Biocontrol of *Fusarium* Moulds and Fumonisin B_1 Production

Mohamed A. Fareid

Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.
mohamedfareid73@yahoo.com

Abstract: The present study was carried out to investigate maize seeds infected by *Fusarium* moulds and fumonisin B_1 production. In order to suppress the growth of fungal infection, and/or fumonisin B_1 production in maize seeds; applicability of *Saccharomyces cerevisiae* as a biocontrol agent as well as detoxification of fumonisin B_1 was investigated. Out of 100 samples of maize investigated 65 (65%) were fungal infection. Of them 38 (58.46%) were *Fusarium moniliforme* others were *F. graminearum* 19 (29.23%) and *F. oxysporum* 8 (12.31%). Growth of *Fusarium moniliforme* and fumonisin B_1 detoxification were negatively correlated with different doses of *S. cerevisiae* while detoxification was positively correlated with the doses. At dose 1, 3, 5 and 7 g of *S. cerevisiae*, *Fusarium* dry weight and detoxification percent of fumonisin B_1 were 5.8, 40.56, 4.3, 77.63, 2.8, 89.52, 0.9 and 100, respectively. The effect of water content as well as different temperatures on fumonisin B_1 productions was investigated. At water content of 50 %, a higher level of fumonisin (16.3 µg/g) was detected in ground maize while in Corn flour and intact grain level of fumonisin was 0.50 and 8.66 µg/g, respectively. At temperature 21°C level of FB_1 was 19.3 µg/g after incubation period for 4 weeks while at temperature 28°C was 9.7 µg/g after the same incubation period and on the same substrate. For growth r = - 0.993, p = 0.001; for detoxification r = 0.927, p = 0.024.

[Mohamed A. Fareid. Biocontrol of *Fusarium* Moulds and Fumonisin B_1 Production. World Rural Observations 2011;3(3):53-61]; ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). http://www.sciencepub.net/rural.

Key words Fumonisins (B_1); *Fusarium moniliforme*; Mycotoxins; Biocontrol

1. Introduction

Maize (*Zea mays*) is a cereal crop grown throughout the world. Maize plays an important role in the diet of millions of African people due to its high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics (Fandohan *et al*., 2003). The total production of Africa in 2001 was estimated to be about 42 million tons (FAO, 2002). In the field as well as in the store, many pests and parasites attack maize during the storage period. Insects are most often considered as the principal cause of grain losses (Gwinner *et al.*, 1996). Surveying harvested maize grains done by many investigators (Samson, 1991; Ominski *et al*., 1994; Munkvold and Desjardins, 1997; Desjardins *et al.*, 2000; Orsi *et al*., 2000; El-Shabrawy, 2001), who reported that *Fusarium spp.* is the major fungal population recovered from the rotted maize ears. *F. verticillioides* and *F. moniliforme* are the most dominant species and earlier colonist of the pre-harvest maize ears before other moulds such as *Penicillium* spp. and *Aspergillus* spp. Kossou and Aho (1993) reported that fungi could cause about 50 – 80 % of damage on farmers' maize during the storage period if conditions are favorable for their development. Experts at the food and Agriculture Organization (FAO) at the United Nation have estimated that over 25 % of worlds food crops are lost each year due to mycotoxin contamination with the *Fusarium* species (Chelkowski, 1998). Fumonisins are mycotoxin produced by a variety of fungi of genus *Fusarium*; *Fusarium monilforme* and other species, fumonisins are natural contaminants of cereal grains worldwide and rare mostly found in corn and products derived from corn (Wan Norhasima *et al*., 2009). Nowadays, a great attention has been focused on the possibility of using natural and safe agents as a biocontrol against different diseases.

Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi (Whipps, 2001). So, application of yeasts as biocontrol agents acts as a new trend against different pathogens. Potential use of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters were recent investigated by El-Tarabily and Sivasithamparam (2006). El-Tarabily (2004) reported that the fungal activities of *Rhizoctonia solani* diseased sugar beet plants were well suppressed by using different types of yeast. Wide variety of yeasts has

been used extensively for the biological control of post-harvest diseases of fruits and vegetables (Punja, 1997; Zheng *et al*., 2003), against moulds of stored grains (Petersson *et al*., 1999) and to control powdery mildews (Urquhart and Punja, 1997). The most investigated fumonisin is FB_1, which can cause severe disorders in animals such as Equine Leukoencephalomalacia (Marasas *et al*., 1988), pulmonary edema syndrome and hydrothorax in pigs (Haschek *et al*., 1992) and it is nephrotoxic, hepatotoxic and hepatocarcinogenic in rats. Furthermore, FB_1 has been implicated to be associated with high rates of human esophageal cancer in South Africa, the United States and China (Rheeder *et al*., 1992). The aim of this study was to suppress the soil-borne pathogenic fungus *Fusarium moniliforme* by using *S. cerevisiae* as biocontrol agent and as fumonisin B_1 production control in maize.

2. Material and Methods

The bio-compound used in this study is active dry yeast of *S. cerevisiae*. Yeast application was conducted as different doses amended to autoclaved, ground maize, using four concentrations of 1, 3, 5 and 7 g/100g.

2.1. Samples

A total of 100 samples of maize were collected from different fields in Al-Dakahliya governorate, Egypt. These samples were kept at -4ºC until *Fusarium* moulds were isolated and fumonisin B_1 analysis was carried out.

Figure 1. Infected maize samples

2.2. Isolation and identification of *Fusarium* moulds

Fusarium moulds in maize seeds were determined by plating seeds on 15 ml of sterile Czapek-Dox agar containing Iprodion and Dichloral (CZID-agar) in Petri-plates (Abildgren *et al*., 1987). The seeds were surface sterilized in 10% sodium hypochlorite for 2 minutes, rinsed in sterilized distilled water and air dried on sterile filter paper. Ten plates of potato dextrose agar media (PDA) were seeded (10 seeds/plate) and incubated at 25ºC for five to seven days. All *Fusarium* isolates were subcultured on PDA, Spezieller Nahrstoffarmer Agar (SNA) and Carnation Leaf Agar (CLA), using a single spore technique (Leslie and Summerell, 2006, and Siddiquee *et al*., 2010). PDA cultures were incubated at 25ºC and CLA and SNA cultures were incubated at 25ºC under near UV light for two to four weeks. Cultural characters were assessed by eye and by microscopic examination. Colony morphology was recorded from cultures grown on PDA. The morphology of macroconidia, microconidia, conidiogenous cells and the chlamydospores was assessed from cultures grown on SNA and CLA. Morphological identifications of isolates were made using the criteria of (Nelson *et al*., 1983, and Leslie and Summerell, 2006).

2.3. Production of fumonisin B_1 by *Fusarium moniliforme*

Mycotoxin production in culture was tested on autoclaved, ground maize (Muthomi *et al*, 2000). One hundred grams of the course ground maize in 250 ml Erlenmeyer flasks were moistened with 75 ml distilled water and autoclaved at 121°C for 20 min in two consecutive days. After cooling, each flask was inoculated with 10 mm diameter agar disks cut from 7 to 14 day-old cultures. One flask used as a control sample while others were amended with *S. cerevisiae* at different concentrations (1, 3, 5, and 7 g). The incubation period was 4 weeks.

2.4. Fumonisin B_1 extraction

The samples were extracted using the method of Muthomi *et al* (2002). In brief, samples were homogenized in methanol-water; 3:1(v/v) for 3 min. After centrifugation, an aliquot of the supernatant was cleaned-up, which were preconditioned with methanol and methanol-water (3:1). After washing with methanol, the fumonisins were eluted with acetic acid-methanol; 1:99 (v/v), then stored at 4ºC to analysis.

2.5. Fumonisin B_1 analysis

Fumonisin B_1 was analyzed by the method of Katta *et al.* (1997) with HPLC. The fumonisin B_1 standard was purchased from Sigma Chemical Company (St. Loius, MO). A standard curve was constructed with levels ranging from 0.02 to 2 ng/µl for fumonisin B_1 and a new standard curve was constructed on each day of analysis and only standard curves with correlation coefficients >0.999 were accepted. Fumonisin B_1 (FB_1) was quantified by correlating peak areas of the sample extracts to that of the standard curves. The sensitivity of the method was 0.025 µg /g for fumonisin B_1.

2.6. Influence of some physical parameters on FB_1 production

Effect of different temperature on FB_1 production by *F. moniliforme* was investigated at different incubation period. Also water content of the medium was investigated to test substrate influence on fumonisin B_1 production according to ASTM D 2216 - Standard Test Method for Laboratory Determination of water (moisture) content of soil, rock, and soil-aggregate mixtures.

2.7. Statistical analysis

The statistical SPSS version 15 was uses in data analysis. The Chi^2 was uses to compare observed and expected frequencies. The Pearson correlation coefficient was used to study the doses of *S. cerevisiae* and fungal growth as well as the detoxification percent of fumonisin B_1. Linear or polynomial regressions were used.

3. Results

Isolation and identification of the fungal strain

The collected seeds were washed in running tap water for 20 minutes, surface sterilized in 10 % sodium hypochlorite for 2 minutes, rinsed in sterilized distilled water and air dried on sterile filter paper. The disinfected seeds were placed on CZID-agar and incubated at 25ºC in the dark. After five to seven days the fungal colonies were observed under a microscope and hyphal tips of *Fusarium*-like fungi were transferred to PDA. The pure cultures of the isolates were obtained using a single-spore culture technique. Species identifications were determined following the descriptions in the manual of Nelson *et al.* (1983) and Leslie and Summerell (2006). Three strains of *Fusarium* viz. *F. moniliforme*, *F. graminearum* and *F. oxysporum* was isolated from infected seeds. Occurrence of isolated *Fusarium* species was 58.46, 29.23 and 12.31 %, respectively (Table 1).

Table 1. Number of *Fusarium* spp. isolates and their occurrence in maize seeds

Fumonisin B_1 (FB_1) detection

Fusarium spp. identified	Maize	
	No. of isolates	Occurrence (%)
F. moniliforme	38	58.46
F. graminearum	19	29.23
F. oxysporum	8	12.31
Total	65	100
p-value (Chi^2)	0.001	

The percentage of *Fusarium* isolates which have the ability to produce fumonisin B_1 was summarized in table 2. Sixty five isolates was collected in the study, of all the isolates two fungal isolates not produce detectable level of FB_1 whereas only one isolate produced FB_1.

Table 2. Toxigenic Fusarium isolates collected from infected maize seeds

Fusarium spp.	No. of collected isolates	No. of toxigenic isolates	Toxigenic isolated (%)
F. moniliforme	38	33	86.8
F. graminearum	19	0	0
F. oxysporum	8	0	0
Total	65	33	86

Effect of *S. cerevisiae* on fungal growth

The effect of different *S. cerevisiae* doses/g on the growth of *Fusarium moniliforme* was detected. Our study revealed that, there was a reverse relationship between increasing *S. cerevisiae* concentration and growth of *Fusarium moniliforme*. Fresh and dry weights of Fusarium *moniliforme* were decreased to 1.9 mg and 0.9 mg in the presence of 7 gm of yeast. Generally, addition of *S. cerevisiae* decreased fresh and dry weight of *F. moniliforme* gradually in comparison to zero dose (control) as shown in table 3 and fig 2.

Table 3. Influence of different S. *cerevisiae* doses on the growth of *Fusarium moniliforme*

S. cerevisiae dose/ (g)	*F. moniliforme*	
	Fresh weight (mg)	Dry weight (mg)
Zero (control)	12.3	7.5
1	8.9	5.8
3	6.1	4.3
5	4.0	2.8
7	1.9	0.9

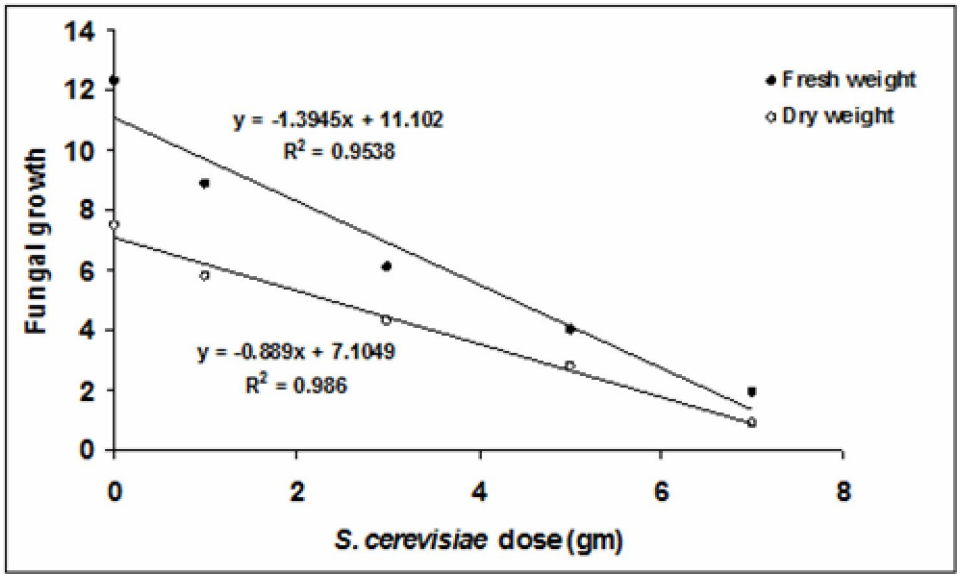

Figure 2. Influence of different *S. cerevisiae* doses on the growth of Fusarium *moniliforme*

Biocontrol of fumonisin B_1

Table 4 and fig 3 represent the effect of *S. cerevisiae* on the production of fumonisin B_1. Application of *S. cerevisiae* at different doses reduces FB_1 production gradually in relation to zero dose (control). The detoxification percent at doses 3 and 5 were 77.63 and 89.52, respectively. Also dose 7 was sufficient for complete detoxification of fumonisin B_1. Table 5 showed the dry weight, fresh weight and detoxification and their correlation with *S. cerevisiae* dose.

Influence of some physical parameters on FB_1 production Effect of different water content

Effect of different water content (30, 40 and 50 %) on production of fumonisin B_1 (FB_1) in sterile ground maize by *Fusarium moniliforme* isolate was determined over period of 6 weeks as shown in table 6. Generally, *F. proliferatum* grew faster with increasing water content. Fumonisin B_1 production depends upon water content and type of substrate. At water content of 50%, a higher level of fumonisin (16.3 µg/g) was detected in ground maize, while in corn flour and intact grain level of fumonisin was 0.50 and 8.66 µg/g, respectively.

Effect of different temperatures on FB_1 production by *F. moniliforme* was investigated as showed in table 7. *F. moniliforme* incubated at different temperatures ranges from 17 to 28°C. Generally our results indicated that level of fumonisin B_1 was increased gradually by increasing temperatures

degrees until reach a high level of production at temperature 21°C and then decreased by increasing temperature degrees. At temperature 21°C level of FB_1 was 19.3 µg/g after incubation period 4 weeks while at temperature 28°C was 9.7 µg/g after the same incubation period and on the same substrate.

Table 4. Effect of *S. cerevisiae* on the production of fumonisin B_1

***S. cerevisiae* dose (g)**									
0		1		3		5		7	
O. (µg/g)	D. (%)	O. (µg/g)	D. (%)	O. (µg/g)	D. (%)	O. (µg/g)	D. (%)	O. (µg/g)	D. (%)
14.3	0	8.5	40.56	3.2	77.63	1.5	89.52	ND	100

Table 5. Correlation between the doses of S. *cerevisiae* and fungal growth as well as the detoxification percent of fumonisin B_1

	Dry weight	Fresh weight	Detoxification
S. cerevisiae dose (g)	r = - 0.993	r = - 0.977	r = 0.927
	p = 0.001	p = 0.004	p = 0.024

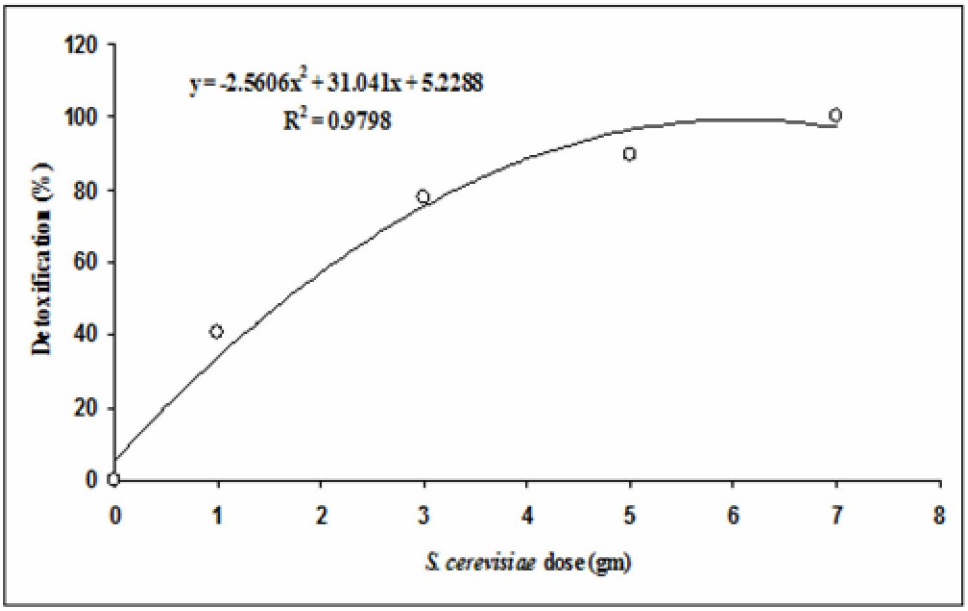

Figure 3. Effect of *S. cerevisiae* on the production of fumonisin B_1

Table 6. Influence of water content on FB_1 production by *F. moniliforme*

	Water content		
	30%	40%	50%
Corn flour	0.70	0.90	0.50
Coarse ground maize	13.25	14.20	16.30
Intact grain	3.43	6.75	8.66

Table 7. Influence of different temperatures on FB_1 production by *F. moniliforme*

Incubation period	Temperatures			
	17 °C	21 °C	25°C	28°C
Week 2	2.5	8.6	4.1	2.2
Week 3	3.9	10.3	5.8	4.1
Week 4	6.4	15.0	11.1	8.7
Week 5	8.5	19.3	14.8	9.7
Week 6	8.3	10.5	9.1	4.9

4. Discussion

Mycotoxins are fungal metabolites capable of having acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic and oestrogenic effects to man and animals (Castella *et al*., 1999). Fumonisins are a group of naturally occurring Mycotoxins produced by *Fusarium moniliforme*, *Fusarium proliferatum* and other related species (Bacon *et al*., 2001 and Miller, 2001). Common mycotoxins in maize include aflatoxins, fumonisins, moniliformin, deoxynivalenol and zearalenone. *Fusarium verticillioides* is the leading fungal species in producing fumonisins that have been shown to cause equine leukoencephalomalacia, pulmonary edema, human esophageal cancer and rat liver cancer (Van Egmond, *et al*., 2007).

Similar to our work Shephard *et al*. (1996) showed that, *F. verticillioides* and *F. proliferatum* are by far the most prolific fumonisin producers. Also Rheeder *et al*. (2002), recorded that, *F. verticillioides* and *F. proliferatum* are produce the highest amounts of toxins: up to 17900 µg/g of FB_1 have been recorded in cultures for the former, and 31000 µg/g FB_1 for the latter.

Our study showed that, *F. moniliforme* produced highest amounts of fumonisin 19.3 µg/g after five weeks incubation period. Also detoxification percent of FB_1 was significantly increased gradually by addition of *S. cerevisiae* while growth of *Fusarium moniliforme* was significantly decreased. Linear regression analysis between *S. cerevisiae* doses and fresh weight and dry weight showed that significant negative correlation was detected. The regression equations for fresh and dry weights were: $Y= -1.3945 x + 11.102$, $R^2 = 0.9538$ and $Y= -0.889x + 7.1049$, $R^2 = 0.986$, respectively.

The detoxification percent at doses 3 and 5 were 77.63 and 89.52, respectively. Also it clear that dose 7 was sufficient for complete detoxification of fumonisin B_1. A similar result was obtained by Paola *et al*. (2010). He showed that, seed treatment with *Bacillus amyloliquefaciens* and *Enterobacter hormaechei* reduced the infection by the fungus and FB_1 contents. Also these results are similar as of El-Tarabily (2004), Madi *et al*. (1997), Moustafa and Mohamed (2008); they reported that *R. solani* and *S. rolfsii* were effectively suppressed by yeasts.

Both temperature and water content of the substrate are factors that are of particular importance for the growth and the mycotoxinogenesis of *Fusarium* species that are mainly mesophilic and hydrophilic fungal strains usually developing on living plants, acting as parasites (Marin *et al*., 2004). Generally our results indicated that, level of fumonisin B_1 was increased gradually by increasing temperatures degrees until reach a high level of production at temperature 21°C and then decreased by increasing temperature degrees. At temperature 21°C level of FB_1 was 19.3 µg/g after incubation period 4 weeks. While at temperature 28°C was 9.7 µg/g after the same incubation period and on the same substrate.

Similarly, Mogensen *et al*. (2009) found that *Fusarium* spp. had the maximal production of FB_1 and FB_2 at 20-25°C. Also, Marin *et al*. (1995 and 1999) showed that both *F. moniliforme* and *F. proliferatum* grew faster with increasing water activity. Similarly, our work showed that, at water content of 50% higher levels of fumonisin (16.3 µg/g) was detected in ground maize, while in corn flour and intact grain level of fumonisin was 0.50 and 8.66 µg/g, respectively. Those results are in agreement with data obtained in other studies (Samapundo *et al*., 2005; Bailly *et al*., 2005 and Narasimha *et al*., 2010). FB_1 production optimal conditions taken off for the end of the study were; 5 weeks at 21°C on sterile coarsely cracked maize with water content of 50 %.

In conclusion, the current work indicates that maize seeds are contaminated to various degrees with *Fusarium* moulds and fumonisin B_1 and the use *S. cerevisiae* as a biocontrol agent can control the *Fusarium* moulds growth as well as fumonisin B_1 production in these seeds. In the present study, we suggest that caution should be used whenever, maize seeds are to be stored prior to use because of the normal occurrence of mycotoxinogenic moulds as part of the natural seed microflora.

Corresponding Author:
Dr. Mohamed A. Fareid
Botany and Microbiology Department
Faculty of Science (Boys)
Al-Azhar University, Cairo, Egypt.
E-mail: mohamedfareid73@yahoo.com

References

1. Abildgren, M. P., Lunf, F., Thrane, U., and Elmholt, S. (1987): Czapek–Dox agar containing iprodione and dichloran as a selective medium for the isolation of *Fusarium* species. *Letters in Applied Microbiology, 5*, 83–86.
2. Bacon, C. W., Tates, I. E., Hinton, D. M., & Meredith, F. (2001): Biological control of *Fusarium moniliforme* in maize. *Environmental Health Perspectives*, 109, 325–332.
3. Bailly, J. D.; Querin, A.; Tardieu, D. and Guerre, P. (2005): Production and purification of fumonisins from a highly toxigenic *Fusarium verticilloides* strain. *Revue Méd. Vét.* 156, 11, 547-554.
4. Castella, G., Bragulat, R.M. and Cabanes, F.J. (1999): Fumonisin production by Fusarium species isolated from cereals and feeds in spain. J. Food Protection, 62(7): 811-813.
5. Chelkowski, J. (1998): Distribution of *Fusarium* species and their mycotoxin in cereal grains. In: Mycotoxin in Agriculture and Food Safety, Sinhaand, K. K. and D. Bhatnagar (Eds.). CRC Press, Marcel Dekker, New York, ISBN: 9780824701925, pp: 520.
6. Desjardins, A. E.; Plattner, R. D. and Gordon, T. R. (2000): *Gibberella fujikuroi* mating population A and *Fusarium subglutinans* from *teosinte* species and maize from Mexico and Central America. *Mycol.Res.*, 104: 865-872.
7. El-Shabrawy, E. M. (2001): Studies on ear and kernel rot of maize caused by *Aspergillus* and *Fusarium* spp. M.Sc. Thesis, Fac. Agric., Tanta Univ. 80 pp.
8. El-Tarabily, K. A. (2004): Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. J Appl Microbiol 96:69-75.
9. El-Tarabily K. A. and Sivasithamparam, K. (2006): Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47:25-35.
10. Fandohan, P., Hell, K., Marasas, W.F.O. and Wingfield M.J. (2003): Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. African Journal of Biotechnology Vol. 2 (12), pp. 570-579.
11. FAO (Food and Agriculture Organization) (2002): FAOSTAT Database, Food and Agriculture Organisation,Roma, Italy. URL: http://apps.fao.org/lim500/nph-wrap.pl. Flamboyant, Benin. 125 p.
12. Gwinner, J., Harnisch, R. and Müch, O. (1996): Manuel sur la manutention et la conservation des grains après-récolte. GTZ, Eschborn, Germany. 368 p. URL: http://www.fao.org/inpho/vlibrary/move_rep/x0298f/x0298F31.htm- 34k.
13. Haschek, W.M., G. Motelin, D.K. Ness, K.S. Harlin, W.F. Hall, R.F. Vesonder, R.E. Peterson and V.R. Beasley, (1992): Characterization of fumonisin toxicity in orally and intravenously dosed swine. Mycopathologia, 117: 83-96. DOI: 10.1007/BF00497283 10.
14. Katta, S. K., Cagampang, A. E., Jackson, L. S., and Bullerman, L. B. (1997): Distribution of *Fusarium* moulds and fumonisins in dry-mulled corn fractions. *Cereal Chemistry, 74*, 858–863.
15. Kossou D.K. and Aho N. (1993): Stockage et conservation des grains alimentaires tropicaux: principes et pratiques. Les Editions du Flamboyant, Benin. 125 p.
16. Leslie J.F., Summerell B.A., (2006): The Fusarium Laboratory Manual. Blackwell Publishing Professional, Ames, IA, USA.
17. Madi, L., Katan, T., Katan, J., and Heins, Y. (1997): Biological control of *Sclerotium rolfsii* and *Verticillium dahaliae* by *Talaromyces flavus* is mediated by different nichanisms. Phytopathol 87(10):1054-1060.
18. Marasas, W.F.O., T.S. Kellerman, W.C.A. Gelderblom, J.A.W. Coetzer, P.G. Thiel and J.J. Van Der Lugt, (1988): Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res., 55: 197-203.
19. Marin, S., Sanchis, V., Vinas, I., Canela, R. and Magan N. (1995): Effect of water activity and temperature on growth and fumonisin B1 and B2 production by *Fusarium proliferatum* and *F. moniliforme* on maize grain. Lett Appl Microbiol. Nov; 21(5):298-301.

20. Marin, S., Magan, N., Belli, N., Ramos, A.J., Canela, R. and Sanchis, V. (1999): Two dimentional profiles of fumonisin B1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize. *Int. J. Food Microbiol.*, 51, 159-167.
21. Marin, S., Magan, N., Ramos, A. J. and Sanchis, V. (2004): Fumonisin producing strains of *Fusarium:* a review of their ecophysiology. *J. Food Protect.*, 67, 1792-1805.
22. Miller, J. D. (2001): Factors that affect the occurrence of fumonisin. *Environmental Health Perspectives*, 109, 321–324.
23. Mogensen, J. M., Nielsen, K. F., Samson, R. A., Frisvad, J. C. and Thrane, U. (2009): Effect of temperature and water activity on the production of fumonisins by *Aspergillus niger* and different *Fusarium* species. BMC Microbiol. Dec 31;9:281.
24. Moustafa, E. S. and Mohamed F. E. (2008): Application of *Saccharomyces cerevisiae* as a biocontrol agent against Fusarium infection of sugar beet plants. Acta Biologica Szegediensis, Volume 52(2):271-275.
25. Munkvold, G.P. and Desjardins, A.E. (1997): Fumonisins in maize can we reduce their occurrence. *Plant Dis.*, 81: 556-565.
26. Muthomi, J. W., A. Schutze, H.-W. Dehne, E. W. Mutitu & E.-C. Oerke (2000): Characterization of *Fusarium culmorum* isolates by mycotoxin production and aggressiveness to winter wheat. Journal of Plant Disease and Protection, 107(2): 113-123.
27. Muthomi, J. W., E.-C. Oerke, E. W. Mutitu, A. Schade-Schuetze & H.-W. Dehne (2002): Susceptibility of Kenyan wheat varieties to head blight, fungal invasion and deoxynivalenol accumulation inoculation with *Fusarium graminearum.* Journal of Phytopathology 150, 30-36.
28. Narasimha, R. K.; Vijaypal R. B.; Girisham, S. and Reddy, S.M. (2010): Factors influencing fumonisins (B_1) production by *Fusarium moniliforme*. Indian Journal of Science and Technology, Vol. 3 No. 2, ISSN: 0974- 6846.
29. Nelson, P. E., Toussoun, T. A. & Marasas, W. F. O. (1983): *Fusarium* species an illustrated manual for identification. The Pennsylvania State University Press, London.
30. Ominski, K.H., Marquardt, R.R., Sinha, R.N. and Abramson, D. (1994): Ecological aspects of growth and mycotoxin production by storage fungi. In: Miller JD, Trenholm HL. eds. Mycotoxins in Grains. Compounds other than Aflatoxin. Eagen Press, USA. p 287–305.
31. Orsi R. B., Corrêa, B., Possi, C. R., Schammass, E. A., Nogueira, J. R., Dias, S.M.C., Malozzi, M. A. B. (2000): Mycoflora and occurrence of fumonisins in freshly harvested and stored hybrid maize. J. Stor. Prod. Res. 36: 75-87.
32. Paola P.; Andrea N.; Carlos C. and Miriam E. (2010): Impact of bacterial biological control agents on fumonisin B_1 content and *Fusarium verticillioides* infection of field-grown maize. Biological Control, Volume 53, Issue 3, Pp. 258-266.
33. Petersson, S., Jonsson, N., Schnürer, J. (1999): *Pichia anomala* as a biocontrol agent during storage of high-moisture feed grain under airtight conditions. Postharvest Biol Technol 15:175-184.
34. Punja, Z. K. (1997): Comparative efficacy of bacteria, fungi and yeasts as biological control agents for disease of vegetable crops. Can J Plant Pathol 19:315-323.
35. Rheeder, J.P., W.F.O. Marasas, P.G. Thiel, E.W. Syndenham, G.S. Shephard and D.J. Van Schalkwyk, (1992): *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkein. Phytopathology, 83: 353-357. DOI: 10.1094/Phyto-82-353.
36. Rheeder, J. P., Marasas, W. F. O. and Vismer, H. F. (2002): Production of fumonisin analogs by *Fusarium* species. Appl. Environ. Microbiol. 68: 2101–2105.
37. Samapundo, S., Devliehgere, F., De Meulenaer, B. and Debevere, J. (2005): Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticilloides* and *Fusarium proliferatum* on corn. *J. Food Protect.* 68, 1054-1059.
38. Samson, R. A. (1991): Identification of food-borne *Penicillium*, *Aspergillus*, and *Fusarium* species. In: Champ BR, Highley E, Hocking AD, Pitt JI. eds. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991.
39. Shephard, G.S., Thiel, P.G., Stockenstrom, S. and Sydenham, E. W. (1996): Worldwide survey of fumonisin contamination of corn and corn-based products. J. AOAC Int. 79: 671–687.
40. Siddiquee, S.; Yusuf, U. K. and Zainudin, N. A. I. M. (2010): Morphological and molecular detection of *Fusarium chlamydosporum* from root endophytes of *Dendrobium crumenatum.* African Journal of Biotechnology Vol. 9(26), pp. 4081-4090.
41. Urquhart, E.J. and Punja, Z. K. (1997): Epiphytic growth and survival of *Tilletiopsis pallescens*, a potential biological control agent of *Sphaerotheca fuliginea*, on cucumber leaves. Can

J Bot 75:892-901.
42. Van Egmond, H.P, C.S. Ronald and A.J. Marco, (2007): Regulations relating to mycotoxins in food. Perspectives in a global and European context. Anal Bioanal Chem., 389: 147-157.
43. Wan Norhasima, W. M., Abdulamir, A. S., Abu bakar, F., Son, R. and Norhafniza, A. (2009): The health and toxic adverse effect Fusarium fungal mycotoxin, fumonisins, in human population. American Journal of Infectious Diseases 5 (4): 273-281.
44. Whipps, J. M. (2001): Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487-511.
45. Zheng, D. X., Sun, Y. P. and Zhang, H. Y. (2003): Yeast application for controlling apple postharvest diseases associated with *Penicillium expansum*. Bot Bull Acad Sin 44:211-216.

5/28/2011