Cryptogamie, Mycologie, 2011, 32 (3): 295-306
© 2011 Adac. Tous droits réservés

Comparative cytotoxicity of the possibly occurring mycotoxins in wheat on the basis of a mycological analysis

Fatma BENSASSI [a,b*], *Amel BOUSLIMI* [a], *Emna EL GOLLI-BENNOUR* [a], *Samia GARGOURI* [b], *Mohamed Rabeh HAJLAOUI* [b] & *Hassen BACHA* [b]

[a] *Laboratory for Research on Biologically Compatible Compounds, Faculty of Dentistry, Rue Avicenne, 5019 Monastir, Tunisia; email: bensassifatma83@yahoo.fr*

[b] *Laboratory of Plant Protection, the National Institute for Agricultural Research, INRA Tunisia, Rue Hedi Karray, 2049 Ariana, Tunisia*

Abstract — A mycological survey was carried out at the Northern part of Tunisia on freshly harvested durum wheat in 2007, as wheat is the basic staple food for the Tunisian population. A total of 65 samples was analysed for their contamination with pathogenic and saprophytic fungal species and isolation frequencies and relative densities of the fungal species were recorded. Our results showed that *Alternaria* species were observed in all the prospected samples and *Fusarium* spp. were present in about 70% of fields whereas the incidence of the genus *Penicillium* was low. In the light of the mycological analysis, we evaluated the cytotoxicity of the possible occurring mycotoxins in such crops using the MTT bioassay. For this purpose, four mycotoxins – Deoxynivalenol (DON), Alternariol (AOH), Ochratoxin A (OTA) and Citrinin (CTN) – were screened for their toxicity in human colon carcinoma cell line HT 29. The viability assay showed significant variation in IC_{50} concentrations between the tested mycotoxins.

Wheat / Tunisia / contamination / mycotoxins / cytotoxicity / MTT assay

INTRODUCTION

Mycotoxins are natural contaminants produced by numerous fungal species. The worldwide contamination of food and feed with these secondary metabolites starts with the infestation of plant materials by the toxigenic fungi either in the field or during storage under suitable conditions (Petzinger & Weindenbach, 2002; Lanier *et al.,* 2009). The accumulation of mycotoxins poses a significant environmental and health problem for both animals and humans (Hussein & Brasel, 2001; Garon *et al.,* 2006; Lanier *et al.,* 2010).

In Tunisia, the agricultural system is based mostly on cereals which are frequently infested by various toxigenic pathogens given the suitable Mediterranean climate of the country. Durum wheat is the most important cereal cultivated in Tunisia and the main source of food for the population. Several fungi are associated with wheat and the common (most important) ones among these that have been reported to produce mycotoxins (Gonzalez *et al.,* 1996, 1999; Saberi *et al.,* 2004) are *Fusarium*, *Alternaria, Penicillium* and *Aspergillus* (Desjardins *et al.*, 1996; Desclaux *et al.,* 2000; Bensassi *et al.,* 2009; Lund & Frisvad, 2003; Bayman *et al.,* 2002).

Fusarium species frequently infect small grain cereals (Doko *et al.,* 1995; Bakan *et al.,* 2002; Bottalico & Perrone, 2002; Westhuizen *et al.,* 2003; Hammond *et al.,*

2004; Zinedine *et al.,* 2006) in the field and cause cereal diseases across the world. The most significant disease in wheat is *Fusarium* head blight (FHB) (Xu *et al.,* 2005) which is predominantly caused by *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *Microdochium nivale (*Edwards, 2004; Glynn *et al.,* 2005). Many of these *Fusarium* pathogens may produce mycotoxins (Joffe, 1986; Cirillo *et al.,* 2003; Jestoi *et al.,* 2004; Desjardins, 2006), and among these especially the trichothecenes have been related to chronic and severe mycotoxicosis of humans and animals (Desjardins & Proctor 2007). Trichothecene deoxynivalenol (DON) or vomitoxin is the most widespread mycotoxin of the fusariotoxin group (Pepeljnjak *et al.,* 1992; Tutelyan, 2004). Exposure to DON can lead to acute and long-term chronic effects resulting in teratogenic, neurotoxic, embryotoxic and immunosuppressive effects (Rotter *et al.,* 1996; Desjardins, 2006).

Species of the genus *Alternaria* are common field fungi that include both plant pathogenic and saprophytic species that may cause extensive spoilage of crops pre or post harvest (Logrieco et al., 1990; Ilhan and Asan, 2001). Some *Alternaria* species have a high toxigenic potential, producing mycotoxins (Woody & Chu, 1992; Logrieco *et al.,* 2003) such as alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins and tenuazonic acid (Bottalico & Logrieco, 1998; Kosiak *et al.,* 2004). These mycotoxins are implicated in several human toxicoses such as hepatocarcinogenicity, oesophageal cancer as well as many digestive complications (Liu *et al.,* 1992; Zureik *et al.,* 2002).

Penicillium and *Aspergillus* species are more commonly found during drying and storage than in the field. They are widespread contaminants of commodities and foods and in particular cereals (Juan *et al.,* 2008). Some species, such as *A. ochraceus* and *P. verrucosum*, are known to produce Ochratoxin A (OTA) (Van der Merwe *et al.,* 1965; Frisvad & Samson, 2004; Peterson, 2008; Zinedine *et al.,* 2006; Amézqueta *et al.,* 2009). This mycotoxin is carcinogenic, nephrotoxic, immunotoxic, genotoxic and teratogenic in all animal species (IARC, 1993; Sava *et al.,* 2006; Pfohl-Leszkowicz *et al.,* 2007). OTA often co-occurs with another mycotoxin Citrinin (CTN) in wheat, barley or oat (Wolff, 2000; Molinié *et al.,* 2005) which is produced chiefly by *P. citrinum* and other species of *Penicillium, Aspergillus* and *Monascus (*Hetherington and Raistrick, 1931; Blanc *et al.,* 2001). Citrinin is also nephrotoxic, embryotoxic, teratogenic and genotoxic (Frisvad & Thrane, 2000; Scott, 2004).

Depending on climatic conditions, all of these fungal toxins can contaminate food and feedstuffs simultaneously. In such a case, humans and animals can be exposed to a cocktail of toxins which may lead to severe toxic effects (Barceloux, 2008).

Numerous *in vitro* cytotoxicity tests can be used for a rapid preliminary screening of mycotoxins in food. *In vitro* MTT cell culture assay is one of the most widely used tests for a preliminary cytotoxicological evaluation (Dombrink-Kurtzman *et al.,* 1994). This colorimetric cell viability assay shows the ability of viable cells to convert MTT tetrazolium salt by mitochondrial succino-dehydrogenase enzyme to formazan. In this study, we have performed our experiments on HT 29 cells, derived from a human colon carcinoma especially that the intestine is the primary target for alimentary intoxications and ingestion of contaminated foods is the main route of exposure to mycotoxins (Shephard *et al.,* 1995; Prelusky *et al.,* 1996; Bouhet & Oswald, 2005).

The aim of this study was to determine the mycoflora associated with wheat grains in the Northern part of Tunisia during the crop year 2007, in order to use this data to evaluate and predict possible effects of the most commonly elaborated mycotoxins by the corresponding isolated fungi towards the human colon carcinoma cells.

MATERIALS AND METHODS

– **Sampling:** During the July 2007 harvest, a total of 65 samples of durum wheat grains were arbitrarily collected from different areas located in the main regions for cereal production in Northern Tunisia. Samples of at least 2 kg in size were taken directly from the fields immediately after the harvest. Samples were packed in sterile hermetic containers and immediately stored at +4°C awaiting analyses.

– **Mycological survey:** From each sample, 200 kernels were surface-disinfected with 1% NaOCl solution for 10 min followed by rinsing twice with sterile water then dried over a filter paper in a sterile laminar flow cabinet. Kernels were plated on potato dextrose agar (PDA), ten kernels per plate and incubated at 25°C for 7 days in darkness. The resulting fungal colonies were subcultured onto PDA and identified based on their macro- and microscopic features (Nelson *et al.,* 1988; Simmons, 1986; Pitt & Hocking, 1997). All isolated fungi were recorded according to Gonzalez *et al.* (1995). The isolation frequency (Fr) and relative density (RD) of species were recorded as follows: Fr(%) = (ns/N) × 100 and RD(%) = (ni/Ni) × 100, with Ns standing for the number of samples where a genus or species was detected, ni for the number of isolates of a genus or species, N for the total number of samples and Ni the number of the total fungal isolates obtained.

– ***In vitro*** **MTT assay:** DON, AOH, OTA and CTN were purchased from Sigma Chemical Company (St. Louis, MO). Dulbecco's modified eagle medium-F12 (DMEM-F12), foetal bovine serum (FBS), phosphate buffer saline (PBS), trypsin–EDTA, penicillin and streptomycin mixture were from GIBCO-BCL (UK), 3-4,5-dimethylthiazol-2-yl, 2,5-diphenyltetrazolium bromide (MTT) was from Sigma–Aldrich, France. All other chemicals used were of analytical grade.

Human colon carcinoma cells (HT 29) were maintained as monolayer culture in DMEM-F12, supplemented with 10% foetal bovine serum (FBS), 1% l-glutamine (200 mM), 1% of mixture penicillin (100 IU/ml) and streptomycin (100 µg/ml), in a humidified incubator at 37°C in an air atmosphere of 5% CO_2 . DON, OTA and CTN were dissolved in pure ethanol whereas AOH was dissolved in pure DMSO (dimethylsulfoxide). To obtain the studied concentrations in the cell culture media, the mycotoxin treatment volume was negligible and represented about 0.025% of the total medium volume. In these conditions, untreated cells and cells receiving this low vehicle volume responded in the same manner. For this reason, we have chosen untreated cells as control.

The cell viability was performed using MTT assay according to Mosmann (Mosmann, 1983) in order to determine the cytotoxicity of DON, AOH, OTA and CTN in HT 29 cells. At 50% confluence, cells were seeded on 96-well culture plates (Polylabo, France). After 24 h of incubation at 37°C in an atmosphere of 5% CO_2/95% air mixture, cells were treated separately with increasing concentrations of DON, AOH, OTA or CTN for 24 h. Then, the MTT test was carried out by discarding the medium and replacing it by 200 µl of medium containing 0.5 mg/ml MTT in PBS. After 3 h of incubation at 37°C, the medium was removed and 100 µl of DMSO was added in each well to ensure total solubility of formazan crystals. Measurement of the absorbance was performed with an ELISA-Reader (Stat Fax 3200—Awareness Technology) at a wavelength of 545 nm. Cell viability was expressed as the relative formazan formation in treated samples in comparison with control cells [(A545 treated cells/A545 control

cells) 100%]. The concentrations inhibiting growth by 50% (IC_{50}) were derived from the plotted data by linear extrapolation.

– **Statistical analyses:** The statistical analysis was performed with the program SPSS 13.0. The different data (RD and Fr) were expressed as percentage and significant differences were compared using non-parametric χ^2 test. Differences were considered significant at $p< 0.05$. Experiments on the cell viability were carried out at least three times separately (in triplicate). Data are expressed as means ± standard deviation (S.D.).

RESULTS

Mycological survey: The results of the mycological analysis showed that the freshly harvested wheat grains in Northern Tunisia during the 2007 harvest were strongly infested by numerous fungal species (Table 1). Although grains were surface-disinfected, we noted a considerable internal fungal invasion. The predominant genus was *Alternaria*, being detected in all samples with a high density of 90% RD. The second most abundant genus in terms of percentage of infested samples was *Fusarium,* which was isolated in about 71% Fr of samples ($p< 0.05$), although the infection level of grains did not exceed 2% (1.8% RD). *F. culmorum* was the most toxigenic species isolated among the *Fusarium* species. The predominance of *Microdochium nivale,* formerly known as *F. nivale*, was also shown. Indeed, it was detected in about 94% Fr of samples with a low relative density compared to *Alternaria* spp. ($p< 0.05$). 11% Fr of wheat samples were positive for the genus *Penicillium*, known to be a storage fungus, with a low

Table 1. Relative density (RD) and isolation frequency (Fr) of the toxigenic fungi isolated in freshy durum wheat grains, collected in 2007 in Northern Tunisia.

	Durum wheat 2007	
Species	*RD (%)*	*Fr (%)*
Alternaria spp.	90	100
Fusarium culmorum	1.1	41.5
Fusarium avenaceum	0.2	21.5
Fusarium pseudograminearum	0.1	13.9
Fusarium sp.	0.4	30.8
Total *Fusarium*	1.8	70.8
Microdochium nivale	7.6	94
Penicillium spp.	0.2	10.8
Other fungi	0.4	12.3

incidence. Some other molds were also isolated in 12% of fields including *Rhizopus, Mucor*, *Eurotium* and *Epiccocum*.

Cytotoxicity assay : Cytotoxic effects of DON, AOH, OTA or CTN on HT 29 cells after 24 h incubation were measured by MTT assay. The results clearly showed a dose-dependent inhibition of cell viability at increasing concentrations of the tested mycotoxins (Figs 1-4). Different cytotoxicities were observed from one mycotoxin to another. The IC_{50} values, in a decreasing order, as estimated after 24 h of cell treatment from the viability curves were about 10 µM, 12 µM, 60 µM and 100 µM for DON, OTA, CTN and AOH respectively (Table 2). AOH was the less toxic mycotoxin, whereas DON was the most toxic one.

DISCUSSION

Tunisia is a Mediterranean country with a warm and humid climate suitable for fungal invasion of crops which are consequently subject to mycotoxin contaminations in the field and during storage. The meteorological conditions of

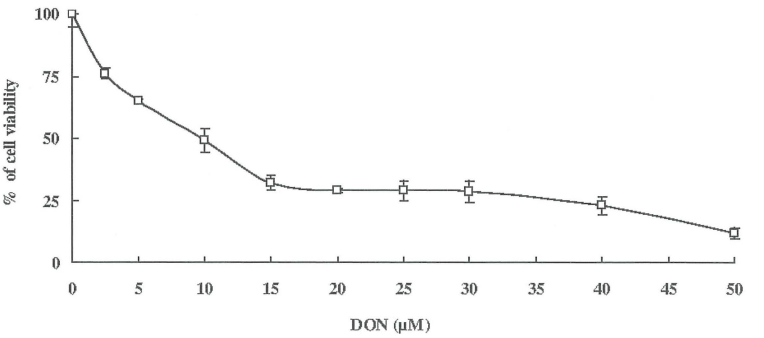

Fig. 1. Cytotoxic effect of DON on HT 29 cells. Cells were treated with DON at the indicated concentrations for 24 h. Cell viability was determined using the MTT assay and expressed as percentages of control. Control value was taken as 100%. Data are expressed as the mean ± S.D. Values are significantly different ($p<0.05$) from control.

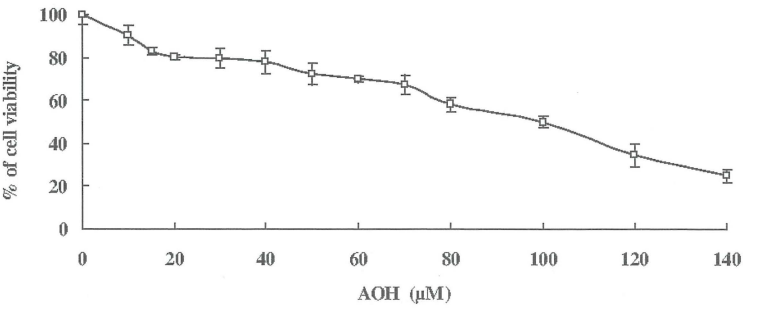

Fig. 2. Cytotoxic effect of AOH on HT 29 cells. Cells were treated with AOH at the indicated concentrations for 24 h. Cell viability was determined using the MTT assay and expressed as percentages of control. Control value was taken as 100%. Data are expressed as the mean ± S.D. Values are significantly different ($p<0.05$) from control.

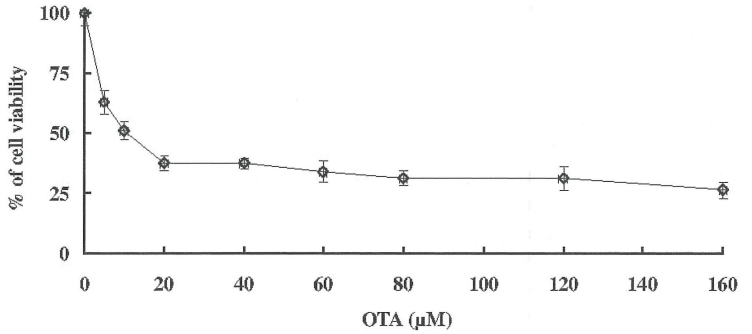

Fig. 3. Cytotoxic effect of OTA on HT 29 cells. Cells were treated with OTA at the indicated concentrations for 24 h. Cell viability was determined using the MTT assay and expressed as percentages of control. Control value was taken as 100%. Data are expressed as the mean ± S.D. Values are significantly different ($p<0.05$) from control.

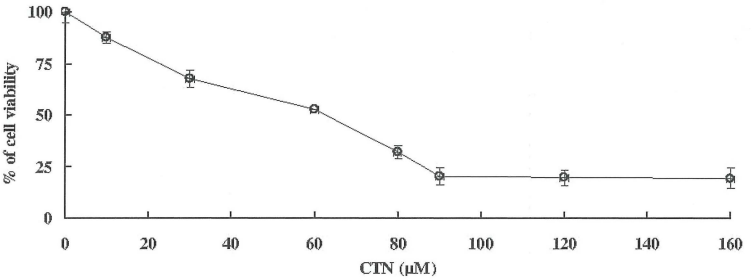

Fig. 4. Cytotoxic effect of CTN on HT 29 cells. Cells were treated with CTN at the indicated concentrations for 24 h. Cell viability was determined using the MTT assay and expressed as percentages of control. Control value was taken as 100%. Data are expressed as the mean ± S.D. Values are significantly different ($p<0.05$) from control.

Table 2. IC_{50} values of DON, AOH, OTA and CTN as assessed by MTT assay on HT 29 cells after 24 h of incubation.

	IC_{50} (µM)
DON	10
OTA	12
CTN	60
AOH	100

the crop year 2007, i.e. favourable temperature and long rains during the harvest, allowed the growth of several fungal species in durum wheat heads. All grain samples from the Northern part of the country were invaded at different degrees with various moulds. Our results clearly showed that the genus *Alternaria* was the main component of the wheat mycobiota. A similar study in Argentina showed that *Alternaria* was also present in all samples with a density of 85% (Patriarca *et*

al., 2007); the same situation was again observed in China (Li & Yoshizawa, 2000). Recently, Bensassi *et al.* (2009) reported that the most predominant *Alternaria* species recovered from Tunisian wheat kernels was *A. alternata* causing particularly black point in Tunisian wheat grains. González *et al.* (1999) found that *A. alternata* was also the most frequent species in Argentinean durum wheat. This species is probably the major mycotoxin-producing species (Li *et al.,* 2001) and possible mycotoxins such as AOH should be considered for toxicity.

In this study, we showed the presence of the fungal complex associated with FHB. In fact, we noted the presence of *F. culmorum* and *F. pseudograminearum* primarily producers of DON (Sudakin, 2003). The incidence of *Microdochium nivale* (94%), a non-toxigenic fungal pathogen of cereals causing FHB, was higher than those observed for *Fusarium* (70,8%), a difference ($p< 0.05$) likely explained by a competitive interaction (Simpson *et al.,* 2001). Bensassi *et al.* (2010) reported that durum wheat harvested in 2007 in Tunisia was target to high amounts of DON. This was chiefly subordinated to the geographic position but owing to this mycological analysis we can assume that DON production is stimulated by the interaction between *M. nivale* and *Fusarium* (Velluti *et al.,* 2000). As previously pointed out, wheat is the main source of food in Tunisia and contamination with high levels of DON presents a potential hazard to both animal and human health.

The detection of *Penicillium* spp. in freshly harvested wheat, even at a low density, was unexpected because this fungus is known to affect mainly stored foods and cereals. Our results corroborated with those obtained in Argentina (González *et al.,* 1999; Broggi *et al.,* 2007). A previous study conducted in Tunisia showed that cereals were contaminated essentially by OTA, at levels higher than the usual norms established by the European commission (Zaied *et al.,* 2009), and also by other mycotoxins such as citrinin (Bacha *et al.,* 1988; Hadidane *et al.,* 1985). Crop contamination by these fungi will be amplified in the post harvest especially when unsuitable storage conditions are employed. In such a case, human contamination by OTA and CTN is very common (Miller & Trenholm, 1994; Frisvad & Samson, 2004; Frisvad *et al.,* 2004; Bragulat *et al.,* 2008).

Results of the mycological survey allowed us to evaluate the individual effect of the discovered mycotoxins. The inclusion of an *in vitro* cytotoxicity test can be used for the preliminary screening of toxicity of mycotoxins in order to elucidate the modes of action. The MTT bioassay for mammalian cells is used on a large scale in a mycotoxin screening assay (Langseth *et al.,* 1999).

The cytotoxicity of DON, AOH, OTA and CTN has been studied in different cell lines and the IC_{50} values weren't similar because of their different sensitivities toward tissues. The cellular model used herein, HT 29 cells, showed a difference in the IC_{50} between the four mycotoxins. It can be stated that DON was the most cytotoxic one among them for intestines. Our result was in accordance with a previous study showing DON's toxicity on Caco-2 cells; another human intestinal cell line; became statistically significant at 10 µM and the IC_{50} was 25 µM at 24 h (Kouadio *et al.,* 2005). Our findings are also in line with what is commonly known about this fusariotoxin concerning gastrointestinal illness (Li *et al.,* 1999; Pestka & Smolinski, 2005). Additionally, DON can cause feed refusal, emesis and vomiting (Prelusky & Trenholm, 1993; Hussein & Brasel, 2001). A similar toxicity was also obtained with the OTA, essentially known to be nephrotoxic. The IC_{50} obtained on HT 29 cells with OTA was lower than the estimated IC_{50} obtained using the same viability assay and at the same exposure time on kidney Vero cells, which was about 37 µM (Bouslimi *et al.,* 2008). This finding may be related to the absorption within the gastrointestinal tract which is the first step directing the entry of OTA in bloodstream and its tissue distribution

(Berger *et al.,* 2003). With respect to OTA, it is clear from our study that its toxicity to HT 29 cells is similar to this observed on Caco-2 cells (Berger *et al.,* 2003). The obtained result with the second nephrotoxic fungal toxin CTN also showed that it's a potent toxin to human intestinal cells comparatively to kidney Vero cells. In fact, Bouslimi *et al.* (2008) reported that the decrease of cell viability with CTN on Vero cells was observed with an IC_{50} value around 220 µM. Another study concerning CTN cytotoxicity has reported the existence of a dose-dependant decrease in Human embryonic kidney cell lines (HEK293) (Chang *et al.,* 2009) treated with 0–100 µM CTN for 24 h with about 92% of viable cells at 100 µM of CTN treatment. Our findings are consistent with previous studies in which OTA was more toxic than CTN to numerous cell lines such as Vero cells, swine kidney cells (SK-6) cells, murine and bovine embryonic cells (Bouslimi *et al.,* 2008; Stec *et al.,* 2007; Terse *et al.,* 1993).

The potency of AOH at a dose inhibiting the viability of HT 29 cells was found to be 10 times lower than that of DON. AOH was found less cytotoxic to all tested mycotoxins, it caused 50% inhibition of proliferation of HT 29 cell line at high concentrations of 100 µM. Lehmann *et al.* (2006), reported that the treatment of Ishikawa cells (an endometrial adenocarcinoma cells), with AOH for 48 or 72 h, generated more than 50% reduction of the cell viability with 10 µM AOH. Our investigation suggests that AOH doesn't target chiefly the intestinal cells. According to the data presented in Table 2, AOH was not the most potent mycotoxin in our studies. This low cytotoxicity was probably due to the fact that AOH was converted to its conjugates which are less toxic than intact AOH form. This finding may correlate with the genotoxic effect of AOH. Indeed, a previous study showed that cells treatment for 24 h, at AOH concentrations ranging from 5 to 50 µM caused a concentration-dependent induction of DNA strand breaks in HepG2 but not HT 29 cells (Pfeiffer *et al.,* 2007). Pfeiffer *et al.* (2007) further indicated that the strand breaking activity resides in the parent toxin and is abolished by conjugation. According to that data, it implies that HT 29 cells are more active for conjugating AOH than other toxins. The genotoxic effect of AOH in HT 29 cells was confirmed by other works which identified this *Alternaria* toxin as a topoisomerase I- and II-poison (Fehr *et al.,* 2007).

Our *in vitro* findings indicate that the HT 29 cell line was found to be very sensitive to the cytotoxic effect of DON, followed by OTA, CTN and AOH after 24 h exposure. This difference may be chiefly explained by the response of the target cell towards the toxins which vary according to the amount of target enzymes, the amount of metabolic enzymes and the efficiency of detoxification mechanisms (Shier *et al.,* 1991). This study highlights that the use of an *in vitro* model may give a more scientific basis to the health risk assessment of mycotoxins in food products.

Acknowledgements. This study was supported by "Le Ministère Tunisien de l'Enseignement Supérieur, de la Recherche Scientifique et de la Technologie". We thank Dr. Mbarek Chetoui (Faculty of Sciences, Tunisia) for his kind advice and help and Dr. Salwa Bacha (University of La Manouba, Tunisia) and B. Buyck (MNHN, Paris) for the correction of the English manuscript.

REFERENCES

AMÉZQUETA S., GONZÁLEZ-PEÑAS E., MURILLO-ARBIZU M. & LÓPEZ DE CERAIN A., 2009 — Ochratoxin A decontamination A review. *Food Control* 20: 326-333.

BACHA H., HADIDANE R., REGNAULT C., ELLOUZ F., CREPPY E.E. & DIRHEIMER G., 1988 — Monitoring and identification of fungal toxins in food products, animals feed and cereals in Tunisia. *Journal of Stored Products Research* 4: 199-206.

BAKAN B., MELCION D., RICHARD-MOLARD D. & CAHAGNIER B., 2002 — Fungal Growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *Journal of Agricultural and Food Chemistry* 50: 728-731.

BARCELOUX D.G., 2008 — Medical Toxicology of natural substances. Foods, Fungi, Medicinal Herbs, Plants and Venomous Animals John Wiley & Sons, USA, ISBN 978-0471-72761-3, pp. 1157.

BAYMAN P., BAKER J.L., DOSTER M.A., MICHAILIDES T.J. & MAHONEY N.E., 2002 — Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*. *Applied and Environmental Microbiology* 68: 2326-2329.

BENSASSI F., ZAIED C., ABID S., HAJLAOUI M.R. & BACHA, H., 2010 — Occurrence of deoxynivalenol in durum wheat in Tunisia. *Food Control* 21: 281-285.

BENSASSI F., ZID M., RHOUMA A., BACHA H. & HAJLAOUI M.R., 2009 — First report of *Alternaria* species associated with black point of wheat in Tunisia. *Annals of Microbiology* 59: 465-467.

BERGER V., GABRIEL A.F., SERGENT T., TROUET A., LARONDELLE Y. & SCHNEIDER Y.J., 2003 — Interaction of ochratoxin A with human intestinal Caco-2 cells: possible implication of a multidrug resistance-associated protein (MRP2). *Toxicology Letters* 140-141: 465-476.

BLANC P.J., LAUSSAC J.P., LE BARS J., LE BARS P., LORET M.O., PAREILLEUX A., PROME D., PROME J.C., SANTERRE A.L. & GOMA G., 2001 — Characterization of monascidin A from *Monascus* as citrinin. *International Journal of Food Microbiology* 27: 201-213.

BOTTALICO A. & LOGRIECO A., 1998 — Toxigenic *Alternaria* species of economic importance. *In*: Sinha KK, Bhatnagar D, editors. *Mycotoxins in Agriculture and Food safety*. New York: Marcel Dekker, Inc, pp. 65-108.

BOTTALICO A. & PERRONE G., 2002 — Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology* 108: 611-624.

BOUHET S. & OSWALD I.P., 2005 — The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response. *Veterinary Immunology and Immunopathology* 108: 199-209.

BOUSLIMI A. & BOUAZIZ C., AYED-BOUSSEMA I., HASSEN W., BACHA H., 2008 — Individual and combined effects of ochratoxin A and citrinin on viability and DNA-fragmentation in cultured Vero cells and on chromosome aberrations in mice bone marrow cells. *Toxicology* 251: 1-7.

BRAGULAT M.R., MARTÍNEZ E., CASTELLÁ G. & CABAÑES F.J., 2008 — Ochratoxin A and citrinin producing species of the genus *Penicillium* from feedstuffs. *International Journal of Food Microbiology* 126: 43-48.

BROGGI L.E., GONZALEZ H.H.L., RESNIK S.L. & PACIN A., 2007 — *Alternaria alternata* prevalence in cereal grains and spybean seeds from Entre Rios, Argentina. *Revista Iberoamericana de Micologa* 24: 47-51.

CHANG C.H., YU F.Y., WANG L.T., LIN Y.S. & LIU B.H., 2009 — Activation of ERK and JNK signaling pathways by mycotoxin citrinin in human cells. *Toxicology and Applied Pharmacology* 237: 281-287.

CIRILLO T., RITIENI A., VISONE M. & COCCHIERI R.A., 2003 — Evaluation of conventional and organic italian foodstuffs for deoxynivalenol and fumonisins B1 and B2. *Journal of Agricultural and Food Chemistry* 51: 8128-8131.

DESCLAUX D., ROYO C., NACHIT M.M., DI FONZO N. & ARAUS J.L., 2000 — Environmental conditions including black point symptom Durum wheat improvement in the Mediterranean region: new challenges. *Proceedings of a Seminar,* Zaragoza, Spain April 12-14.

DESJARDINS A.E., PROCTOR R.H., BAI G., MC CORMICK S.P., SHANER G., BUECHLEY G. & HOHN T.M., 1996 — Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Molecular Plant-Microbe Interactions* 9: 775-781.

DESJARDINS A.E. & PROCTOR R.H., 2007 — Molecular biology of *Fusarium* mycotoxins. *International Journal of Food Microbiology* 119: 47-50.

DESJARDINS A.E., 2006 — *Fusarium* mycotoxins: chemistry, genetics, and biology. *APS Press,* St.Paul, Minnesota, USA.

DOKO M.B., RAPIOR S., VISCONTI A. & SCHJOTH J.E., 1995 — Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *Journal of Agricultural and Food Chemistry* 43: 429-434.

DOMBRINK-KURTZMAN M.A., BENNETT G.A. & RICHARD J.L., 1994 — An optimized MTT bioassay for determination of cytotoxicity of fumonisins in turkey lymphocytes. *Journal of AOAC International* 77: 512-516.

EDWARDS S.G., 2004 — Influence of agricultural practices on *Fusarium* infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. *Toxicology Letters* 153: 29-35.

FEHR M., PAHLKE G., FRITZ J. & MARKO D., 2007 Altemariol acts as a topoisomerase poison. *29th Mycotoxin Workshop*, Fellbach, Germany: Abstract Book, pp. 123.

FRISVAD J.C. & SAMSON R.A., 2004 — Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. *Studies in Mycology* 49: 1-173.

FRISVAD J.C., SMEDSGAARD J., LARSEN T.O. & SAMSON R.A., 2004 — Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus Penicillium. *Studies in Mycology* 49: 201-242.

FRISVAD J.C. & THRANE U., 2000 — Mycotoxin production by common filamentous fungi. *In*: Samson RA, Hoekstra S, Frisvad JC, Filtenborg O, editors. *Introduction to Food and Airbone Fungi.* Utrecht: Centraalbureau voor Schimmelcultures, pp. 321-331.

GARON D., RICHARD E., SAGE L., BOUCHART V., POTTIER D. & LEBAILLY P., 2006 — Mycoflora and Multimycotoxin Detection in Corn Silage: Experimental Study. *Journal of Agricultural and Food Chemistry*, 54: 3479-3484.

GLYNN N.C., HARE M.C., PARRY D.W. & EDWARDS S.G., 2005 — Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties *majus* and *nivale* to species status. *Mycological Research* 109: 872-880.

GONZALEZ H.H.L., MARTINEZ E.G., PACÍN A. & RESNIK S.L., 1999 — Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurence on Argentinean durum wheat. *Mycopathologia* 144: 97-102.

GONZALEZ H.H.L., PACÍN A., RESNIK S.L. & MARTINEZ E.G., 1996 — Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinean wheat in 1993. *Mycopathologia* 135: 129-134.

GONZALEZ H.H.L., RESNIK S.L., BOCA R.T. & MARASAS W.F.O., 1995 — Mycoflora of Argentinean corn harvested in the main production area in 1990. *Mycopathologia*130: 29-36.

HADIDANE R., ROGER-REGNAULT C., ELLOUZ F., BACHA H., CREPPY E.E. & DIRHEIMER G., 1985 — Correlation between alimentary mycotoxin contamination and specific diseases. *Human Toxicology* 4: 491-501.

HAMMOND B.G., CAMPBELL K.W., PILCHER C.D., DEGOOVER T.A. & ROBINSON A.E., 2004 — Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the United states in 2000-2002. *Journal of Agricultural and Food Chemistry* 52: 1390-1397.

HETHERINGTON A. & RAISTRICK C., 1931 — Studies in biochemistry of microorganism XI on the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by *Penicillium citrinum*. Thom Philosophical Transactions of the Royal Society of London Series B-*Biological Sciences* 220: 269-297.

HUSSEIN S.H. & BRASEL J.M., 2001 — Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 167: 101-134.

ILHAN S. & ASAN A., 2001 — Soilborne fungi in wheat fields of Kirka vicinity (Turkey). *Biologia, Bratislava* 56: 263-71.

INTERNATIONAL AGENCY for RESEARCH on CANCER (IARC)., 1993 — Monographs on the evaluation of carcinogenic risk to humans. Lyon: *IARC* 1: 489.

JESTOI M., RETIENI A. & RIZZO A., 2004 — Analysis of the *Fusarium* mycotoxins fusaproliferin and trichothecenes in grains using chromatography - mass spectrometry. *Journal of Agricultural and Food Chemistry* 52: 1464-1469.

JOFFE A.Z., 1986 — *Fusarium* species. Their biology and toxicology. Ed. John Wiley & Sons, New York, NY, USA,.; ISBN 0-471-827332-0, pp. 173.

JUAN C., MOLTÓ J.C., LINO C.M. & MAÑES J., 2008 — Determination of ochratoxin A in organic and non-organic cereals. *Food Chemistry* 107: 525-530.

KOSIAK B., TORP M., SKJERVE E. & ANDERSEN B., 2004 — *Alternaria* and *Fusarium* in Norwegian grains of reduced quality-a matched pair sample study. *International Journal of Food Microbiology* 93: 51-62.

KOUADIO J.H., MOBIO T.A., BAUDRIMONT I., MOUKHA S., DANO S. & CREPPY E.E., 2005 — Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. *Toxicology* 213: 56-65.

LANGSETH W., BERNHOFT A., RUNDBERGET T., KOSIAK B. & GAREIS M., 1999 — Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. *Mycopathologia* 144: 103-113.

LANIER C., HEUTTE N., RICHARD E., BOUCHART V., LEBAILLY P. & GARON D., 2009 — Mycoflora and Mycotoxin Production in Oilseed Cakes during Farm Storage. *Journal of Agricultural and Food Chemistry 57:* 1640-1645.

LANIER C., RICHARD E., HEUTTE N., PICQUET R., BOUCHART V. & GARON D., 2010 — Airborne molds and mycotoxins associated with handling of corn silage and oilseed cakes in agricultural environment. *Atmospheric Environment* 44: 1980-1986.

LEHMANN L., WAGNER J. & METZLER M., 2006 — Estrogenic and clastogenic potential of the mycotoxin alternariol in cultured mammalian cells. *Food and Chemical Toxicology* 44: 398-408.

LI F. & YOSHIZAWA T., 2000 — *Alternaria* mycotoxins in weathered wheat from China. *Journal of Agricultural and Food Chemistry* 48: 2920-2924.

LI F.Q., LUO X.Y. & YOSHIZAWA T., 1999 — Mycotoxins (trichothecenes, zearalenone and fumonisins) in cereals associated with human red-mold intoxications stored since 1989 and 1991 in China. *Natural Toxins* 7: 93-97.

LI F.Q., TOYAZAKI N. & YOSHIZAWA Y., 2001 — Production of *Alternaria* mycotoxins by *Alternaria alternata* isolated from weather-damaged wheat. *Journal of Food Protection* 64: 567-571.

LIU G., QIAN Y., ZHANG P., DONG W., QI Y. & GUO H.T., 1992 — Etiological role of *Alternaria alternata* in human oesophageal cancer. *Chinese Medical Journal* 105: 394-400.

LOGRIECO A., BOTTALICO A., MULÉ M., MORETTI A. & PERRONE G., 2003 — Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. *European Journal of Plant Pathology* 109: 645-667.

LOGRIECO A., BOTTALICO A., SOLFRIZZO M. & MULE G., 1990 — Incidence of *Alternaria* species in grains from Mediterranean countries and their ability to produce mycotoxins. *Mycologia* 82: 501-505.

LUND F. & FRISVAD J.C., 2003 — *Penicillium verrucosum* in wheat and barley indicates presence of ochratoxin A. *Journal of Applied Microbiology* 95: 1117-1123.

MILLER J.D. & TRENHOLM H.L., 1994 — Mycotoxins in grain. Compounds Other than aflatoxin, *Eagan Press*, USA, ISBN 0-9624407-5-2. pp. 552.

MOLINIÉ A., FAUCET V., CASTEGNARO M. & PFOHL-LESZKOWICZ A., 2005 — Analysis of some breakfast cereals on the fresh market for their contents of ochratoxin A, citrinin and fumonisin B1; development of a method for simultaneous extraction of ochratoxin A and citrinin. *Food Chemistry* 92: 391-400.

MOSMANN T., 1983 — Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65: 55-63.

NELSON P.E., TOUSSON T.A. & MARASAS W.F.O., 1983 — *Fusarium* Species. *An Illustrated Manual for Identification.* The Pennsylvania State University Press: University Park.

PATRIARCA A., AZCARATE M.P., TERMINIELLO L. & FERNÁNDEZ PINTO V., 2007 — Mycotoxin production by *Alternaria* strains isolated from Argentinean wheat. *International Journal of Food Microbiology* 119: 219-222.

PEPELJNJAK S., UENO Y. & TANAKA T., 1992 — Mycological situation and occurrence of fusariotoxins in cereals from endemic nephropathy region in Croatia. *Microbiologie, aliments, nutrition* 10: 191-197.

PESTKA J. & SMOLINSKI A., 2005 — Deoxynivalenol: toxicology and potential effects on humans. *Journal of Toxicology and Environmental Health* 8: 39-69.

PETERSON S.W., 2008 — Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* 100: 205-226.

PETZINGER E. & WEINDENBACH A., 2002 — Mycotoxins in the food chain: the role of ochratoxins. *Livestock Production Science* 76: 245-250.

PFEIFFER E., ESCHBACH S. & METZLER M., 2007 — *Alternaria* toxins: DNA strand-breaking activity in mammalian cells *in vitro*. *Mycotoxin Research* 23: 152-57.

PFOHL-LESZKOWICZ A. & MANDERVILLE R.A., 2007 — Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. *Molecular Nutrition & Food Research* 51: 61-99.

PITT J.I. & HOCKING A.D., 1997 — Fungi and food spoilage. New York: *Blackie Academic and Professional*.

PRELUSKY D.B., TRENHOLM H.L., ROTTER B.A., MILLER J.D., SAVARD M.E., YEUNG J.M. & SCOTT P.M., 1996 — Biological fate of fumonisin B1 in food-producing animals. *Advances in Experimental Medicine and Biology* 392: 265-278.

PRELUSKY D.B. & TRENHOLM H.L., 1993 — The efficacy of various classes of anti-emetics in preventing deoxynivalenol induced vomiting in pigs. *Natural Toxins* 1: 296- 302.

ROTTER B.A., PRELUSKY D.B. & PESTKA J.J., 1996 — Toxicology of deoxynivalenol (vomitoxin). *Journal of Toxicology and Environmental Health* 48: 1-34.

SABERI R., JAVAN-NIKKHAH M., HEIDARIAN R., HOSSENI S. & SOLEIMANI P., 2004 — Detection of fungal infectious agent of wheat grains in store-pits of Markazi province, Iran. *Agricultural and Applied Biological Sciences* 67: 418-23.

SAVA V., REUNOVA O., VELASQUEZ A., HARBISON R. & SANCHEZ-RAMOS J., 2006 — Acute neurotoxic effects of the fungal metabolite ochratoxin-A. *Neurotoxicology* 27: 82-92.

SCOTT P.M., 2004 — Other mycotoxins. *In*: Magan N, Olsen M, editors. *Mycotoxins in Food.* Boca Raton: Wood head Publishing Ltd and CRC Press, pp. 406-440.

SHEPHARD G.S., THIEL P.G., SYDENHAM E.W. & SAVARD M.E., 1995 — Fate of a single dose of 14C-labelled fumonisin B1 in vervet monkeys. *Natural Toxins* 3: 145-150.

SHIER W.T., ABBAS H.K. & MIROCHA C.J., 1991 — Toxicity of mycotoxins fumosin B1 and B2 and *Alternaria alternaria lycopersici* toxin (AAL) in cultured mammalian cells. *Mycopathology* 116: 97-104.

SIMMONS E.G., 1986 — *Alternaria* themes and variations (22-26). *Mycotaxon* 25: 287-308.

SIMPSON D.R., WESTON G.E., TURNER J.A., JENNINGS P. & NICHOLSON P., 2001 — Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *European Journal of Plant Pathology* 107: 421-431.

STEC J., SZCZOTKA M. & KUZMAK J., 2007 — Cytotoxicity of feed-borne mycotoxins to animal cell lines in vitro using the MTT assay. *Bulletin of the Veterinary Institute in Pulawy* 51: 679-684.

SUDAKIN D.L., 2003 — Trichothecenes in the environment: Relevance to human health. *Toxicology Letters* 143: 97-107.

TERSE P.S., MADHYASTHA M.S., ZUROVAC O., STRINGFELLOW D., MASQUARDT R.R. & KEMPPAINEN B.W., 1993 — Comparison *in vitro* and *in vivo* biological activity of mycotoxins. *Toxicon* 31: 913-919.

TUTELYAN V.A., 2004 — Deoxynivalenol in cereals in Russia. *Toxicology Letters* 153: 173-179.

VAN DER MERWE K.J., STEYN P.S. & FOURIE L., 1965 — Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. *Nature* 205: 1112-1113.

VELLUTI A., MARIN S., BETTUCCI L., RAMOS A.J. & SANCHIS V., 2000 — The effect of fungal competition on colonization of maize grain by *Fusarium moniliforme*, *F. proliferatum* and *F. graminearum* and on fumonisin B1 and zearalenone formation. *International Journal of Food Microbiology* 59: 59-66.

WESTHUIZEN L., SHEPARD G.S., SCUSSEL V.M., COSTA L.L.F., VISMER H.F., RHEEDER J.P. & MARASAS W.F.O., 2003 — Fumonisin contamination and *Fusarium* incidence in corn from Santa Catarina, Brazil.. *Journal of Agricultural and Food Chemistry* 51:5574-5578.

WOLFF J., 2000 — Ochratoxin A in cereal and cereal products. *Archives fur Lebensmittelhygiene* 51: 81-88.

WOODY M.A. & CHU F.S., 1992 — Toxicology of *Alternaria* mycotoxins. *In*: Chelkowsky J, Visconti A, editors. *Alternaria Biology, Plant Diseases and Metabolites.* Amsterdam, Netherlands: Elsevier Science Publishers pp. 409-434.

XU X.M., PARRY D., NICHOLSON P., SIMPSON D., EDWARDS S., COOKE B., DOOHAN F., BRENNAN J., MONAGHAN S., MORETTI A., TOCCO G., MULE G., HORNOK L., GICZEY G. & TATNELL J., 2005 — Predominance and association of pathogenic species causing *Fusarium* ear blight in wheat. *European Journal of Plant Pathology* 112: 143-154.

ZAIED C., ABID S., ZORGUI L., BOUAZIZ C., CHOUCHANE S., JOMAA M. & BACHA H., 2009 — Natural occurrence of ochratoxin A in Tunisian cereals. *Food Control*; 20: 218-222.

ZINEDINE A., BRERA C., ELAKHDARI S., CATANO C.F., DEBEGNACH F., ANGELINI S DE SANTIS B., FAID M., BENLEMLIH M., MINARDI V. & MIRAGLIA M., 2006 — Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food control* 17: 868-874.

ZUREIK M., NEULCK C., LEYNNART B., LIARD R., BAUSQUET J. & NEUKIRAH F., 2002 — Sanitization to airborne mould and severity of asthma: Cross sectional study from European Community respiratory health survey. *British Medical Journal* 325: 411.