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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (11): 1386-1389, 2001

Mycotoxins and Invertase Enzyme of the Mycoflora of Molasses in Upper Egypt

A. H. M. El-Said
Botany Department, Faculty of Science, South Valley University, Qena, Egypt

Abstract: Ninety- two species and one species variety belonging to 12 genera were isolated from 30 samples of molasses on 1% glucose (10 genera, 22 species and one variety) and 50 % sucrose (7, 21 and one) Czapek's agar at 25 °C media. *Aspergillus, Mucor, Mycosphaerella* and *Penicillium* were the most common genera on the two types of media. From the above genera the most prevalent species were: *Aspergillus flavus, A. fumigatus, A. niger, Mycosphaerella tassiana, Penicillium chrysogenum, P. oxalicum* and *P. purpurogenum*. Also, some species were only isolated on 50% sucrose such as *Eurotium amstelodami, E. chevalieri E. repens, Humicola fuscoatra, Penicillium aurantiogriseum* and *P. puberulum*. About 65 fungal isolates were isolated from 50% sucrose agar tested for their ability to produce invertase enzyme in liquid medium and 93. 8% of the isolates could produce this enzyme. From the positive isolates, 32 showed high invertase activity, 21 had moderate activity and the remaining 8 isolates were of weak activity. 60 isolates of *Aspergillus, Emericella, Eurotium, Mycosphaerella* and *Penicillium* from the preceding study were screened for the presence of their respective mycotoxins. Larva of brine shrimp (*Artema salina* L.) were used for toxicity test of the fungal isolates crude extracts. With 3 isolates out of 60 tested being toxic. Using thin-layer chromatographic technique, 5 different known mycotoxin were detected as aflatoxins : B1, B2, G1, G2 and citrinin.

Key words: Mycoflora of molasses, invertase enzyme and mycotoxins

Introduction

Molasses are considered to be one of the most important consumed food in Upper Egypt. Molasses has traditionally been marketed as a popular and public food (meal) especially for kids due to its richment with energy and minerals. Molasses is easily invaded by microorganisms. Scott (1989) considered that prolonged storage could permit mould growth particularly if the package is opened. These moulds exhibit the potential to produce toxic metabolites (Northolt *et al.*, 1995) and therefore proliferation of the organisms is to be regarded as a potential health hazard. Many studies were made in the uses of microorganisms in transformation and fermentation of molasses into ethanolic and organic acid products (Rohr *et al.*, 1983, Galazzo and Bailely, 1990, Nolan *et al.*, 1994, Nigam *et al.*, 1997, Parvez *et al.*, 1998 and Sheoran *et al.*, 1998).

Several fungal isolates are able to produce extracellular enzymes in the substrate such as invertase enzyme (Abdel-Sater and Ismail, 1993; Abdel-Sater and Saber, 1999).

Moulds contamination on molasses affects the yielded quality and nutritional value of the products, molasses may contain toxic moulds metabolites (Barakat *et al.*, 1999) as a result of mould growth. The secondary metabolites of fungi known as mycotoxins have gained considerable importance during the past two decades as health hazards to animals and man (Purchase, 1974). Toxicity and toxin production by members of the genera *Aspergillus, Emericella, Eurotium, Mycosphaerella* and *Penicillium* were extensively investigated in this laboratory (El-kady and Abdel-Hafez, 1981; El-Maraghy and Zohri, 1988, Abdel-Mallek *et al.*, 1993, El-Kady *et al.*, 1994, Abdel-Sater *et al.*, 1996, Abdel-Sater and Saber, 1999 and Barakat *et al.*, 1999).

No information has been reported on fungal flora and mycotoxins of molasses in Upper Egypt. So, the objective of this study was to investigate the mycoflora of molasses. In addition the ability of these fungi for production of invertase enzyme and the potentialities of the isolates fungi for mycotoxins production.

Materials and Methods

Collection of samples: Thirty random samples of molasses were collected from different markets during the winter months 2000 from some governorates in Upper Egypt (Sohage, Qena and Aswan). Samples were transferred to the laboratory and kept at 4 °C in a refrigerator till fungal analysis.

Determination of fungi : The dilution -plate method was used for determination of molasses moulds (Johnson and Curl, 1972). Two types of media were used: glucose-and 50% sucrose Czapek's Dox agar for isolation of glucophilic and osmophilic (or osmotolerant) fungi, respectively. Rose bengal (30 mg\ L) and chloramphenicol (200 mg \ L) were added to the above media as bacteriostatic agents. Six plates were used for each examined samples (3 plates for each medium). Plates were incubated at 25 °C for 7 days in the case of glucose and 15 days for sucrose plates. The developing fungal colonies were counted, identified and the numbers were calculated per ml of molasses.

Screening for invertase production: Sucrose hydrolysis by the isolates recovered on 50% sucrose were tested on liquid medium of 50% sucrose-Cazpek's. After the incubation at 28 °C for 10 days the sucrase (invertase) activity was determined in the culture filtrate using Fehling's solution, a positive result was indicated by a yellow, green or brown precipitate.

Cultivation and extraction of the fungal toxins: The test fungi were collected from the molasses samples on 50% sucrose in this study. All isolates were cultivated on Czapek's liquid medium fortified by 2 g yeast extract and 10 g peptone and incubated at 28 °C for 10 days at static cultures.

After the incubation period, the content of each flask (medium + mycelium) were homogenized for 5 min in a high speed blender (1600 r. p. m.) with 100 ml chloroform. The extract procedure was repeated three times. The combined chloroform extracts were washed with equal volume of distilled water, dried over anhydrous sodium sulphate, filtered then concentrated under vacuum to near dryness, and diluted to 1 ml with chloroform.

Thin- layer chromatographic separation of mycotoxins: For qualitative identification, a thin- layer chromatographic technique was employed, and mycotoxins were identified by comparison with appropriate reference standards (Moss, 1971 Wilson, 1971; Gimeno, 1979 and Van Egmond *et al.*, 1980).

Bioassay method for mycotoxins: The immature brine shrimp (*Artemia salina* L.) was used for mycotoxins bioassay. The test has been used for aflatoxins (Biji *et al.*, 1981) and for other mycotoxins (Scott *et al.*, 1980).

Results and Discussion

Twenty-nine species and one variety of *A. terreus* var. *aureus* belonging to 12 genera were collected from 30 samples of molasses on 1% glucose and 50% sucrose Czapek's agar at 25 °C (Table 1). In this respect Barakat *et al.* (1999) isolated 56 species and 3 varieties appertaining to 25 genera from 36 Gallab samples on 1% glucose and 40% sucrose Czapek's agar at 28 °C. In this investigation the most common genera were *Aspergillus* (8 species + 1 var.), *Mucor* (2), *Mycosphaerella* (1) and *Penicillium* (8) on the two types of media. They were encountered in 26. 7 - 83. 3 % and 26. 7 - 83. 3 % of the samples comprising 2. 02 - 79. 1 % and 1. 3 - 53. 4 % of total fungi, respectively (Table 1). These results agreed with those observed by Higgy *et al.* (1977), Sandhu & Sidhu(1980),Olufolaji(1986),Abdel-Sater and Ismail (1993), Abdel-Hafez *et al.* (1995), Muhsin and Abdel-Kader (1995), and Abdel-Sater and Saber (1999). Barakat *et al.* (1999) recorded that *Aspergillus, Mucor, Mycosphaerella* and *Penicillium* were the most prevalent genera in Gallab at upper Egypt using 1% glucose and 40% sucrose-Czapek's agar at 25 °C. The most prevalent species from the above genera were: *Aspergillus flavus, A. fumigatus, A. niger, Mucor circinelloides, M. racemosus, Mycosphaerella tassiana, Penicillium chrysogenum, P. oxalicum* and *P. purpurogenum*. They occurred in 13. 3 - 73. 3 % and 13. 3 - 83. 3% of the samples constituting 1. 1 - 39. 5 % and 0. 7 - 23. 6 % of the total fungi on the two types of media, respectively (Table 1). *Eurotium* (3 species) was frequently recovered only on 50% sucrose, emerging in 53. 3 % of the samples contributing 8. 2 % of total fungi (Table 1). *E. chevalieri* (53. 3 % of the samples and 6. 1 % of total fungi) was the most common species, while *E. amstelodami* (20 and 1. 6 %) was isolated in low frequency of occurrence. *E. repens* (10 and 0. 5%) was rarely isolated (Table 1). These results almost agree with those obtained by Abdel-Sater and Ismail (1993), Abdel-Sater and Saber (1999) and Barakat *et al.* (1999). They reported that *E. amstelodami* and *E. chevalieri* were the most common *Eurotium* species on biscuits, Dried fruits and Gallab. This genus was also isolated with variable frequencies and populations from substrates with

Table 1: Total counts (TC per ml), number of cases of isolation (NCI, out of 30 samples), occurrence remark, (OR) and percentage frequency (F) of fungal genera and species recovered from molasses on glucose and 50% sucrose-Czapek's agar at 25°C.

Genera and species	1% Glucose				50% Sucrose			
	TC	% F	NCI&OR	% F	TC	% F	NCI&OR	% F
Aspergillus	12675	79.1	25H	83.3	8150	53.4	25H	83.3
A. flavus Link	6325	39.5	22H	73.3	3600	23.6	25H	83.3
A. fumigatus Fresenius	1175	7.3	17H	56.7	925	6.1	18H	60
A. niger Van. Tieghem	4025	25.1	20H	66.7	3125	20.5	21H	70
A. ochraceus Wilhelm	125	0.78	4L	13.3	-	-	-	-
A. sydowii (Bain&Sart.)Thom& Church	275	1.7	4L	13.3	75	0.5	2R	6.7
A. terreus Thomt	375	2.3	5L	16.7	250	1.6	6L	20
A. terreus var. *aureus* Thom	125	0.8	4L	13.3	50	0.3	2R	6.7
A. ustus (Bainier) Thom & Church	125	0.8	4L	13.3	-	-	-	-
A. versicolor (Vuill.)Tiraboschi	125	0.8	4L	13.3	125	0.8	2R	6.7
Emericella nidulans (Edam.) Vuill	150	0.94	3R	10	250	1.6	5L	16.7
Eurotium	-	-	-	-	1250	8.2	16H	53.3
E. amstelodami Mangin	-	-	-	-	250	1.6	6L	20
E. chevalieri Mangin	-	-	-	-	925	6.1	16H	53.3
E. repens De Bary	-	-	-	-	75	0.5	3R	10
Humicola fuscoatra Traaen	-	-	-	-	550	3.6	2R	6.7
Mucor	325	2.02	8M	26.7	200	1.3	8M	26.7
M. circinelloides Van Tiegh	175	1.1	5L	16.7	100	0.7	4L	13.3
M. racemosus Fresenius	150	0.94	4L	13.3	100	0.7	4L	13.3
Mycosphaerella tassiana (de Not.) Johanson	350	2.2	8M	26.7	2125	13.9	9M	30
Penicillium	1975	12.3	19H	63.3	2750	18	19H	63.3
P. aurantiogriseum Dierckx	-	-	-	-	100	0.7	3R	10
P. chrysogenum Thom	800	4.9	12M	40	1825	11.9	11M	36.7
P. citrinum Thom	100	0.62	4L	13.3	-	-	-	-
P. corylophilum Dierckx	475	2.9	5L	16.7	175	1.1	5L	16.7
P. funiculosum Thom	-	-	-	-	75	0.5	3R	10
P. oxalium Currie & Thom	225	1.4	8M	26.7	200	1.3	8M	26.7
P. puberulum Bainier	-	-	-	-	150	0.98	4L	13.3
P. purpurogenum Stoll	375	2.3	9M	30	225	1.5	5L	16.7
Rhizopus stolonifer (Fhrenb) Lindt	200	1.2	3R	10	-	-	-	-
Scopulariopsis brevicaulis (Sacc.) Bainier	100	0.62	3R	10	-	-	-	-
Sterile mycelia (white & dark colour)	75	0.5	2R	6.7	-	-	-	-
Torula herbarum (Pers.) link	25	0.2	1R	3.3	-	-	-	-
Trichoderma viride Pers. ex S. F. Gray	150	0.94	3R	10	-	-	-	-
Total counts	16025				15275			
Number of genera = 12 genera	10				7			
Number of species = 29 + 1var.	22 + 1				21 + 1			

H = High occurrence, between 15-30 cases (out of 30).
M = Moderate occurrence, between 8-14. L = Low occurrence, between 4-7. R = Rare occurrence, between 1-3.

Table 2: Ability of fungal species isolated from molasses to produce invertase

Organisms	W	M	H	P	NI
Aspergillus flavus	1	4	3	8	10
A. fumigatus	-	3	5	8	8
A. niger	-	2	7	9	10
A. sydowii	-	-	1	1	1
A. terreus	-	1	2	3	4
A. terreus var. *aureus*	-	-	1	1	1
A. versicolor	1	-	-	1	1
Emericella nidulans	-	1	-	1	1
Eurotium amstelodami	-	-	2	2	2
E. chevalieri	1	2	3	6	6
E. repens	-	-	1	1	1
Humicola fuscoatra	-	1	1	2	2
Mucor circinelloides	-	1	-	1	1
M. racemosus	-	1	-	1	
1*Mycosphaerella tassiana*	1	1	1	3	3
Penicillium aurantiogriseum	1	-	-	1	1
P. chrysogenum	1	1	3	5	5
P. corylophilum	-	1	-	1	1
P. funiculosum	-	1	-	1	1
P. oxalicum	1	-	-	1	1
P. puberulum	-	1	2	2	3
P. purpurogenum	1	-	-	1	1
Total isolates	8	2 1	32	61	65

NI = Number of isolates tested. P = Positive isolates
H = High producers. M = Moderate producers.
W = Weak producers.

high osmotic potential and using sucrose or sodium chloride-Czapek's agar as an isolation medium (Hocking and Pitt, 1980; Nassar, 1986; Kozakiewicz, 1989; Khayria Abdel-Gawad and Zohri 1993 and Abdel-Hafez *et al.*, 1995).

The remaining genera and species were isolated in low or rare frequency of occurrence (Table 1). Sixty five isolates, representing 21 species and one variety of *A. terreus* var. *aureus* were tested for their ability to produce invertase enzyme in liquid medium. It was observed that the invertase production was greatly variable not only among different species, but also among different isolates of the same species (Table 2). Of the isolates tested, about 93. 8 % (61 isolates) could produce invertase enzyme. From the positive isolates 61 isolates belonging to *Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Eurotium amstelodami, E. chevalieri, Penicillium chrysogenum* and *P. puberulum* (52. 5%) had high invertase activity. Some 34. 4% of the tested isolates showed moderate invertase activity which related to *Aspergillus flavus, A. fumigatus, A. niger* and *Eurotium chevalieri* and some other isolates. The remaining 8 isolates (13. 1%) were found to be weak invertase-producers and these were related to *Aspergillus flavus* and *Mycosphaerella tassiana* and other isolates (Table 2). Abdel-Sater and Ismail (1993) tested 69 isolates, recovered from food materials (biscuits) for invertase production and noticed that all isolates tested could produce this enzyme. Also, Abdel-Sater and Saber (1999) tested 92 isolates recovered from dried fruits for invertase production and noticed that 80 isolates could produce this enzyme. Many plant species infected with pathogenic fungi have been shown to have significant increase in invertase activity (Long *et al.*, 1975, Benhamou *et al.*, 1991). In respect, Bhaskaran and Smith (1993) noticed that, in the absence of the substrate, the pathogen may not produce the enzyme. When the pathogen is studied in association with its host plant, extracellular release of the enzyme by the pathogen into the host tissues can be mistaken for an enzyme of plant origin. Also, in the absence of the host plant but in the presence of defined carbohydrates clearly show that invertase is produced by the fungus tested.

Sixty different isolates of fungi belonging to *Aspergillus* 24 (*A. flavus* 10 isolates; *A. fumigatus*, 4; *A. terreus*, 8 and *A. versicolor*, 2), *Emericella nidulans*, 8; *Eurotium*, 10., (*E. amstelodami* 4; *E. chevalieri*, 4 and *E. repens*, 2) *Mycosphaerella tassiana*, 7 and *Penicillium*, 12 isolates (*P. chrysogenum*, 6; *P. corylophilum*, 2; *P. puberulum*, 2 and *P. purpurogenum*, 2) were screened for their ability to produce mycotoxins. These fungi were collected in this study from molasses samples. Results in Table 3 show that the crude extract of one isolate out of ten tested contained aflatoxin B1, B2, G1 and G2. The crude extract of two out of eight of *Aspergillus terreus* contain citrinin as revealed from the thin layer chromatographic analysis. The crude extract of one isolate of *Aspergillus flavus* tested was highly toxic to the brine shrimp larva and 2 and 7 were moderate and of low toxicity, respectively. On the other hand the crude extract of 1, 1, 2 and 4 isolates of *A. terreus* tested were high, moderate, low and non toxic to the larvae tested, respectively (Table 3). Above results, show that *Aspergillus flavus* and *A. terreus* produced aflatoxin B1, B2, G1, G 2 and citrinin. This result agrees with those obtained previously by Sargeant *et al.* (1961), Youssef (1986), Abdel-Mallek *et al.* (1993), El-Kady *et al.* (1994), Abdel-Sater and Saber (1999) and Barakat *et al.* (1999). Of the known mycotoxins, the most important from the viewpoint of direct hazards to human health are aflatoxins (Scott, 1973). They are also the most extensively investigated mycotoxins. Aflatoxins are mutagenic, carcinogenic, teratogenic and actually toxic to most experimental and domesticated animals and man (Davis and Dienes, 1978).

In conclusion, several saprobes such as *Aspergillus, Euratium, Penicillium* and others are associated with molasses causing

Table 3: Toxin produced by different isolates of *Aspergillus*

Genera & species	Toxicity*					Mycotoxins produced
	No. of isolates produced mycotoxin	High	Moderate	Low	None	No. of isolates Produced mycotoxin
A. flavus	10	1	2	7	0	1	aflatoxin B1&B2, G1&G2
A. terreus	8	1	1	2	4	2	citrinin

* Toxicity to brine shrimp (*Artemia salina* L.)
High toxicity = More than 75% mortality of larva tested.
Moderate toxicity = More than 50-75% mortality of larva tested.
Low toxicity = More than 25-49% mortality of larva tested.
None toxicity = Less than 75 % mortality of larva tested.

deterioration of molasses. Also mycotoxins specially aflatoxins, one of the most carcinogenic substance known, therefore, it is important to take in consideration the different methods to prevent the fungal growth and mycotoxin accumulation which are hazardous to health.

Acknowledgment

The authors are deeply indebted to Prof. S. I. I. Abdel-Hafez (Professor of Microbiology, Faculty of Science, Assiut University) for their kind help.

References

Abdel-Hafez, S. I. I., A. H. El-Said and Y. A. M. H. Gherbawy, 1995. Mycoflora of leaf surface, stem, bagasse and juice of adult sugarcane (*Saccharum officinarum*) plant and cellulolytic ability in Egypt. Bull. Fac. Sci. Assiut Univ., 24: 113-130.

Abdel-Mallek, A. Y., S. S. M. El-Maraghy and A. H. Hasan, 1993. Mycotoxin-Producing potential isolates found on corn grains and sunflower seeds in Egypt. J. Islamic Acad. Sci., 6: 189-192.

Abdel-Sater, M. A. and M.A. Ismail, 1993. Ecological and enzymatic studies on fungi associated with biscuits in Egypt. International Biodeterioration and Biodegradation, 31: 277-292.

Abdel-Sater, M. A. and S. M. Saber, 1999. Mycoflora and mycotoxin in some Egyptian dried fruit. Bull. Fac. Sci. Assiut Univ., 28 :91-107.

Abdel-Sater, M. A., A. A. Zohri and M. A. Ismail, 1996. Natural contamination of some fruit juices and drinks by mycoflora and mycotoxins IX IUPAC, Int. Symp. Mycotoxins & Phytotoxins, Rome, 27-31 May.

Barakat, A., N. F. Abo-Dahab and A. H. El-Said, 1999. Mycoflora and mycotoxin associated with gallab (public sweet) in Upper Egypt. The African J. Mycol. Biotechnol. (under press)

Benhamou, N., J. Grenier and M. J. Chrispeels, 1991. Accumulation of B-fructosidase in the cell walls of tomato roots following infection by a fungal wilt pathogen. Pl. Physiol., 97: 739-750.

Bhaskaran, S. and R. H. Smith, 1993. Carbohydrates, invertase activity, growth and dimorphism in *Sporisorium reilianum*. Mycopathologia, 122: 35-41.

Biji, J., D. Dive and C. Van Pelegbeim, 1981. Comparison of some bioassay methods for mycotoxin studies. Env. Pollut. Ecol. Biol., 26:173-162.

Davis, N. D. and U. L. Diener, 1978. Mycotoxins. In Beuchat, L. R. (ed.), Food and Beverge Mycology. AVI Publishing Co., Westport, Connecticut, pp: 397-470.

El-Kady, I. A. and S. S. I. Abdel-Hafez, 1981. Production of sterigmatocystin by some species and varieties of *Aspergillus nidulans* group. Crypto. Mycol., 2: 239-244.

El-Kady, I. A., S. S. M. El-Maraghy and A. A. Zohri, 1994. Mycotoxin producing potential of some isolates of *Aspergillus flavus* and *Eurotium* group from meat products. Microbiol. Res., 149: 297-307.

El-Maraghy, S. S. M. and A. A. Zohri, 1988. Mycotoxin - production potential of *Aspergillus* and *Penicillia* of broad beans in Egypt. Bull. Fac. Sci. Assiut. Univ., 17: 91-102.

Galazzo, J. L. and J. E. Bailey, 1990. Fermentation pathway kinetics and metabolic flux control in suspended and immobilized *S. Cerevisiae*. Enz. Microbiol. Technol., 12: 162-172.

Gimeno, A., 1979. Thin-layer chromatographic determination of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxy scripenol, penicillic acid, Patulin and penitrem. J. AOAC., 62 : 579-585.

Higgy, A. H., A. A. Abdel-Razik and H. M. Rushdi, 1977. Occurrence of pokkah being disease of sugarcane in ARE. 155 CT. XVI-Congress Brazil, Pl. Pathol. Sec., 1: 473-481.

Hocking, A. D. and J. I. Pitt, 1980. Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. Appl. Env. Microbiol., 39: 488-492.

Johnson, L. F. and E. A. Curl, 1972. Methods for research on Ecology of soil-borne pathogens. Burgess Publishing Co., Minneapolis, MN, USA.

Khayria, M. Abdel-Gawad and A. A. Zohri, 1993. Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. Mycopathologia, 124: 55-64.

Kozakiewicz, Z., 1989. *Aspergillus* species on stored products (Mycological Paper No. 161). C. A. B. International Mycological Institute, Kew, Surrey, UK.

Long, D. E., A. K. Fung, E. E. M. McGee, R. C. Cooke and D. M. Lewis, 1975. The activity of invertase and its relevance to the accumulation of storage polysaccharides in leaves infected by biotrophic fungi. New Phytol., 74: 173-183.

Moss, M. O., 1971. The rubratoxins, toxin metabolites of *Penicillium rubrum*. Stoll. In Ciegler, A, radis, S. ;and Ajl, S. J. (eds). Microbiol. Toxins, Academic Press, New York, 6: 381-408.

Muhsin, T. and M. Abdel-Kader, 1995. Ecology of fungi associated with *Phragmites australis* in Iraq. Abhath Al-Yarmouk, 4 :31-50.

Nassar, M. S. M., 1986. Mycoflora associated with dates in Aswan Area. M. Sc. Thesis, Bot. Dep. Fac. Sci. Aswan, Assiut Univ. Assiut, Egypt.

Nigam, P., I. M. Banat, D. Singh, A. P. Michale and R. Marchant, 1997. Continuous ethanol production by thermotolerant Kluyveromyces marxianus IMB3 immobilized on mineral kissiris at 45° C. W. j. Microbiol. Biotechnol., 13: 283-288.

Nolan, A. M., N. Barron, D. Brady, T. Mearee, L. Michale and A. P. Michale, 1994. Ethanol production at 45°°C by an alginate immobilized thermotolerant strain of Kluyveromyces marxianus following growth on glucose containing media. Biotechnol. Lett., 16: 849-852.

Northolt, M. D., J. C. Frisvad and R. A. Samson, 1995. Occurrence of food borne fungi and factors for growth. In: Sanson. R. A. ; Hoekstra, E. S. ; Frisvad, J. C. and Filtenborg, O. (eds.). Introduction to food borne fungi, pp: 243-250, Centraalbureau voor Shimmelcultures, Baarn, The Netherlands.

Olufolaji, D. B., 1986. *Curvularia* leaf spot of sugarcane, a new disease. Sugarcane, 2 :1-2.

Parvez, S., M. I. Rajoka, M. N. Ahmed, F. Latif, R. Shahid and K. A. Malik, 1998. Citric acid production from sugarcane molasses by 2-Deoxyglucose resistant mutant strains of *Aspergillus niger*. Folia Microbiol., 43 :59-62.

Purchase, I. F. H., 1974. Mycotoxins, Elsevier Scientific Co. Amsterdam, pp: 433.

Rohr, M., C. P. Kubicek and J. Kominek, 1983. Citric acid. In H. J. Rehman and G. Reed (eds). Biotechnol., 3: 419-454, Verlag Chemie, Weinheim.

Sandhu, D. K. and M. S. Sidhu, 1980. Fungal succession on decomposing sugarcane bagasse. Trans. Br. Mycol. Soc., 75: 281-286.

Sargeant, K., A. Sheridan, J. O'kelly and R. B. A. Carranghan, 1961. Toxicity associated with samples of ground nuts. Nature (London) 192: 1096-1097.

Scott, P. M., 1973. Mycotoxins in stored grains, feeds and other cereal products. In Sinha, R. N. and Muir, W. E. (eds), Grain Storage. AVI Publishing Co., Westport, Connecticut.

Scott, P. M., 1989. Mycotoxigenic fungal contaminations of cheese and other dairy products. In : H. P. Van Egmond (ed.), Mycotoxin in dairy products. pp: 193-260, Elsevier Applied Science, London.

Scott, P. M., J. Harwing and B. J. Btanchlield, 1980. Screening *Fusarium* strains isolated from over wintered Canadian grains for trichothecene. Mycopathologia, 7: 66-85.

Sheoran, A., B. S. Yadav, P. Nigam and D. Singh, 1998. Continuous ethanol production from sugarcane molasses using a column reactor of immobilized *Saccharomyces cerevisiae* HAU-1. J. Basic Microbiol., 38: 123-128.

Van Egmond, H. P., W. E. Paulsch, E. Deijill and P. L. Schuller, 1980. Thin -layer chromatographic method for analysis and chemical confirmation of sterigmatocystin in cheese. J. AOAC., 63: 110-114.

Wilson, B. J., 1971. Miscellaneous *Aspergillus* toxins In Gegler, A., Kadis, S. ; and Ajl, S. J. (eds). Microbiol. Toxins., Vol. 6. Academic Press, New York, 6: 207-295.

Youssef, M. S., 1986. Mycoflora and mycotoxins of soybean seeds in Egypt. M. Sc. Thesis, Bot. Dept. Fac. Sci. Assiut Univ. Assiut. Egypt.