TITLE: Visual, instrumental, mycological and mycotoxicological characterization of wheat inoculated with and protected against *Alternaria spp.*

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Visual, instrumental, mycological and mycotoxicological characterization of wheat inoculated with and protected against *Alternaria* spp.

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Abstract

The aim of this work was to characterize visual properties, instrumentally measured colour properties, field fungi presence and *Alternaria* toxins levels in wheat samples grown under conditions aimed at inhibition and stimulation of wheat infection with fungi from the *Alternaria* genus. Experiment was carried out on the wheat treated by fungicide and wheat inoculated by *Alternaria* spp., while non treated wheat was used as a control. Statistically significant difference was observed between all three treatments using visual scale. Protected wheat samples were significantly different from other samples in terms of all measured colour parameters while inoculated and control wheat samples were significantly different in terms of lightness and dominant wavelength. Identification of field fungi in the all examined wheat samples showed that the dominant mycotoxigenic fungus was *Alternaria* spp., followed by *Fusarium* spp. The content of *Alternaria* toxins in samples of wheat hulls and dehulled kernels indicated higher concentrations of *Alternaria* toxins in hulls than in dehulled kernels.

Keywords: colour, wheat ears, wheat kernels, field fungi, *Alternaria* toxins.

Available online at the Journal website: http://www.ache.org.rs/HI/

SCIENTIFIC PAPER

UDC 633.11–248:66

Hem. Ind. **70** (0) 000–000 (2016)

doi: 10.2298/HEMIND141114031J

Cereal grains are the primary source of energy in the human diet, with wheat being the third most produced grain worldwide. Recent studies have shown that besides *Fusarium* spp., fungi of the genus *Alternaria* became the dominant source of wheat kernels contamination [1]. The two major features of *Alternaria* species are the production of melanin, especially in the spores, and the production of host-specific toxins in the case of pathogenic species [2]. Apart from a role in conidial development [3], melanin appears to have an indirect as well as a direct function in virulence. It acts as 'body ~~armor~~', protecting fungi against environmental stress or unfavourable conditions like extreme temperatures, UV-radiation and compounds secreted by microbial antagonists, thus adding to longevity and survival [3,4]. In addition, the melanin rapidly reacts with free oxygen radicals which are components of the host defense system against the penetration of the pathogen, thus increasing the susceptibility of the host [5]. The usual symptom of infection with *Alternaria* spp. is darkening of the cereal ears (Figure 1a) before harvest [6]. There are various types of discolouration that can affect common (*Triticum aestivum* L.) wheat kernels. Black point and dark smudge, mostly associated with *Alternaria alternata* (Fr.) Keissl., and *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dast. (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker) [7] are common discolourations of cereal seed, which occur in most regions where these crop species are grown. The condition in wheat or barley known as black point is a dark discoloration at the embryo end of the kernel (Figure 1b), resulting in downgrading of the grain quality. In severe cases, the discoloration occurs in the outer pericarp and inner seed coat tissue, and may extend along the groove on the ventral side of the grain [8,9].

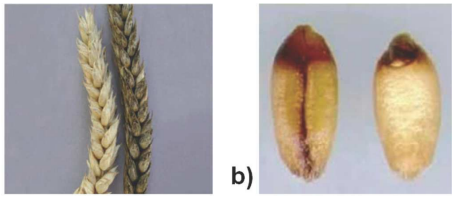

Figure 1. Symptoms of infection with Alternaria spp. on wheat ear (right) (a, taken from URL: http://www.hgca.com/, accessed 06/05/2015) and on grain (b, taken from URL: http:// //agropedia.iitk.ac.in/content/black-point-disease-wheat/, accessed 06/05/2015).

These types of kernel discolouration vary significantly in incidence and severity depending on environmental conditions during kernel maturation. Numerous other studies indicate that black point may be a result of abiotic stresses, as symptoms are more likely to occur after extreme environmental conditions such as heavy rain, high humidity and extremes of temperature [7,10–15]. However, a recent study showed that

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Paper received: 14 November, 2014
Paper accepted: 1 June, 2015

although abiotic factors, such as high humidity levels, can promote the occasional development of black point or dark smudge on durum wheat kernels under controlled-environment conditions, fungal infection by *C. sativus* or *A. alternata* was the main factor associated with their development [16]. Besides pathogenicity and reduction of quality of kernels, several *Alternaria* spp. are known producers of toxic secondary metabolites, *Alternaria* mycotoxins tenuazonic acid, alternariol, alternariol monomethyl ether (Figure 2a–c), altenuen and altertoxins, which might be harmful for human and animal health [1].

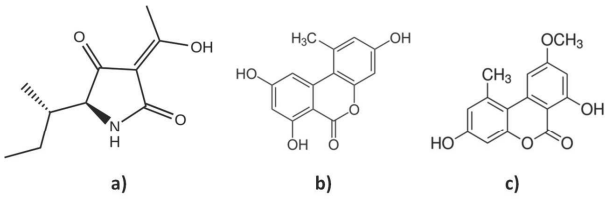

Figure 2. Structures of examined Alternaria toxins: tenuazonic acid (a), alternariol (b) and alternariol monomethyl ether (c).

In this context, the aim of this work was to explore the possibility of determining the intensity of field fungi infestation by visual scale application and instrumental measurement of wheat ears and kernels colour and to determine field fungi presence and *Alternaria* toxins levels in wheat samples grown under conditions aimed at inhibition and stimulation of infection of wheat with fungi from the *Alternaria* genus.

MATERIAL AND METHODS

Material and climate conditions

Experiment was carried out in the 2012/2013 season in the region of Vojvodina, north Serbia on the wheat (*Triticum aestivum* cv. Sirtaki) protected by fungicide and wheat inoculated by *Alternaria* spp., while non treated wheat was used as a control. May 2013 was characterized by weather fluctuations, warmer and more humid weather conditions than multiannual average, with surplus precipitation (ΣPrec. 125 mm). According to Standardized Precipitation Index (SPI), *Z* index, and Palmer Drought Severity Index (*PDSI*) values (1.9, 4.8 and 4.0, respectively), this month had extreme humid weather conditions.

Fungus culture and inoculation

The isolates of *A. tenuissima* were multiplied in 8 L Chapek medium at room temperature (25 °C) with daylight cycles for 14 days. Medium was aerated during the isolate multiplication. For the purpose of inoculation, a conidial suspension was sprayed on the plants with a hand atomizer. Before spraying, flasks were shaken vigorously and 1 mL suspension was poured in to a haemocytometer and the number of infective particles was counted in ten replications. The average concentration of *A. tenuissima* conidia was 0.13025×10^6 infective particles/mL. At the full flowering stage inoculation was performed with 8 L of aqueous suspension of fungal isolates. Inoculated spikes were immediately covered with plant protection cover (Stocker, Italy) for 48 h. ~~None treated~~ spikes ~~was~~ used as control ~~object~~. In the full ripeness stage spikes from each plot were cut by hands and used for next analysis.

Chemicals and reagents

Alternariol (AOH, purity 99.0%), alternariol monomethyl ether (AME) (purity 99.5%) and tenuazonic acid (TeA, purity 99.5%) standards were purchased from Sigma-Aldrich (St Louis, MO, USA). Stock solutions of AOH (2500 μg mL^{-1}), AME (2500 μg mL^{-1}) and TeA (10000 μg mL^{-1}) were prepared in methanol and stored at –20 °C. The following solvents were used: methanol (MeOH, J.T.Baker, Deventer, Netherlands) and ethyl acetate (EtOAc, Sigma-Aldrich (Saint Louis, USA), all LC–MS grade, formic acid (FA, purity 99.9%, Carlo Erba, Italy), and fuming HCl (37%, pa, Merck, Darmstadt, Germany). Deionized water was sourced from a Millipore Simplicity UV water purification system (Bedford, MA, USA).

Visual scale establishing and colour measurement

Visual scale (1–6) of wheat ear colour was established, where 1 represented the lightest sample and 6 represented the darkest sample. These samples were also measured instrumentally in order to explore the correlations between these two methods. The colour of wheat kernels was only instrumentally measured.

The colour of all samples was measured with Konica Minolta Chroma Meter CR-400, using different attachments; for the measurement of wheat ears colour, Light Protection Tube CR-A33f was used, while the colour of wheat kernels and hulls was measured using Granular Attachment CR-A50. The CIE L* (lightness), CIE a* (red–green) and CIE b* (yellow–blue), and dominant wavelength (DWL) were read using a D_{65} light source and the observer angle at 2°. The tristimulus values of CIE L*, a* and b* readings were calibrated against a standard white plate (Y = 84.8; x = 0.3199; y = 0.3377). Each wheat ear sample was divided in four subgroups, and colour of one hundred ears from each subgroup (400 ears from one sample) was measured on 6–8 locations, depending on the ear size. Each wheat kernel sample was also divided in four subgroups, and ten replications were measured from each subgroup (40 replications per sample in total).

Percent of kernel infection

According to the method proposed by Pitt and Hocking [17], 100 wheat kernels were randomly sel-

ected from each treatment. The samples were disinfected with 0.4% NaClO, rinsed with water for 2 min and placed on Petri plates in four repetitions (25 kernels per plate). Incubation was conducted at 25 °C and after seven days intensity of infection was assessed. Confirmation of fungi genera was carried out by microscopic examination on potato dextrose agar (PDA) and malt extract agar (MEA) media after 7 days of incubation at 25 °C.

LC–MS/MS analysis

The Agilent 1200 series liquid chromatography, consisting of vacuum degasser, binary pump, autosampler and thermostated column compartment was used for separation of analytes, whose detection was carried out by means of Agilent series 6410A triple-quad mass spectrometer with electrospray ionization (ESI). MassHunter ver. B.03.01 software (Agilent Technologies Inc., USA) was used for instruments control and data analysis. The separation was achieved using a Zorbax Eclipse XDB-C18 column (50 mm×4.6 mm i.d., 1.8 µm) (Agilent Technologies) with a column compartment temperature of 50 °C. The binary mobile phase consisted of 0.05% aqueous formic acid (A) and methanol (B) and was delivered at a flow rate of 1 mL/min. Components were eluted in gradient mode, starting with 30% B, followed by a linear gradient reaching 70% B in 6.0 min, then by a linear gradient reaching 100% B in 9.0 min and holding for 3.0 min, with post-time of 3.0 min. The entire effluent was transferred to mass spectrometer, without flow splitting. The injection volume for all samples was 15 µL. ESI parameters were as follows: drying gas (N_2) temperature 350 °C, flow 10 L/min, nebulizer gas pressure 50 psi, and capillary voltage 4 kV. Compounds were quantified in negative ionization dynamic selected reactions monitoring mode. Each compound was monitored at determined retention time ±1.5 min. Fragmentor voltage and collision energies were optimized for each analyte during infusion of the pure standard, and the most abundant fragment ions were chosen for the selected reaction monitoring. The utilized MRM transitions (*m/z* mother ion→quantifier/qualifier) were *m/z* 196.2→139.0 (fragmentor 170 V, collision energy 15V)/112.0 (fragmentor 170 V, collision energy 20 V) for TeA, *m/z* 257.2→213.0 (fragmentor 180 V, collision energy 20 V) /215.0 (fragmentor 180 V, collision energy 25 V) for AOH and *m/z* 271.3→256.0 (fragmentor 130 V, collision energy 20 V)/228.0 (fragmentor 160 V, collision energy 30 V) for AME. The mean retention times (n = 20) were 4.14 min for TeA, 5.03 min for AOH and 7.01 for AME.

Sample preparation

Method of sample preparation by Siegel *et al.* [18] was slightly modified. Samples (500 g) were ground to a 1 mm particle size using laboratory mill (KnifetecTM 1095 mill, Foss, Hoganas, Sweden). After that, approximately 1 g (exact weights known) of homogenized samples were mixed with 7 mL water. Subsequently, 2 mL of 2 mol/L aq. HCl and 5 mL of EtOAc were added. The resulting ternary phase systems were shaken on shaker (Griffin and George, Wembley, England) for 45 min, ultrasonicated for 10 min (ATM40-3LCD, Madrid, Spain) and shaken again for 45 min. Then, the extracts were transferred into glass cuvettes and centrifuged (Tehtnica, Železniki, Yugoslavia) at ~~4200~~*g* for 15 min to achieve complete phase separation. Thereafter, 2 mL of the upper EtOAc layers were transferred into another glass cuvette, and evaporated under a stream of nitrogen (Reacti-Therm I #18821, Thermo Scientific, USA). The dry residue was dissolved in 1 mL of LC/MC grade MeOH, and transferred to an HPLC vial through the Econofilter PTFE (13 mm, 0.2 µm) syringe filter (Agilent Technologies, China) and stored at –20 °C until analysis.

Validation of method

The developed method was validated by in-house quality control procedure following the guidelines of Commission Decision 2002/657/EC [19]. Method validation was performed in terms of matrix effects, linearity, trueness, precision (repeatability), limit of detection (LOD) and limit of quantification (LOQ). The calibration curves for all of the compounds in pure solvent and in matrix were obtained by plotting the peak areas against the concentrations of the corresponding calibration standards at five calibration levels ranging from 25.0 to 100.0 µg/kg for TeA and 2.5 to 10.0 µg/kg for AOH and AME. The linearity of calibration curves was expressed by the correlation coefficient (r^2). For the matrix-matched calibration curves (MMC), the blank wheat samples were enriched with working standard solutions at the final reconstitution step providing linearity over the range from the 25.0 to 100.0 µg/kg for TeA and from the 2.5 to 10.0 µg/kg for AOH and AME (five-point MMC). For overall method recovery, the blank wheat samples were spiked prior to sample preparation, providing linearity also over the range from the 25.0 to 100.0 µg/kg for TeA and from 2.5 to 10.0 µg/kg for AOH and AME (five-point R_A) in three replicates. Spiked samples were left overnight at room temperature to allow solvent evaporation and equilibration between analytes and matrix. To differentiate between extraction efficiency and matrix-induced signal suppression/enhancement, the slope ratios of the linear calibration functions were calculated to yield the apparent recovery (R_A), *i.e.*, the overall method recovery and the signal suppression/enhancement (SSE) due to matrix effects. The recovery of the extraction step (R_E), *i.e.*, sample preparation recovery was calculated by dividing the overall recovery by the matrix effect as follows (modified after Matuszewski *et al.* [20]):

R_A(%) =
= 100×slope spiked sample/slope liquid standard (1)

SSE(%) =100×slope matrix-matched standard/slope liquid standard (2)

R_E(%) = 100×slope spiked sample/slope matrix-matched standard (3)

The precision of the method was expressed in terms of repeatability, *i.e.*, as relative standard deviation (*%RSD*) of 6 replicates at three concentration levels (25.0, 50.0 and 100.0 µg/kg for TeA and 2.5, 5.0 and 10.0 µg/kg for AOH and AME) using the spiked blank wheat samples prior to analysis using the MMC curve.

The limit of detection (*LOD*) and limit of quantification (*LOQ*) were estimated by injecting decreasing concentrations of matrix-matched standards and measuring the response at a signal-to-noise ratio (*S/N*) of ≥3 and ≥10 for the *LOD* and *LOQ*, respectively.

Statistical analysis

Pearson correlation coefficients on different significance levels (5, 1 and 0.1%) between visual scale and measured parameters were calculated. Analysis of variance (ANOVA) and Duncan's multiple range tests were applied to compare means at 5% significance level. Principal Component Analysis (PCA) was applied to explore the relationships among the colour parameters and to group the wheat ears used for visual scale establishing. Data analysis was performed using the statistical data analysis software system Statistica, version 12.0.

RESULTS AND DISCUSSION

Obtained results are discussed in the scope of the colour properties of wheat ears, kernel colour properties, presence and distribution of the field fungi infecting wheat and presence and concentration of *Alternaria* toxins.

Visual and instrumental assessment of wheat ear colour

The results obtained by application of visual evaluation of wheat ears and their instrumental colour measurement (Table 1) indicate that all three treatments differed significantly among each other by scores obtained using visual scale, with protected wheat sample assessed with the lowest scores, and inoculated wheat sample with the highest scores. Protected wheat samples were significantly different from other samples in terms of all measured colour parameters (L*, a*, b* and *DWL*). Inoculated and control wheat samples were significantly different in terms of lightness and dominant wavelength.

Regarding the correlations between methods of visual and instrumental assessment of colour of Pearson correlation coefficients between visual scale and instrumental measurement showed that L* values were in the highest negative correlation (–0.97, $p < 0.001$) with the visual scale. Other colour parameters (b*and *DWL*) were also in high correlation ($p < 0.001$) with the visual scale (–0.72 and +0.74, respectively). Colour parameter a* was also significantly positively correlated with the visual scale at 5% significance level. Six wheat ears used for visual scale establishing differed significantly among each other only by L* (lightness) values.

PCA was performed for the colour values the wheat samples used for establishment of visual scale, and the results showed that high percentage of total variance is explained by the first two components (95.66%, Figure 3a).

Lightness (L*) and yellow tone (b*) were close to the circle line and close to each other, which indicated high contribution of these two parameter to the total variance as well as high correlation (r close to +1) between them. Red tone (a*) and dominant wavelength (*DWL*) were also close to the circle line which pointed out their importance in explanation of variance between the wheat samples.

Wheat ears that comprised the visual scale were completely separated by colour parameters (Figure 3b). Wheat ears assessed as ~~1 – 3~~ were distinguished mostly by lightness and yellow tone. Wheat ear assessed as 6 was almost in opposite to the wheat ear marked as 3, indicating dark colour of this sample. Higher values of *DWL* and a* caused differentiation of wheat ears marked as 4 and 5, which could be explained by more prominent red hue of these samples. Measured colour parameters were in most cases in accordance with appearance of wheat ears used for visual scale establishing.

Table 1. Colour parameters of differently treated wheat ears; 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample. Results are presented as mean±standard deviation (n ≈ 2400). Values with the different superscript within the same column are statistically different (p < 0.05)

Sample	L*	a*	b*	*DWL* / nm	Visual scale
0	60.91±3.05^b	2.89±0.70^a	24.07±3.89^b	578.33±0.52^a	2.90±0.50^b
1	63.16±3.46^a	2.49±0.69^c	25.41±3.32^a	577.94±0.49^c	2.35±0.74^c
2	60.09±3.87^c	2.81±0.71^b	24.11±4.01^b	578.29±0.59^b	3.01±0.75^a

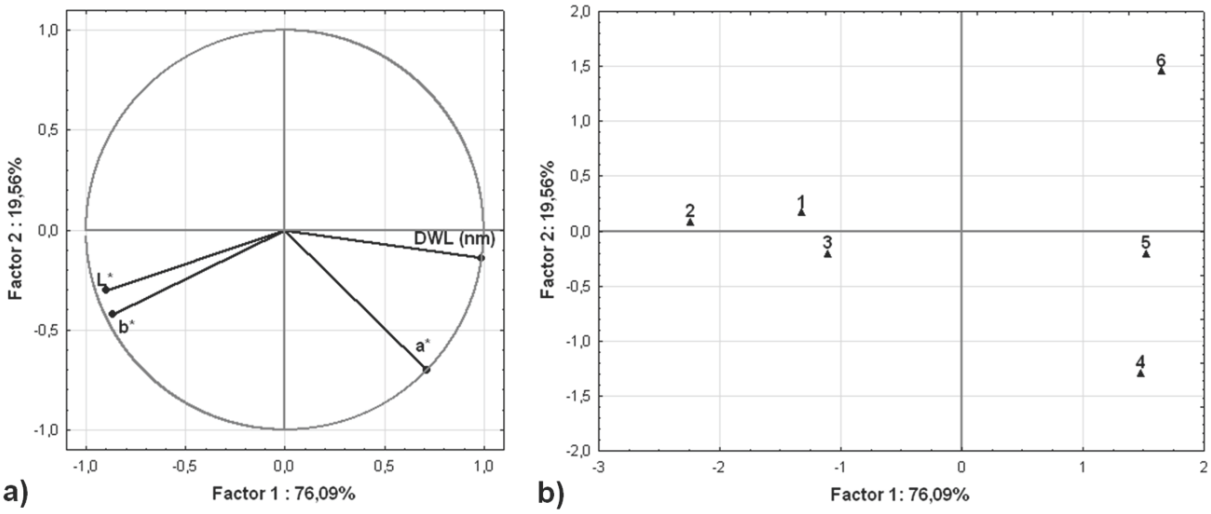

Figure 3. Projection of variables (a) and cases (b) on the factor plane.

Instrumental assessment of wheat kernel colour

Results of kernel colour measurement were slightly different: kernels of inoculated wheat differed significantly from other samples in terms of L*, a* and b* parameters (Table 2), whereas all samples belonged to the same homogenous group by dominant wavelength values.

Table 2. Colour parameters of differently treated wheat kernels ; 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample. Results are presented as mean±standard deviation (n = 40). Values with the different superscript within the same column are statistically different (p < 0.05)

Sample	L*	a*	b*	*DWL* / nm
0	53.05±1.63^{a}	8.73±0.91^{a}	23.81±1.60^{a}	582.64±0.41^{a}
1	53.34±1.88^{a}	8.87±0.88^{a}	24.09±1.84^{a}	582.68±0.37^{a}
2	52.20±1.53^{b}	8.30±0.96^{b}	22.25±1.84^{b}	582.67±0.40^{a}

Considering all mentioned above, it can be concluded that infection entered the kernel in greater extent in inoculated wheat samples, while in non-treated samples it was less prominent. Protected wheat samples were generally characterized by higher lightness and more prominent yellow tone. Results obtained by instrumental colour measurement are in accordance with the visual scale assessment of treated wheat samples.

Field fungi distribution

Identification of field fungi in ~~the~~ all examined wheat samples showed (Table 3) that the dominant field fungi was *Rhizopus* spp., while of mycotoxigenic fungus *Alternaria* spp. was dominant, followed by *Fusarium* spp.

Percent of infection by *Alternaria* spp. was the highest for inoculated samples as expected. This caused the differentiation of this wheat sample by its dark colour as determined both by application of visual scale and instrumental measurement, which was expected due to the fact that *Alternaria* spp. produce melanin pigments.

Presence of *Alternaria* toxins

Presence of *Alternaria* toxins in examined hulls and dehulled wheat samples for the applied treatments was analyzed by high performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (HPLC-ESI–MS/MS). The validation data of the employed analytical method for the determination of selected *Alternaria* toxins are given in Table 4, while precision, expressed as repeatability gave *RSD* values of 15.0, 10.2 and 9.3% for AOH, 19.9, 10.4 and 15.8% for AME and 10.7, 8.3 and 8.9% for TeA. Linearity, gave values of correlation coefficients (r^2) above 0.9903 for all three *Alternaria* toxins for standard and matix-matched calibration curves and calib-

Table 3. Presence of certain genera of fungi in wheat samples; 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample

Sample	Fungi genus, %				
	Fusarium	*Alternaria*	*Cladosporium*	*Rhizopus*	*Penicillium*
0	2	31.5	1	65.5	–
1	2	29	–	69	–
2	4	44.5	–	50	1.5

ration curves of spiked samples in the concentration range from 25.0 to 100.0 µg/kg for TeA and from 2.5 to 10.0 µg/kg for AOH and AME.

Table 4. Limit of detection, limit of quantification, matrix effects, overall method recovery and sample preparation recovery data of the employed analytical method; LOD – limit of detection; LOQ – limit of quantification ; SSE – slope ratio of matrix-mached calibration curve and standard calibration curve for selected analytes; R_A – overall method recovery (slope ratio of calibration curve of spiked samples and standard calibration curve for selected analytes ; R_E – sample preparation recovery (slope ratio of calibration curve of spiked samples and matrix-mached calibration curve for selected analytes)

Alternaria toxin	*LOD/LOQ* µg/kg	SSE %	R_A %	R_E %
AOH	0.75/2.5	99.6	71.4	71.7
AME	0.1/0.3	92.7	70.7	76.2
TeA	2.5/7.5	125.3	87.7	70.0

Based on obtained validation parameters, the developed method was successfully validated according to the criteria specified in Commission Decision 2002/657/EC for quantitative confirmation method [19].

The results on presence of *Alternaria* toxins in examined samples are given in Table 5.

Table 5. Content of alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) in hulls and dehulled wheat samples for the applied treatments (µg/kg); 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample

Sample	AOH	AME	TeA
0 – hulls	10.6	0.99	189
1 – hulls	–	0.54	105
2 – hulls	21.1	1.25	227
0 – dehulled wheat	–	–	59.5
1 – dehulled wheat	–	–	30.7
2 – dehulled wheat	–	0.46	96.6

It should be noted that the *Alternaria* toxins were quantified by external matrix-matched calibration procedure. Also, the presented results are corrected with samples preparation recovery and it was recalculated on the dry matter.

TeA was the predominant toxin quantified in all analysed cases. In the hulls of treated and non-treated wheat samples all three examined *Alternaria* toxins were detected, while in hulls of protected wheat sample AOH was not detected. Also, it can be seen that ~~in sample~~ of dehulled wheat ~~kernels~~ the highest concentration of TeA was detected in inoculated wheat kernels. The detected levels of TeA were about three times higher in hulls compared to kernels from all treatments. It should be noted that concentration of AOH was doubled in hulls of inoculated wheat sample compared to hulls of non-treated wheat sample, while AOH was not detected in dehulled wheat samples in any of analysed cases. The detected level of AME was about three times higher in hulls compared to dehulled kernels in inoculated wheat sample. Hulls might be considered as physical barriers with a protective effect from pathogens on kernels, which has been proved in hulled barley and oat varieties [21,22]. There are several studies dealing with the protective role of hulls against mycotoxins produced by *Fusarium* species [21––24], but data on the ability of hulls to protect wheat kernels from the *Alternaria* toxins is still scarce. Similarly to our findings, Vučković *et al*. [25] showed the protective effect of hulls on the occurrence of *Alternaria* toxins in spelt wheat.

On the basis of obtained results, visual scale or instrumental measurement of wheat ear colour could be applied for the fast determination of infection degree by *Alternaria* spp. in the field. Anyhow, these methods should be validated with larger number of samples, cultivars and production years. Methodology of sampling should be also further elaborated in order to obtain representative results. One of the further directions of research should be examination of possible relation between colour and content of *Alternaria* toxins as well as prediction of toxin content by colour measurements performed in field. In addition, possible protective effect of hulls could be important for organic farming.

CONCLUSIONS

Results obtained for the non-treated, protected and inoculated wheat samples showed that instrumentally measured colour parameters are in accordance with the visual scale assessment. Pearson correlation coefficients between visual scale and instrumental measurement showed that L* values were in the highest negative correlation with the visual scale; moreover, samples used for visual scale establishing differed significantly among each other only by L* values. Identification of field fungi in the all examined wheat samples showed that the dominant mycotoxigenic fungus was *Alternaria* spp., followed by *Fusarium* spp. It can be concluded that higher degree of infection by *Alternaria* spp., higher score on visual scale and lower L* (lightness) values were directly related due to the production of melanin pigments by this genus of fungi. Obtained results indicate the higher concentrations of *Alternaria* toxins in hulls than in dehulled kernels which implicate the possible protective effect of wheat hulls.

Acknowledgements

This paper is a result of the researches within the projects III 46001 and III 46005, financed by the Min-

istry of Education, Science and Technological Development, Republic of Serbia.

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IZVOD

VIZUELNA, INSTRUMENTALNA, MIKOLOŠKA I MIKOTOKSIKOLOŠKA KARAKTERIZACIJA PŠENICE GAJENE U USLOVIMA STIMULACIJE I INHIBICIJE ZARAŽENOSTI SA *Alternaria spp.*

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Žita predstavljaju primarni izvor energije u ljudskoj ishrani, pri čemu u svetskoj proizvodnji pšenica zauzima treće mesto po proizvedenoj količini. Skorašnja istraživanja su pokazala da su pored *Fusarium* spp. gljive iz roda *Alternaria* postale dominantni zagađivači pšeničnog zrna. Pored patogenosti i narušavanja kvaliteta pšeničnog zrna, pojedine vrste roda *Alternaria* su poznate kao proizvođači sekundarnih toksičnih metabolita, *Alternaria* toksina, koji mogu biti štetni po zdravlje ljudi i životinja. Cilj ovog rada je bio karakterizacija vizuelno i instrumentalno merenih svojstava boje, prisustva i zastupljenosti poljskih fitopatogenih gljiva i *Alternaria* toksina u uzorcima pšenice gajenih u uslovima usmerenim na inhibiciju i stimulaciju razvoja gljiva iz roda *Alternaria*. Eksperiment je izveden na uzorcima pšenice tretirane fungicidom i na uzorcima pšenice inokulisane sa *Alternaria* spp., dok je netretirana pšenica korišćena kao kontrola. Boja klasova pšenice koji su predstavljali vizuelnu skalu je instrumentalno izmerena radi utvrđivanja korelacije između ove dve metode. Grupisanje pšeničnih klasova po vrednostima svetloće (L*) bilo je u skladu sa korišćenom skalom za vizuelnu ocenu boje. Štaviše, vrednosti svetloće su bile u najvećoj negativnoj korelaciji (-0,97, $p < 0,001$) sa vizuelnom skalom. Pri vizuelnoj oceni boje pšeničnih klasova utvrđena je statistički značajna razlika između tri tretmana. Uzorci pšenice zaštićene fungicidom su se statistički značajno razlikovali od ostalih uzoraka u pogledu svih izmerenih parametara boje (L*, a*, b* i dominantna talasna dužina). Uzorci inokulisane i kontrolne pšenice su se međusobno statistički značajno razlikovali u pogledu svetloće klasova i dominantne talasne dužine. Boja pšeničnog zrna je ispitana samo primenom instrumentalne ocene boje. Boja inokulisanog pšeničnog zrna se statistički značajno razlikovala od boje zrna ostalih uzoraka u pogledu L*, a* i b* parametara boje. Identifikacija poljskih gljiva u svim ispitivanim uzorcima pšenice je pokazala da je dominantna gljiva koja produkuje mikotoksine upravo *Alternaria* spp., a zatim sledi *Fusarium* spp. Dodatno je ispitan sadržaj *Alternaria* toksina u uzorcima pšenične plevice i u pšeničnom zrnu za primenjene tretmane primenom visoko performansne tečne hromatografije spregnute sa masenim detektorom. Dobijeni rezultati ukazuju na veće koncentracije *Alternaria* toksina u pšeničnoj plevici u odnosu na pšenično zrno bez plevice ukazujući na mogući zaštitni efekat plevice pšenice.

Ključne reči: Boja • Pšenični klasovi • Pšenično zrno • Fitopatogene gljive • *Alternaria* toksini