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Ecological and Trophic Features of Toxigenic Fungi Contaminating of Fodders

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Abstract

Feed grain contamination with mycotoxins occurs in the field following infection of plants with particular microscopic fungi, characterized by the ability to synthesize toxic substances of various chemical composition that are hazardous to the health of humans and animals. In the present investigation epiphytic and endophytic microbiota of winter wheat ear collected from different stages of its formation were studied. Isolation and identification of microorganisms, and their antimicrobial activity were made with using of general mycological and microbiological methods. Forty eight epiphyte and thirty four endophyte species of fungi were classified. It was found that micromycetes from genera Alternaria, Aspergillus, Fusarium and Penicillium were dominant. Fungi isolated are known as producers of toxins that contaminate fodder and can be a reason of acute, and chronicle diseases of animal and human. Strains Fusarium sambucinum 139, Fusarium oxysporum 1806, F. culmorum 216, F. graminearum 986, Aspergillus flavus 8799, A. fumigatus 276, Penicillium granulatum 2898, Alternaria tenuissima 2706 characterized by wide spectrum of antimicrobial activity. Five strains of Bacillus subtilis were isolated from samples of winter wheat ear characterized by antagonistic activity towards plant pathogenic fungi. The field experiments showed positive results of its using for biological control of winter wheat diseases. Ecological and trophic features, of toxigenic fungi contaminating fodders define by selective action of plants during vegetation period and antagonistic interactions at the wheat ear colonization. Monitoring of toxigenic fungi development under field conditions is very important for prevention of mycotoxin accumulation in fodders and plant products. Results received will be used to controlling mycotoxin contamination by reducing fungal infection during plant vegetation and suppression of plant diseases through biological agents.

Keywords: epiphytic and endophytic mycobiota, winter wheat, toxins, antagonism

Introduction

The last decades were characterized by total deterioration in mycotoxicological situation in the world because of ecological imbalance as result of intensive technologies of crop production, environment pollution and climate changes (Baliukoniene, 2003; Lugauskas 2006). Products of fungal metabolism – mycotoxins have special place

in group of toxicants. Mycotoxins penetrate into animal and man's organisms with plant fodder and food and can be a reason of acute, and chronicle diseases.

Today, it is known more than 250 species of toxigenic fungi that affect agricultural plants and more than 400 toxic substances of different chemical origin and mechanism of biological action on organism (Bhatnagar, 2000). Mainly soil and rhizosphere micromycetes transferred with dust and insects are the source of fodder infection and contamination by mycotoxins (Kharchenko, 2003, 2007; Hentosh, 2012).

The representatives of genera Fusarium Link, Aspergillus P. Micheli, Penicillium Link are known as the main producers of mycotoxins contaminated fodders (Koval 2014). Additionally, microorganisms colonize vegetative plants and develop intensively on their roots, stems, leaves, seeds (grain). Plant pathogens such as Fusarium, Aspergillus, defeated vegetative plants can continue development after harvest as saprotrophs on grain, stems, straw etc. Therefore, it is necessary to control fodder quality not only at harvest and storage, but also during vegetation period. Studying of plant pathogenic fungi are actually, because of their mycotoxins involved in the trophic cycle of biosphere.

Materials and Method

The species structure of epiphytic and endophytic microbiota was studied at different stages of winter wheat ear development. A total 864 samples were analyzed.
Colonies of epiphytic microorganisms were obtained by direct plating of plant pieces on Potato Dextrose agar (PDA). Winter wheat ear were surface sterilized by placing in 70% ethanol for 2 min, 2% sodium hypochlorite and washed in sterile water. After sterilization ear parts were cut and placed to PDA plates. All plates were incubated at 25°C±2 for 7 days. As microorganisms emerged, they were transferred to PDA slants to obtain pure cultures. Pure isolates of bacteria were identified on the basis of their cultural, morphological and physiological characteristics. Identification of micromycetes species was done with light microscopy.

Antimicrobial activity of fungal strains was studied by paper disk method. Similarity coefficient of Jakar was used for characterization of epiphytic and endophytic mycobiota at different wheat ear formation (Methods of experimental mycology, 1982):

$$Kj = \frac{c}{a+b-c} \cdot 100\%,$$

a – number of species isolated at one ear development stage; b – number of species isolated at another ear development stage; c – number of total species.

Results and discussion

The results showed that winter wheat ear at different development stages was contaminated by fungi of a wide spectrum. Epiphytic mycobiota were represented by 48 species of micromycetes belonged to 3 phylums: Zygomycota (6 species), Ascomycota (2 species) and Anamorphic fungi (40 species). The following fungal species were most frequently isolated Alternaria alternata, A. tenuissima, Aspergillus flavus,

A. fumigatus, A. nidulans, A. niger, A. terreus, Fusarium avenaceum, F. culmorum, F. gibbosum, F. graminearum, F. sambucinum, F. solani, F. sporotrichioides, F. oxysporum, F. verticillioides, Penicillium aurantiogriseum, P. canescens, P. chrysogenum, P. commune, P. corylophylum, P. cyclopium, P. funiculosum, P. griseofulvum, P. purpurogenum, P. restrictum, P. solitum, P. verrucosum. Many of these fungi are able to synthesize various toxic secondary metabolites (Baliukoniene 2003; Bhatnagar 2000; Lugauskas, 2006).

Flowering and milk stages were characterized by the highest number of fungal species isolated, 44 and 37 species accordingly, but the lowest number of fungi observed at the heading stage (29).

Table 1: *Dynamic of epiphytic mycobiota of winter wheat ear during vegetation peri-*

Stage of ear formation	Family and genera of fungi						
	Mucoraceae	*Dematiaceae*	*Fusarium*	*Aspergillus*	*Penicillium*	*Trichoderma*	*Trichothecium*
Heading	5,3	25,3	12,8	8,6	20,2	0	11,0
Flowering	7,6	25,5	35,3	8,5	11,5	3,0	8,6
Milk ripening	5,4	36,2	24,0	7,8	4,0	5,9	16,0
Wax ripening	0	68,9	23,7	0	2,8	0	4,6

od, % from total number of isolated species

Dark colored fungi (Dematiaceae family) dominated during all ear formation stages studied (Table 1). Fusarium species were the most numerous mostly at flowering and milk ripening. High variety of Penicillium species was observed at heading.

The maximal similarity of species structure of epiphytic fungi was found between stages of flowering and milk (Kj 76,1%) and milk and wax ripening of grain (Kj 74,4%). Stages of heading and wax ripening had the biggest difference in species structure – Kj 20,0%. This is due to the fact that different plant parts vary in their chemical compounds during ontogenesis (Wang 2015).

Endophytic mycobiota of winter wheat ear was represented by 3 phylums, where the group of Anamorphic fungi was the most numeral too. Totally 34 species of fungi were isolated.

Species structure of endophytes characterized by less variety. Micromycetes of genera Alternaria, Aspergillus, Fusarium and Penicillium prevailed.

The stages of flowering and milk ripening characterized by the highest number of fungal species isolated too. It is 20 and 21 species accordingly, the lowest number of fungi observed at stage of heading (16).

Stage of ear formation	Family and genera of fungi						
	Mucoraceae	*Dematiaceae*	*Fusarium*	*Aspergillus*	*Penicillium*	*Trichoderma*	*Trichothecium*
Heading	2,2	45,1	26,3	6,1	16,0	0	3,6
Flowering	2,9	54,2	15,2	14,3	10,3	0	3,2
Milk ripening	0	31,5	36,1	0,1	19,4	0	0
Wax ripening	0	46,7	24,8	0	20,3	0	4,2

Table 2: Dynamic of endophytic mycobiota of winter wheat ear during vegetation period, % from total number of isolated species

Dark colored micromycetes dominated by species number at most stages of ear formation (Table 2). Fungi of genus Fusarium were prevalent at the milk stage.

The highest similarity of endophyte species structure was found between milk and wax ripening of grain (Kj 58,3%). The biggest difference was between species structure of flowering and wax stages (Kj 19,4%).

Filamentous fungi are well known for their production of substances with antimicrobial activities, several of which have formed the basis for the development of new clinically important antimicrobial agents (Kharchenko, 1986, 2003, 2007; Svahn 2012). Therefore, colonization of plant substrate by fungi not only depends on their biochemical features such as presence of certain enzyme complex and also ability to produce substances toxic to other organisms. Fungal toxins have function to provide surviving in competitive fight for substrate. Mycotoxins are similar with antibiotics, because of they specific action on microorganism metabolism (Kharchenko, 1986).

The antimicrobial activity of 27 species (74 strains) of fungi, isolated from winter wheat ear was studied. Strains Fusarium sambucinum 139, Fusarium oxysporum 1806, F. culmorum 216, F. graminearum 986, Aspergillus flavus 8799, A. fumigatus 276, Penicillium granulatum 2898, Alternaria tenuissima 2706 characterized by wide spectrum of antimicrobial activity.

Results of our research confirmed the statement of some authors that micromycetes of the Fusarium genus most frequently infect grain in the field and regarded as the most active toxin producers (Lugauskas 2007; Muthomi 2000; Webley 1998). During the research five strains of Bacillus subtilis were isolated from samples of winter wheat ear at different stages of plant development. Bacteria of this genus can be used as biocontrol agents of plant diseases (Cavaglieri 2005; Cawoy 2011; Schmiedeknecht 2001). Our experiments showed their antagonistic activity towards plant pathogenic fungi. The strains were tested under the field conditions as biocontrol agents and positive results were obtained.

Conclusions

The research has shown that Alternaria, Aspergillus, Fusarium and Penicillium species isolated from winter wheat ear at different development stages are known as potential toxins producers and contaminants of grain feed (Baliukoniene 2003; Lugauskas 2006, 2007; Sapsai 2016; Shukla 2015). Fungi from Dematiaceae family prevailed during all ear development stages. The maximal similarity of epiphytic and endophytic mycobiota structure was found between stages of flowering and milk. Fungal isolates characterized by wide spectrum of antimicrobial activity. Strains of Bacillus subtilis with antifungal activity were found among of isolated microorganisms. Thus, ecological and trophic features, of toxigenic fungi contaminating fodders define by selective action of plants during vegetation period and concurrent microbial interactions at the wheat ear colonization. Monitoring of toxigenic fungi development under field conditions is very important for prevention of mycotoxin accumulation in fodders and plant products. Results received will be used to controlling mycotoxin contamination by reducing fungal infection during plant vegetation and suppression of plant diseases through biological agents.

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