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DETECTION OF AIR-BORNE MYCOTOXIN LEVELS BY IMMUNOBIOSENSOR

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ABSTRACT

The main purpose of this article is to investigate the possibility of detection of the level of mycotoxins in air samples by a surface plasmon resonance (SPR) immunobiosensor. Air samples of 0.25-0.5 m^3 volume collected using a special miniature air pump device were passed through 20% acetonitrile as an absorption liquid, and 20 µl aliquots of the extract were analyzed by an SPR immunobiosensor. Comparative analyses by the determination of T2 mycotoxin content were carried out in air and in grain samples upon dispersion in a phytotron. Moreover, mycotoxin content was investigated also under field conditions in air and in vegetable matrix, in the absence and presence of *Fusarium fungi*, and results were shown to have similar characteristics: the level of mycotoxins detected in air samples correlated with that in grain samples. Thus, results of air-borne mycotoxin detection were correlate with mycotoxin levels detected in environmental samples and may used for screening purposes.

KEYWORDS: immunobiosensor, T2 mycotoxin, control of level, air samples.

1. INTRODUCTION

Mycotoxins are very widely occurring toxins of natural fungal origin, being produced by more than 200 species of micromycetes producing approximately 400 low molecular weight substances.[1,2] The vast majority of these secondary fungal metabolites exert toxicity to

living organisms, and that is why different analytical methods including thin-layer chromatography, gas or liquid chromatography, enzyme linked immunosorbent assays (ELISAs) and different types of immunobiosensors are developed for their detection.[3-8] To provide successful analysis of the presence of these substances in environmental samples, it is of utmost importance to choose a highly effective method that is both sufficiently sensitive, rapid, economic and feasible under field conditions. All variants of the immunobiosensors we developed,[8-11] meet these requirements. Detection performance, as widely known in analytical chemistry, also depends on sample preparation techniques, mostly relying on extraction of the mycotoxin analytes from solid samples. We have previously described some of these methods,[12] yet it has to be underlined that sample preparation protocols for detection of mycotoxins in air samples are to date lacking. This study aimed towards two approaches: a) on-line analysis of plant and air samples under cultivation conditions for preliminary assessment of crop infection by fungus on the one hand, and for possible further utilization in crop cultivation on the other hand; and b) control of toxic substances in given atmospheric samples. The approach was demonstrated on T2 mycotoxin as main target analyte, certain toxic effects of which exceeded the action of mustard gas or Lewisite.[13] and came into the focus of concern related to bioterrorism. Taken the above-mentioned factors into consideration, the main purpose of this report is to indicate the possibility of rapid determination of mycotoxin contents in air samples by immunobiosensor technology.

2. MATERIALS AND METHODS

All reagents were obtained from Millipore–Sigma-Aldrich (St. Louis, MO, USA). Mycotoxin levels were determined by our surface plasmon resonance (SPR) immunobiosensor produced in the V.M. Glushkov Institute of Cybernetics of the National Academy of Sciences of Ukraine (Kyiv, Ukraine). To obtain specific antibodies to *Fusarium* fungus, rabbits were immunized according to standard procedure. Fraction of G the antibodies obtained was isolated by ammonium sulfate precipitation.[14]

Air sample preparation was carried out by bubbling 0, 25-0,5 m^3 through a 20% solution of acetonitrile in water by a special mini-pump device. Plant samples were subjected to direct extraction with 20% acetonitrile, as our previous investigations,[12] indicated that this solvent is optimal for the extraction of mycotoxins from various solid samples. A drop (approximately 20µl volume) of this aqueous acetonitrile extract was placed on the transducer sensor surface preliminary devised by the formation of several successive layers

from: a) polyallylamine hydrochloride; b) specific antibodies and c) bovine serum albumin, using an optimized SPR immunobiosensor analysis protocol.[12]

In model experiments T2 mycotoxin in acetonitrile solution (at concentrations of 10-1000 µg/ml) was dispersed locally in a special air chamber. Experiments with wheat at the physiological stage of spikelet formation, spiked similarly with T2 mycotoxin or infected with *Fusarium* fungi, were carried out in a small phytotron (Silver box evolution) and also under field conditions. Plants and ambient air were systematically sampled, processed sample extracts were subjected to SPR immunobiosensor analysis, with parallel determination of T2 mycotoxin levels in air and plants samples in all cases.

3. RESULTS AND DISCUSSION

T2 mycotoxin was detected both in plant and air samples within one day following its arbitrary dispersion (in a volume of 5 ml at a concentration of 5 µg/ml) in the special phytotron: its concentration ranged 5-10 ng/g in the wheat samples and 60-80 ng/m^3 in the air samples.

In field monitoring, T2 mycotoxin levels correlated not only in the in the plant itself and in ambient air, but also the level of some *Fusarium* structures in the air, which are able stimulate specific response of the immune biosensor. The calibration curve of the dependence of the immune biosensor response on the concentration of some *Fusarium* structures is given in Fig.1.

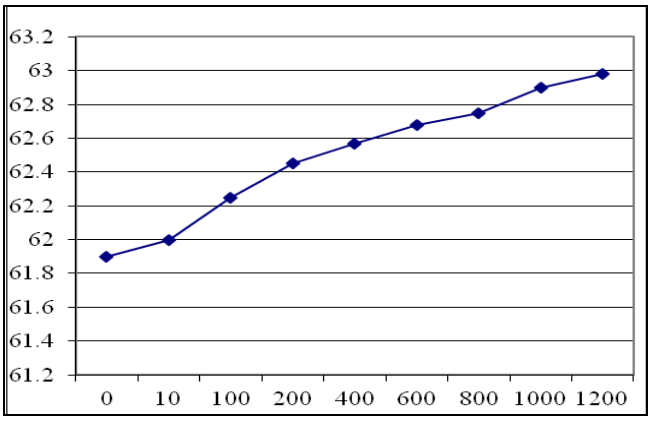

Fig. 1: The dependence of the SPR immune biosensor response on the concentration of some *Fusarium* structures in the solution Ordinate - changes of resonant angle (angle/ sec), abscise - ng/mL).

The analysis in field conditions indicate that some structures of fungi were present in real, of course they do not produce mycotoxin at full level when yet immature (Table 1). Nonetheless, their presence or absence should signal about possible levels of mycotoxin currently or at a later stage. Thus, such a comparison is justified as the very presence of fungi detected can predict long-term mycotoxin occurrence in the grain, especially during storage. The results obtained unambiguously testify their unidirectional nature, which definitely indicates that taking the level of mycotoxins in the air in the cultivation area of infected plants into attention allows conclusions regarding the state of the future grain and a possibility to take appropriate measures. To confirm this, T2 mycotoxin levels were determined similarly in parallel during grain sifting in the crop plant infected with fungi and containing a different amount of mycotoxin and in ambient air (Table 2). The results obtained fully prove that air sample analysis provides reliable information regarding the mycotoxin level and therefore, the fungal infection state of the grain processed. As a simple and rapid analytical method current variants of the immunobiosensor proposed by us earlier[8-11] is available for the screening estimation of mycotoxin content in the grain of what.

Table 1. The comparison of the levels of the content mycotoxins in air, vegetable and the presence of *Fusarium* fungi in air.

Number of the observed field	Level of mycotoxins in the crop plant and in the air		Relative units of some *Fusarium* structures in the air
	In wheat (ng/g)	In the air (ng/m^3)	
1	97	140	120
2	78	100	101
3	20	20	42
4	9	10	15
5	> 5	< 10	~

Table 2: The results of the analysis air from the grains containing mycotoxin.

Samples	Levels of T2 mycotoxin	
	After extraction from grain (ng/g)	In air during grain sifting (ng/m^3)
1	75.0	92.0
2	27.0	26.8
3	12.0	< 20.0
4	5.0-10.0	~

4. CONCLUSIONS

The results obtained strongly indicate that screening control of T2 mycotoxin in crop commodities may be carried out not only by analysing crop plant samples, but by

determination of mycotoxin levels in ambient air samples as well. Detection of air-borne mycotoxins allows solutions to a number of principal and technical problems. A main advantage of the approach proposed roots in the simplicity of the method for the determination of mycotoxin level, allowing the assessment under field conditions of the infection status of cultivated plants. In addition, the procedure makes the estimation of the overall level of mycotoxin in corn possible without plant sampling and extraction. Moreover, monitoring mycotoxins in ambient air offers a field of application of outstanding importance, namely monitoring the presence of air-borne toxic agents, released by accidental or deliberate (e.g., terrorist actions) means.

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