ISSN : 0974 - 7435 Volume 5 Issue 1

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 5(1), 2011 [1-4]

Incidence of toxigenic fungi in rotting fruits of banana

Supriya Sarkar*, Penugonda Shilpa, Sivadevuni Girisham, Solipuram Madhusudan Reddy

Dept. of Microbiology, Kakatiya University, Warangal - 506 009, (INDIA)

E-mail : supriya.sarkar4@gmail.com

Received: 17th April, 2010 ; Accepted: 27th April, 2010

INTRODUCTION

Banana (*Musa paradisiaca* L.) is an important commercial fruit crop of India, accounting for about 37% of the total fruit production and provides livelihood security to millions of people. The banana fruits being succulent are liable to damage and deterioration during harvesting, transportation, marketing, storage and consumption, if not properly handled. The deterioration mainly results due to physical injuries and enzymatic action by the attack of microorganisms. There are several reports of post-harvest diseases of banana from different countries[7,17,19,33]. Similarly Roy et al.[28], Chandra[5], Rawal and Summanwar[27], Jamaluddin et al.[16], Chitra and Arun[6] and Pawar and Papdiwal[21] have reported several post-harvest diseases of banana from India.

Several fungi associated with agricultural commodities are reported to produce variety of biologically active secondary metabolites which are channelised to animal and human systems, incite different health hazards and cause diseases known as mycotoxicoses. Among the thousands of species of fungi, only about 100 belonging to genera *Aspergillus*, *Fusarium* and *Penicillium* are known to produce mycotoxins[4]. Though there are several reviews of contamination of various food commodities[2,20,23,34-36], only scattered reports of mycotoxin contamination of either fresh or dried fruits are available[9,10,14,30,37]. However, little or no such reports on the incidence of mycotoxigenic fungi in rotting fruits of banana are available from Warangal (Andhra Pradesh), India. Hence, in the present investigation fungal infected banana fruits were analysed for the presence of different mycotoxins.

MATERIALS AND METHODS

Isolation of fungi

A regular survey of fruit godowns of wholesale and retail markets of Warangal (A.P.) was undertaken during Nov 2005 to Oct 2006 for studying the post-harvest fungal diseases of banana (*Musa paradisiaca* L.) and the diseased fruits were collected separately in polythene bags to avoid contamination. Isolations were made from the juncture of healthy and diseased regions on the peel of the infected banana fruits. The diseased tissues were surface sterilized with 90% ethyl alcohol and transferred aseptically to Asthana and Hawker's medium slants (5g glucose; 3.5g KNO_3; 1.75 g KH_2PO_4; 0.75 g $MgSO_4.7H_2O$; 15g agar-agar and 1000 ml of distilled water).The pH of the medium was adjusted to 6.5 with 0.1N HCL or 0.1 N NaOH. The inoculated slants were incubated at room temperature (28 $\pm$ 2°C) for about 5-6 days. The fungal colonies were isolated from the third day until the seventh day and identified by standard monographs[3,11,29]. The fungus was designated as a pathogen only after satisfying Koch's postulates. The percentage of incidence of individual fungi was calculated using the following formula.

$$\text{Percent incidence} = \frac{\text{No. of colonies of a species in all the plates}}{\text{Total no. of colonies of all the soecies in all the plates}} \times 100$$

Chemicals

Agar-agar, ethyl acetate, glucose and all other chemicals of the highest available purity were obtained

FULL PAPER

from Himedia, Mumbai, India.

Identification of mycotoxins

The possible production of mycotoxins by some of the fruit-rot fungi was assessed by employing Thin Layer Chromatography (TLC). The naturally infested and inoculated fruits were homogenized and the mycotoxins were extracted with chloroform/ethylacetate. Subsequently they were passed through Na_2SO_4 (anhydrous) bed to remove moisture and evaporated to dryness before dissolving in suitable solvent mixtures and spotting onto the TLC plates.

The mycotoxin extract was spotted on TLC plate along the imaginary line (approximately 2 cm above the bottom edge of the plate) by using microlitre syringe. The spotted plates were developed in a suitable solvent by ascending chromatography.

The toxins were identified by spraying the plates with different spray reagents (TABLE 1) and the compounds thus separated were identified based on the color of the fluorescence of the spot and by the R_f values, as compared with standards .The R_f value was

TABLE 1 : Detection of different mycotoxins produced by species of *Aspergillus, Penicillium* and *Fusarium*

Name of the mycotoxin	Solvent system	Spray reagent	Detection		Reference
			UV	Visible	
Aflatoxin (B_1, B_2, G_1, G_2)	C: A (95 : 5)	--	Blue and green	--	Stack and Pohland (1975)
Ochratoxin A	T : Ea : F (6 : 3 : 1)	1	Bright blue	--	Gimeno (1979)
Patulin	T : Ea : F (6 : 3 : 1)	2	--	Yellow	Subramanian (1982)
Sterigmatocystin	C : M : A (1 : 1 : 1)	1	Yellow	--	Adye and Mateless (1964)
Citrinin	T : Ea : F (6 : 3 : 1)	3,4,5	--	y, by, lb	Gorst-Allman and Steyn (1979)
Cyclopiazonic acid	T : Ea : F (6 : 3 : 1)	3,4,5,6	--	b, rb, br, p	Rathinavelu and Shanmugasundaram (1984)
Ochratoxin A	T : Ea : F (6 : 3 : 1)	4,5,7	-, -, b	y, pb, -	Piskorska and Jusckiewicz(1977)
Penitrem A	C : M (97 : 3)	3,4,5	--	p, p, g	Gorst-Allman and Steyn (1979)
Deoxynivalenol (DON)	C : M (97 : 3)	8,10,12	-, ch, bl	y, -, -	Ramakrishna and Bhatt (1987)
Diacetoxy scirpenol (DAS)	C : M (97 : 3)	10,11	Bg , -	-, br	Ramakrishna and Bhatt (1987)
Fusarenone - X	C : M (97 : 3)	1	bl	--	Kamimura *et al.* (1981)
Nivalenol (NIV)	C : M (97 : 3)	9,10,12	-, ch, bl	y, -, -	Ramakrishna and Bhatt (1987)
T-2 Toxin	C : M (97 : 3)	10, 11	bg, -	-, p	Ramakrishna and Bhatt (1987)
Zearalenone	C : M (97 : 3)	3,4,5,9,10,12	-, -, -, br, ch, bl	br, do, lp,-,-,bg	Gorst-Allman and Steyn (1979)
Trichothecin	C : M (98 : 2)	13	--	Pink	Rao *et al.* (1985)
Trichodermin	C : M (98 : 2)	13	--	pink	Rao *et al.* (1985)

Solvent systems: A = Acetone; C = Chloroform; F = Formic acid; Ea = Ethyl acetate; M = Methanol; T = Toluene
Spray reagents: 1= 20% $AlCl_3$; 2= $_2$% Phenyl hydrazine hydrochloride: 3 = $Ce(SO_4)_2$ 1% in 6 N H_2SO_4; 4 = 2,4-DNP; 5 = $FeCl_3$ 3% in ethanol; 6 = 1% p-dimethylaminobenzaldehyde in n-butanol and HCl fumes; 7 = Ammonia fumes; 8 = p-anisaldehyde; 9 = H_2SO_4 ; 10= 20% H_2SO_4; 11 = Chromatropic acid; 12 = 50% H_2SO_4 in methanol; 13 = Phloroglucinol
Detection colours : bl = Blue ; Ch = Charring; Y = Yellow ; bg = Blue green ; br = Brown ; P = Purple ; do = Dark orange ; lp = Light purple ; by = Brown yellow ; b = Black; rb = Red brown; pb = Purple brown ; g = Grey ; bg = blue green

calculated by using the formula:

$$\mathbf{Rf = \frac{Distance\ traveled\ by\ the\ compound}{Distance\ traveled\ by\ the\ solvent}}$$

RESULTS AND DISCUSSION

From TABLE 2 it is clear that a considerable percentage of fruits of banana were getting spoiled by the time it reaches to the consumer. The loss caused due to the fungal infection was maximum during August to February, probably because of high humidity and favourable temperature for the fungal attack. Based on the percentage of incidence *Fusarium moniliforme, F.oxysporum, Aspergillus flavus* and *Penicillium citrinum* occurred considerably with highest percentage. Since majority of these fungi are reported to be mycotoxigenic, one has to examine for the mycotoxin contamination. Incidence of some mycotoxigenic species such as *Aspergillus flavus, A. nidulans, A. ochraceus, A.terreus*, species of *Fusarium* and *Penicillium* suggests the possibility of elaboration of mycotoxins during their infestation and may pose a health hazard.

TABLE 3 reveals that *Aspergillus flavus, A.nidulans, A.ochraceus* and *A.terreus* were found to elaborate aflatoxins, sterigmatocystin, ochratoxin A and patulin respectively in rotting fruits of banana.

Harrison[15] and Harjeet and Geeta[14] have reported the incidence of species of *Penicillium* and *Aspergillus* and their toxins in apple products and dehydrated vegetables. Similarly Sangeeta et al.[30] have also assessed the mycotoxin producing potential of pathogenic fungi causing vegetable rots. Out of different *Fusarium* species isolated from banana fruits only *Fusarium moniliforme, F. oxysporum* and *F.poae* have elaborated zearalenone and T-2 toxin respectively in infected banana fruits. Dilip Chakrabarti and Ghosal[8] have detected trichothecolones and zearalenone contamination in banana fruits infected by *F.moniliforme*. Similarly *Trichoderma viride* and *Trichothecium roseum* elaborated Trichodermin and Trichothecin toxins respectively.

TABLE 2 : Incidence (in percentage) of toxigenic fungi in rotting fruits of banana during 2005-2006

Name of the fungus	2005		2006									
	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Aspergillus flavus	20.0	23.3	--	11.0	16.7	10.0	17.0	16.7	9.1	15.4	16.7	12.5
A.nidulans	19.2	10.3	--	--	8.3	--	--	--	7.2	--	--	17.5
A. ochraceus	--	--	4.1	20.0	--	--	--	--	14.2	--	--	--
A. terreus	--	--	--	--	19.9	10.0	--	--	--	--	5.6	--
F. moniliforme	10.0	18.2	33.2	22.3	8.3	10.0	--	17.6	27.2	15.4	16.7	12.5
F. oxysporum	20.0	27.2	16.7	--	16.7	20.6	17.0	--	--	7.7	5.6	--
F. poae	--	--	15.6	--	--	10.0	--	--	8.3	--	--	12.5
Penicillium citrinum	--	9.1	--	20.0	20.0	--	19.9	19.4	9.1	20.4	12.3	21.2
Trichoderma viride	--	--	11.1	--	--	--	--	16.7	--	--	--	--
Trichothecium roseum	--	--	--	--	--	20.0	16.4	17.2	9.1	11.1	5.6	--
Other fungi	29.6	12.2	18.8	27.2	23.3	11.4	29.8	12.5	14.5	31.1	33.2	25.0

(—) = not detected

TABLE 3 : Mycotoxins detected in rotting fruits of banana

Name of the fungus	No. of strains examined	No. of toxin producing strains	R_f value	Mycotoxin
Aspergillus flavus	15	B_1, B_2 3	0.39	Aflatoxins
		G_1, G_2 2	0.28	
A. nidulans	7	3	0.91	Sterigmatocystin
A. ochraceus	9	1	0.60	Ochratoxin A
A. terreus	8	3	0.45	Patulin
Fusarium moniliforme	11	3	0.73	Zearalenone
F. oxysporum	10	3	0.80	Zearalenone
F. poae	8	1	0.40	T-2 toxin
Penicillium citrinum	9	5	0.96	Citrinin
Trichoderma viride	2	1	0.97	Trichodermin
Trichothecium roseum	3	1	0.97	Trichothecin

CONCLUSION

Mycotoxins, secondary metabolites of storage moulds, have been receiving increasing attention in view of their undisputed role in public health. People in tropical countries consume sizable amounts of banana fruits as such and in the form of processed food. Fungal infections of banana fruits, being of wide occurrence in the world, could cause serious health problems in humans when the infected fruits are ingested for a prolonged period of time. From the present study it can be concluded that there is a need for regular monitoring of banana fruits during storage and marketing especially during warm and humid months when fungal infestation and concomitant production of mycotoxins is at the maximum. Further more, fungal infection and elaboration of mycotoxins should also be monitored periodically.

Thus the present attempt was made to assess the mycotoxin contamination in banana fruits at the post-harvest stage and to project the future problems in the light of changing conditions with special reference to tropical countries. But the task of this magnitude cannot be accomplished unless and until help and encouragement is received from different corners to probe further in this direction to get better insight.

ACKNOWLEDGEMENT

Thanks are due to Head, Department of Microbiology, Kakatiya University for providing necessary facilities.

REFERENCES

[1] J.Adye, R.C.Mateless; Biochem.Biophy.Acta, **86**, 418 **(1964)**.

FULL PAPER

[2] Anonymous, 'Maximal Acceptable Mycotoxin Concentrations In Foods of National and International Origin', Italian Ministry of Health, Circular dated June 9th, **(1999)**.

[3] H.L.Barnett, B.B.Hunter; 'Illustrated Genera of Imperfect Fungi', Burgess Publishing Company, U.S.A., **(1972)**.

[4] J.R.Barrett; Environ Health Prospects Focus A, 20-23 **(2000)**.

[5] K.J.Chandra; 'Status of Banana Diseases in India', In Banana diseases in Asia and the Pacific, Proc.Tech.Meet., Diseases Affecting Banana and Plantain in Asia and Pacific Brisbane, Australia, R.V.Valmayor, B.E.Umali, P.C.Bejosano, Eds.; INIBAP, Montpellier, France, 38-43 **(1991)**.

[6] A.Chitra, A.Arun; Aerobiologia, **23**, 283-289 **(2007)**.

[7] Z.J.M.Cordeiro, A.P.D.Matos; Informe Agropecuario, **26**, 12-16 **(2005)**.

[8] K.Dilip Chakrabarti, S.Ghosal; Applied and Environmental Microbiology, **51**, 217-219 **(1986)**.

[9] K.M.Dombrink; 'Economic Aspects of Mycotoxins in Fruits and Vegetables', In: Barkai R.Golan, P.Nachman, Ed,; Mycotoxins in Fruits and Vegetables, New York, Academic Press. Chapter 2, 27-44 **(2008)**.

[10] S.Drusch, W.Ragab; Food Control, **9**, 119-126 **(1998)**.

[11] M.B.Ellis; 'More Dematiaceous Hyphomycetes', Commonwealth Mycological Institute, Kew, Survey, England, **(1976)**.

[12] A.Gimeno; J.Assoc.off.Anal.Chem., **63**, 579-585 **(1979)**.

[13] C.P.Gorst-Allman, P.S.Steyn; J.Chromatogr., **175**, 325-331 **(1979)**.

[14] K.Harjeet, S.Geeta; Indian Phytopath., **59**, 460-466 **(2006)**.

[15] M.A.Harrison; J.Food Safety, **9**, 147-153 **(1989)**.

[16] S.Jamaluddin, M.G.Goswami, B.M.Ojha; 'Fungi of India', (1989-2001), M/S Scientific Publ., Jodhpur, India, **(2004)**.

[17] D.R.Jones, R.H.Stover; 'Fungal Diseases of Banana Fruit', Pre-harvest Diseases, In: Diseases of Banana, Abaca, D.R.Enset, Jones (Ed.), Wallingford, UK, CABI Publishing, 173-190 **(2000)**.

[18] H.Kamimura, M.Nishijima, K.Yasuda, K.Saito, A.Ibe, T.Nagayama, H.Yoshiyama, Y.Naoi; J.Assoc.off.Anal.Chem., **64**, 1067-1073 **(1981)**.

[19] M.B.Llyas, M.U.Ghazanfar, M.A.Khan, C.A.Khan, M.A.R.Bhatti; Pak.J.Agri.Sci., **44**, 534-539 **(2007)**.

[20] R.Neeta, S.Singh; J.Ind.Bot.Soc., **75**, 283-285 **(1996)**.

[21] B.T.Pawar, P.B.Papdiwal; Bioinfolet., **5**, 37-39 **(2008)**.

[22] P.Piskorska, T.Jusckiewicz; Arch.Inst.Pasteur.Tunis, **3-4**, 279-288 **(1977)**.

[23] A.Pittet; Review Medicine Veterinaire, **149**, 479-492 **(1998)**.

[24] Y.Ramakrishna, R.V.Bhatt; Curr.Sci., **56**, 524-526 **(1987)**.

[25] G.V.Rao, P.S.Rao, S.Girisham, S.M.Reddy; Curr.Sci., **54**, 507-509 **(1985)**.

[26] A.Rathinavella, E.R.B.Shanmugasundaram; J.Assoc.off.Anal.Chem., **67**, 38-40 **(1984)**.

[27] R.D.Rawal, A.S.Summanwar; 'Diseases of Banana and Their Management', In: Diseases of Fruits and Vegetables and Their Management, (Ed.: T.S.Thind), Kalyani Publishers, 111-127 **(2001)**.

[28] A.K.Roy, C.P.Singh, D.K.Singh; Indian Phytopathology, **42**, 202-203 **(1989)**.

[29] R.A.Samson, E.Moekstra, C.N.Van; 'Introduction to Food Borne Fungi', Instiute of Royal Netherlands Academy of Arts and Science, **(1984)**.

[30] G.Sangeeta, R.Aggarwal, P.Ahluwalia; Indian Phytopath., **62**, 137-143 **(2009)**.

[31] M.E.Stack, A.E.Pohland; J.Assoc.off.Anal.Chem., **58**, 110-113 **(1975)**.

[32] T.Subramanian; J.Assoc.off.Anal.Chem., **65**, 5-7 **(1982)**.

[33] A.Sulali, A.Krishanthi, D.Ranjith, W.Shant, A.Luxshmi; Mycopathologia, **157**, 91-97 **(2004)**.

[34] Trucksess; J.AOAC, **84(1)**, **(2001)**.

[35] R.J.Verma, A.S.Kolhe, H.C.Dube; Proc.Nat.Acad. Sci., **65**, 167-170 **(1995)**.

[36] T.Vrabcheva, E.Usleber, R.Dietrich, E.Martlbauer; J.Agric.Food Chem., **4**, 2483-2488 **(2000)**.

[37] A.A.Zohri, K.M.Abdel-Gawad; Journal of Basic Microbiology, **33**, 279-288 **(1993)**.