100	0	100	0	100	90	0	45	50
Control	90	-	90	-	90	-	90	-	90	-
CD (p = 0.5)	1.6	-	-	-	1.4	-	0	-	2.9	-
CV (%)	3.6	-	-	-	3.1	-	0	-	2.3	-

RG- Radial growth (mm), INH- % Inhibition over control

Among the other test formulations, propineb was found highly effective at 750 ppm, where it had shown 100% inhibition of the test Aspergilli. However, the efficacy of tricyclazole and bitertinol was only in the range of 53 to 100% on *A. ochraceus* at the highest concentration tested. The fungicidal effect was more pronounced in all the test fungicides with any increase in concentration from 100 to 1000 ppm. Among the chemicals benzoic acid were completely inhibited the mycelial growth at 0.1% followed by acetic acid at 0.2% concentration (Table 7).

DISCUSSION

The data on survey revealed *A. ochraceus* in 46 rice samples. The infection of *A. ochraceus* dominated in the samples collected from Pusa. These results confirm the earlier observations of Trung *et al*. (2001) had also reported the *A. ochraceus* as one of the most predominant fungi in the rice samples collected from mills of Mekong delta. Canton, Vietman. Reddy (1990) had also reported *Aspergillus* sp. as one of the most predominant fungus in the grain samples of flood affected paddy variety NLR 9672 collected from standing crop, threshing floors and storage sites in Nellore district of Andhra Pradesh.

Eucaris *et al*. (1982) used a TLC based technique for the estimation and quantification of Ochratoxin A in rice and other vegetable foods. Golinski and Grabarkiew (1984) developed TLC based technique for the confirmation of Ochratoxin A on TLC by spraying of NaOH. Santosh and Vargas (2002), Fredrik *et al*. (1981) also developed TLC for detection of OTA by using different solvent systems. We also used the Eucaris method for extraction and Fredrik method for identification of OTA produced by *Aspergillus ochraceus* isolates.

Arun *et al*. (1995) reported the fungitoxic effect of garlic against *Aspergillus niger* and *Fusarium pallidoroseum* in pigeon pea. Our study on effect of plant extracts on *A. ochraceus* showed that garlic bulb at 10% concentration was the most effective treatment in reducing the growth compared to other plant products. The fungitoxicity of the garlic bulb extract in this study might be due to antifungal substances present in garlic namely sulphur containing compounds like allicin, allylpropyl disulphide or diallyl disulphide, or due to an enzyme allinase (Radha *et al*., 1998).

The studies on antagonistic effect of culture filtrates of five *Trichoderma* species against *A. ochraceus* showed that at 15% concentration, *Trichoderma virens* was the most effective in reducing the growth of test Aspergilli compared to other culture filtrates. Calistru *et al*. (1997) has also reported a similar potential for biological control of *A. ochraceus* using *Trichoderma* sp., based on *in vitro* results.

Shekhawat *et al*. (1986) had reported the effective control of the seed borne inoculum in groundnut following the seed treatment with a higher dose of carbendazim (2.5 g kg^{-1}). This study however, showed that carbendazim even at 100 ppm concentration was the most effective fungicide in reducing the growth of *A. ochraceus* compared to the other fungicides. Chipley and Uraih (1980)

had reported the control of *Aspergillus* sp. and Aflatoxin release by derivarives of benzoic acid. In this study we found that the benzoic acid were completely inhibited the mycelial growth of *A. ochraceus* at 0.1% concentration.

Present study had shown that plant extract like garlic bulb, biological agents like *Trichoderma virens* and *Pseudomonas fluorescens*, fungicide like carbendazim and chemical like benzoic acid prevent or drastically reduce the incidence of *A. ochraceus* on rice/grains during storage. Therefore, further studies are warranted to deploy them usefully as seed/grain treatment to avoid the occurrence of OTA contamination in grains and chemical characterization of OTA and development of antibodies and ELISA technique to check the OTA contamination by qualitatively and quantitatively in rice grains.

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REFERENCES

Arun, A., R. Chuhan and C. Arya, 1995. Effect of allicin and extracts of garlic and *Bignonia* on two fungi. J. Mycol. Plant Pathol., 25: 316-318.

Bars, J. and P. Bars, 1992. Fungal contamination of aromatic herbs, aflatoxinogenesis and residues in infusions. Microbiol. Aliment. Nutr., 10: 267-271.

Begum, F. and N. Samajpati, 2000. Mycotoxin production on rice, pulses and oil seeds. N aturwissenschaffen, 87: 275-277.

Calistru, C., M. McLean and P. Berjak, 1997. *In vitro* studies on the potential for biological control of *Aspergiulls flavus* and *Fusarium moniliforme* by *Trichoderma* species. 1. Macroscopical and microscopical observations of fungal interactions. Mycopathologia, 139: 115-121.

Carlos, A.P., L.B. Reinaldo, O.D. Paulo and B. Correa, 2000. Mycoflora and aflatoxigenic species in derivatives of milled rice Ciênc. Tecnol. Aliment. , 20 : http://www.scielo.br/scielo.php.

Chipley, J.R. and N. Uraih, 1980. Inhibition of *Aspergillus* growth and Aflatoxin release by derivatives of benzoic acid. Applied Environ. Microbiol., 40: 352-357.

Cotty, P.J., 1994. Comparison of four media for the isolation of Aspergillus group fungi. Mycopathologia, 125: 57-62.

Eucaris, A., I. Sarmiento and D. Klaus, 1982. Quantitative determination of Ochratoxin A in vegetable foods. Anal. Bioanal. Chem., 331: 511-513.

Fredrik, C., C. Stromer, E. Hansen, I. Pedersen, H. Vistendahl and J. Aasen, 1981. Formation of (4R)- and (4S)-4-hydroxyochratoxin a from ochratoxin a by liver microsomes from various species. Applied Environ. Microbiol., 42: 1051-1056.

Golinski, P. and J. Grabarkiew, 1984. Chemical confirmatory tests for Ochratoxin A, citrinin, penicillic acid, sterigmatosystin and zearalenone performed directly on thin layer chromatographic plates. J. Assoc. Off. Anal. Chem., 67: 1108-1110.

Nene, Y.L. and P.N. Thapliyal, 1971. Fungicides in Plant Disease Control. (Oxford and IBH Publishing Co. pvt. Ltd. New Delhi).

Radha, M., S.R. Mala, G. Revathy and A.R. Solayappan, 1998. *In vitro* antifungal properties of garlic (*Allium sativum* L.) extracts against *Colletotrichum falcatum* Went. J. Biol. Cont., 12: 81-83.

Reddy, C.S., 1990. Effect of seed dressing fungicides on seed borne infection in rice. Extended Summary. Proc. Int. Symp. Rice Research: New Frontiers, 1990, Directorate of Rice Research, Hyderabad, pp: 247-248.

Reddy, C.S., K.R.N. Reddy, N. Raja Kumar, G.S. Laha and K. Muralidharan, 2004. Exploration of aflatoxin contamination and its management in rice. J. Mycol. Plant Pathol., 34: 816-820.

Santos, E.A. and E.A. Vargas, 2002. Immunoaffinity column cleanup and thin layer chromatography for determination of ochratoxin a in green coffee. Food. Addit. Contam., 19: 447-458.

Shekhawat, K.S., C.P. Verma and V.N. Pathak, 1986. Effect of seed-dressing fungicides on collar rot of groundnut caused by *Aspergillus niger*. Summa Phytopathologica, 12: 207-216.

Shetty, H.S., C.M. Usha, K.L. Patkar and J. Lacey, 1994. Mycotoxin contamination in developing seeds of rice, sorghum and groundnut in Mysore. Seed Res., 22: 31-38.

Trung, Y., J.D. Bailly, A. Querin, P. Lebars and P. Guerre, 2001. Fungal contamination of rice from south vietman, mycotoxinogenesis of selected strains and residues in rice. Rev. Med. Vet., 7: 555-560.

Zinedine, A., J.M. Soriano, C. Juan, B. Mojemmi, J.C. Moltó, A. Bouklouze, Y. Cherrah, L. Idrissi, R.E. Aouad and J. Mañes, 2007. Incidence of ochratoxin A in rice and dried fruits from Rabat and Sale area, Morocco. Food Add. Contam., 24: 285-291.