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RESEARCH REGARDING THE CONTAMINATION WITH *Fusarium* spp. OF THE WHEAT GRAINS FROM THE VARIETY *Triticum aestivum* ssp. *spelta* BEFORE AND AFTER THE TREATMENT WITH BIO-FUNGICIDE - CASE STUDY

Otilia COTUNA[1, 2], Mirela PARASCHIVU[3], Veronica SĂRĂȚEANU[1], Marinel Nicolae HORABLAGA[1,2], Carmen Claudia DURĂU[1]

[1]Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania
[2]Agricultural Research and Development Station Lovrin, Timis County, Romania
[3]University of Craiova, Faculty of Agronomy, Department of Agriculture and Forestry Technologies, Craiova, Romania

Corresponding author email: paraschivumirela@yahoo.com

Abstract

In the last 20 years, the demand for spelt wheat has increased rapidly and was anticipated that it will continuate to increase with a rate of about 5% annually. The demand is determined by the consumers perceptions and by the scientific proofs that highlight that spelt wheat has a higher nutritional value compared with common wheat. The purpose of this research was to determinate the Fusarium spp. contamination level of the spelt wheat grains treated with bio-fungicide in comparison with the non-treated variants. The biological material used in this research was the spelt winter wheat variety Rokosz. The obtained results after the incubation of the samples show the presence of the fungus Fusarium spp. in the spelt wheat grains from all the variants. The contamination index determined was 73% in the non-treated variant and 80% in the treated one. The insignificant difference was considered to be influenced by the fact that spelt wheat grains are covered with hull, that being the reason why the bio-fungicide doesn't adhere to the seed surface.

Key words: *Fusarium sp., Triticum spelta, bio-fungicide, spelt wheat, fungal contamination.*

INTRODUCTION

Spelt wheat (*Triticum aestivum* ssp. s*pelta*) is an ancient wheat with the grain covered with hulls, that was cultivated still from the year 7000-8000 BC (Dvorak et al., 2012; Packa et al., 2019). This wheat subspecies is suitable for cultivation in different climatic and pedological conditions, sometimes even harsher compared to common wheat, it being able to grow in mountainous areas and in nutrient-poor soils too (Bonafaccia et al., 2000; Pospišil et al., 2011). Spelt wheat has been disadvantaged in cultivation for long time in the favour of common wheat, mainly due to the low yields (Krawczyk et al., 2008). However, the ability of spelt wheat to tolerate unfavourable environmental conditions and the valuable nutrients contained in the grains has caught the attention of the farmers as well as the consumers attention. (Haliniarz et al., 2020). Spelt wheat has a higher content of minerals and proteins in comparison with common wheat (Capouchova, 2001; Lacko M., 2010; Suchowilska et al., 2012).

Most experts point out that in the last 20 years, the area cultivated with spelt wheat has increased. There is an annual increase of the areas cultivated in the conventional farming system, but the increase is greater in the ecological farming area with about 5%; thus, there was noticed the expansion of this crop in areas where it wasn't cultivated before (Lacko-Bartošová et al., 2010; Koutroubas et al., 2012; Wang et al., 2021). *Triticum spelta* is nowadays an attractive crop for cultivation in ecological farming system due to its natural resistance to the attack of the pathogens and pests determined by the hulls that adhere tight to the caryopses and which acts as a protective barrier against all the external factors (Wiwart et Suchowilska, 2009). In addition to diseases and pests, invasive species of weeds are a threat for wheat (Ștef et al., 2013; Vîrteiu et al., 2015).

Spelt wheat cultivated in ecological farming system doesn't needs application of fertilizers and phytosanitary treatments as is necessary for the common wheat and durum wheat (Radomski et al., 2007; Kohajdova et Karovicova, 2008; Krawczyk et al., 2008; Wilson et al., 2008; Zielinski et al., 2008).
Spelt wheat is suitable for organic agriculture where the application of synthesis fertilizers and pesticides are forbidden due to its obvious qualities (valuable nutrients, tolerance for diseases and pests and for unfavourable soil and climate conditions) (Finch et al., 2006; Sinkevičienė et al., 2019).
In Romania, the areas cultivated with spelt wheat in conventional system and organic system are increasing (Eurostat, 2022).
Fusarium head blight is a very dangerous disease of wheat produced by different *Fusarium* species which in the favourable years determinates important harvest loses, both quantitative and qualitative (Becher et al., 2013; Cotuna et al., 2013; Paraschivu et al., 2014). Climate changes from the last years have leaded to the repeated appearance of the fusarium head blight epidemics in the great wheat-growing areas; they have damaged the harvests due to the mycotoxins present in grains (Aboukhaddour et al., 2020). While many aspects of common wheat resistance to fusarium head blight are known nowadays, there are fewer researches referring to the resistance levels and sources of resistance in spelt wheat (Buerstmayr et al., 2020; Zhu et al., 2019; Miedaner et al., 2019).
In these researches was tested a low number of spelt wheat genotypes (Chrpová et al., 2013; Wiwart et al., 2016; Góral și Ochodzki, 2017). Chrpova et al. (2021) shows that most of the modern spelt wheat varieties are susceptible and very susceptible to fusarium head blight and to the contamination with deoxynivalenol (DON).
The researches regarding the use of the biological agents for the control of fusarium head blight are few. Sinkevičienė et Pekarskas (2019) highlight the efficiency of some bio-products (medicinal plants extracts, bio-humus and volatile oils) on some grain pathogens from the experiments carried out on spring wheat varieties. The same authors show that the efficiency of some bio-products against the fungus *Fusarium* was even 50% in some variants. Couto et al. (2021) shows in research that the treatment with *Trichoderma* spp. applied to the common wheat grains had the capacity to prevent and even to control the fungi from the genus *Fusarium*, the number of grains free of fungi being increased after treatment in comparison with the non-treated control.
The researchers are even less in the case of spelt wheat, mostly for the cultivation in ecological cropping system where the seeds shall be treated with bio-fungicides.
The relaunch of the spelt wheat crop, especially in the organic cultivation system, requires researches on the effectiveness of bio-fungicides against to the pathogens that infect the seed.
In the present research, samples of organic spelt wheat seed of the Rokosz variety (from Poland) were analysed in the laboratory before and after the treatment with a bio-fungicide based on *Trichoderma* spp. The seeds of spelt wheat were designated for the establishment of an organic crop. The spelt wheat variety Rokosz was analysed to determinate if it is contaminated with *Fusarium* spp. The aim of the study was to assess the fusarium head blight contamination index of spelt wheat before and after treatment; other aspect analysed was the treatment efficiency.

MATERIALS AND METHODS

In this experiment was used as biological material a variety of spelt winter wheat (*Triticum aestivum* ssp. *spelta*) produced in Poland and approved in the year 2012 named Rokosz. This spelt wheat variety is suitable for ecological cropping system due to its features. In laboratory were brought two samples of one kilogram each, one treated and one non-treated with bio-fungicide, to be analysed from the point of view of the contamination with *Fusarium* spp. the two samples were collected from a batch of seeds that was brought from Poland in order to be cultivated in an organic farm from Timiș County. The bio-fungicide used for the spelt wheat seeds is one allowed in ecological agriculture. The product used has the commercial name Micover WP and is formulated as wettable powder. This bio-product contains mycorrhizae (*Rhizophagus irregularis,* AGF 630, 20% (1 x 10^3 prop./g), rhizobacteria (*Bacillus* sp. AGF 527 1 x 10^6 CFU/g) and spores of the antagonist fungus *Trichoderma harzianum* AGF 276 1 x 10^8 UFC/g. The seeds were treated with the dose of 1 kg/t Micover WP in mixture with a product based on marine algae *Ascophyllum nodosum* - 3 l/t (Superfifty). The contamination index of the seeds with *Fusarium* spp. was assessed with the classical method that consists in placing of the seeds in Petri plates on agar culture medium. The culture medium used in this experiment was simple agar that was prepared in laboratory. The preparation of the spelt wheat seeds for the culture medium was done respecting the following preparatory stages: seeds washing with tap water; seeds disinfection in ethanol 96% for 1 minute; the successive washing of the caryopsis disinfected in two sterile water baths; drying of the washed seeds on sterile filter paper. There were prepared three variants with three replicates (about 41 caryopsis/ replicate): non-sterilized and non-treated (V1), sterilized and non-treated (V2) and treated with bio-fungicide (V3). After the placing of the seeds on the culture medium the Petri plates were incubated at the temperature of 24 ºC for 7 days.

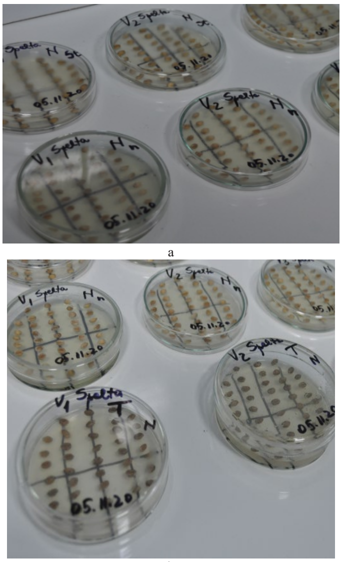

Figure 1. a) and b) Spelt wheat seeds from the variety Rokosz placed on agar culture medium for the stimulation of pathogens growth (Cotuna Otilia, 2020)

The samples were evaluated after 7 days from incubation. The examination of the mycelia growth on seeds was realised at stereomicroscope, being completed with microscopic evaluation for the accurate identification of the fungi that have growth on the seed surface. The obtained data were used for the calculation of the fungal contamination index (ICS%). This index for the spelt wheat was calculated with the formula: *ICS% = contaminated seeds no./total seeds no. on plates x 100* (Doolotkeldieva, 2010)**.** The statistical analysis was performed with the software JASP (Version 0.14) (2020).

RESULTS AND DISCUSSIONS

The use of bio-products based on *Trichoderma* spp. for the wheat seed treatment is mentioned in very few studies nowadays. There are necessary numerous researches to show the efficiency of the *Trichoderma* species against the seed pathogens and to indicate the precise doses for a good control. In the present study, the bio-product used for the seeds of spelt wheat contains *Trichoderma harzianum* spores in a concentration of 1x10^8 UFC/g. The spelt wheat seeds from the V1 weren't treated with bio-fungicide and weren't sterilized before the placing on the culture medium for the observation of the mycelia growing from the exterior and from the interior of the seeds. The three replicates of this variant were contaminated with *Fusarium* spp. in rates comprised between 68% and 78%. The average of the fungal contamination of the variant was 72.35% (Figure 2).

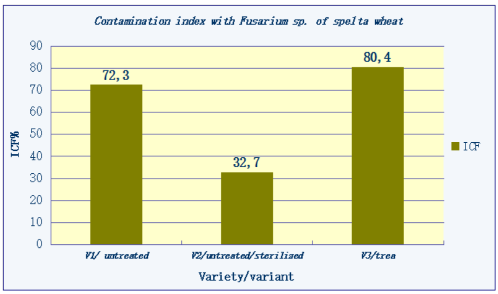

Figure 2. Comparative presentation of the contamination index with *Fusarium* spp. of spelt wheat seeds from variety Rokosz (Cotuna Otilia, 2020)

In V2 the spelt wheat seeds weren't treated with bio-fungicide, but they were sterilized with alcohol 96% for 2 minutes to observe the mycelia growing from the interior of the seeds. The contamination rate with *Fusarium* spp. of this variant was comprised between 25% and 41%. The variant average was 32.72%, much lower in comparison with the V1 in which the seeds weren't sterilized with alcohol.

In V3 the seeds were treated with bio-fungicide, they were left to dry and after were placed on the culture medium. The index of fungal contamination from this variant was comprised between 60% and 93%. The average contamination rate of the sample was 80.48%, the highest from the experiment, even the seeds were treated with bio-fungicide (Figure 2)

After the incubation of the samples, at the surface of the seeds were grew white-pinkish mycelia. The analysis at stereomicroscope revealed the presence of the mycelia with cottony like white-pink mycelia specific to the fungus *Fusarium* spp. on the surface of the spelt wheat seeds in all the analysed variants in different rates (Figures 3-5).

Identification of the pathogen *Fusarium* spp. was done in a first stage visually by analysing the mycelia at the stereomicroscope and second after the fungus was identified using the microscope.

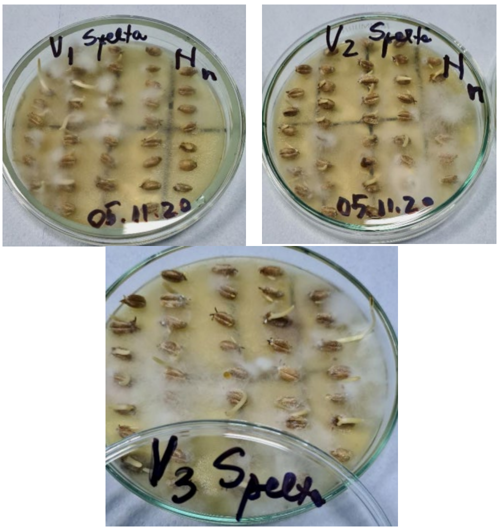

Figure 3. *Fusarium* spp. mycelia grown at the surface of spelt wheat seeds from V1, non-treated and non-sterilized (Cotuna Otilia, 2020)

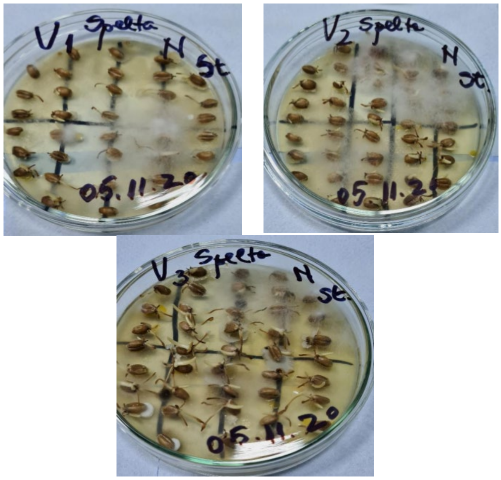

Figure 4. *Fusarium* spp. mycelia grown on spelt wheat seeds surface from V2, non-treated but sterilized with ethanol 96% (Cotuna Otilia, 2020)

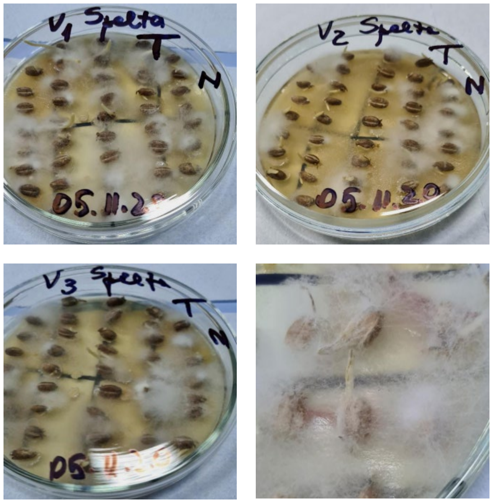

Figure 5. *Fusarium* spp. mycelia grown on spelt wheat seeds surface from V3, treated with cu bio-fungicide based on *Trichoderma sp.* (Cotuna Otilia, 2020)

The microscopic slides prepared for the identification of the fungus were stained with lactophenol blue. Typical *Fusarium* spp. conidia, mycelia and chlamydospores were highlighted at microscope (Figure 6).

The obtained results were processed by variance analysis (ANOVA), respectively F test and Tukey Test. The comparative statistical analysis of the data shows that the difference is insignificant between the V1 non-treated and the V3 treated with bio-fungicide (Table 3).

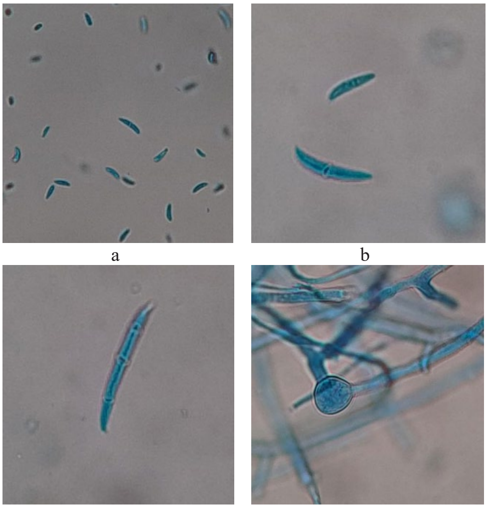

Figure 6. a, b, c) Micro and macroconidia of *Fusarium* spp.; d) mycelium hyphae and chlamydospore of *Fusarium spp.* (Photo at microscope x40, Cotuna Otilia, 2020)

Same statistical significance was registered between V1 (non-treated and sterilized with ethanol) and V2 (non-treated and sterilized with ethanol) (Table 3). The descriptive analysis of the data regarding the contamination index with *Fusarium* spp. (standard deviation, averages and F test) are presented in Table 1 and Table 2, and in Figure 7.

Table 1. Averages and standard deviations for contamination index with *Fusarium* spp. of spelt wheat variety Rokosz

	Fusarium spp. contamination index		
	V1/non-treated control	V2/ non-treated / sterilized	V3/treated
Valid	3	3	3
Missing	0	0	0
Mean	72.353	26.053	80.483
Std. Deviation	5.074	5.201	17.074
Minimum	68.290	21.460	60.970
Maximum	78.040	31.700	92.680

Efficiency of the bio-products on the spelt wheat seeds is quite less studied. In the case of spelt wheat (with hulled grains), the researches are very few and the results are divert, sometimes contradictory, depending by the biological agents used in experiments. The treatment of the wheat seeds with bio-products based on mycorrhizae, fungi and antagonist bacteria could improve a lot their phytosanitary condition and other features too, mostly in the ecological cultivation systems where the chemical substances cannot be applied.

Table 2. Variance analysis (ANOVA) for contamination index with *Fusarium* spp. of spelt wheat variety Rokosz

ANOVA - *Fusarium* contamination index						
Homogeneity Correction	Cases	Sum of Squares	df	Mean Square	F	p
Brown-Forsythe	Sample	5172.412	2.000	2586.206	22.533	0.020
	Residual	688.640	2.745	250.885		
Welch	Sample	5172.412	2.000	2586.206	55.439	0.002
	Residual	688.640	3.653	188.490		

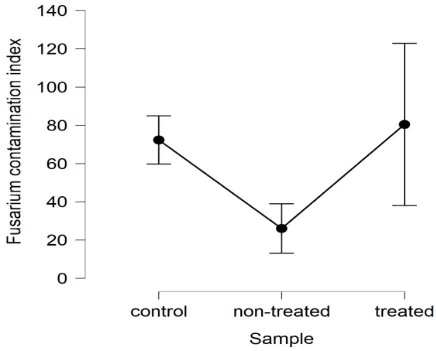

Figure 7. Contamination index with *Fusarium* spp. of spelt wheat grians variety Rokosz (control = V1 non-treated/unsterilized; non - treated = V2 non-treated/sterilized; treated = treated with bio-fungicide)

Table 3. Post Hoc comparison analysis (Tukey test) for the contamination index with *Fusarium* spp. of the spelt wheat variety Rokosz experimental variants

Post Hoc Comparisons - Sample							
			95% CI for Mean Difference				
		Mean Difference	Lower	Upper	SE	t	p_{tukey}
V1/ control	V2/ non-treated/ sterilized	46.300	19.461	73.139	8.747	5.293	0.004**
	V3/ treated	-8.130	-34.969	18.709	8.747	-0.929	0.643
V2 non-treated	V3 treated	-54.430	-81.269	-27.591	8.747	-6.222	0.002**
** p < 0.01							
Note. P-value and confidence intervals adjusted for comparing a family of 3 estimates (confidence intervals corrected using the Tukey method).							

Couto et al. (2021) shows the treatment of common wheat seeds with bio-products based on *Trichoderma* spp., bacteria and mycorrhizae gives good results in comparison with the chemical treatments. With all of these, there can be seen differences depending by the species *Trichoderma* used and the applied doses. In their study (Couto et al., 2021) *Trichoderma asperellum* has inhibited the growth of the fungi on common wheat seeds in all the treated varieties, while in case *T. harzianum* only in a single variety was noticed the efficiency of the treatment. The rate of seeds without fungi has increased linearly with the increase of the doses of *T. harzianum*, the most efficient proved to be the dose of 2 x 10^{12} CFU (Couto et al., 2021).

In our research, the efficiency of the bio-product based on mycorrhizae, bacteria and *T. harzianum* 2 x 10^{8} CFU wasn't the expected one, the difference between non-treated and treated being not significant from statistical point of view. *Fusarium* spp. grew on the spelt wheat seeds, the lowest incidence of the seeds with mycelia being determined in the variant non-treated but sterilized with alcohol (Figure 2). In the other two variants the incidence passed over 70%. Such results were reported by other authors too which have highlighted that spelt wheat can be colonized by numerous fungi, but predominant is *Fusarium* spp. (Haliniartz et al., 2020). In contrast, in the tests carried out on common wheat seeds, Sinkevičienė and Pekarskas (2019) show that some bio-products have diminished the contamination with *Fusarium* spp. up to 50%.

Our opinion is that the obtained result in the present study was influenced by at least to factors: the presence of the hulls adhered on the spelt wheat grains which haven't allowed to the bio-product to be in direct contact with the grain and the bio-fungicide applied dose. These aspects are making difficult the control of the pathogens from the seed mostly in the case of the ecological crops, increasing the susceptibility of the plants to disease during the growing season. On the other hand, if the seeds are not infected, the coating hulls provide some protection against the pathogens and pests (Chrpová, 2021). According with Vinale et al. (2009) the capacity of the *Trichoderma* species to control the pathogens can be oscillating. Even there are numerous studies which highlight the resistance of spelt wheat to the fungal pathogens of the seeds given by the hulled seeds (Suchy et al., 2018), however, the seeds of the variety Rokosz used in this research were contaminated with *Fusarium* spp. in a very high rate. Chrpova et al. (2021) say that most of the modern varieties of spelt wheat are susceptible to be infected with *Fusarium* spp. The introduction of spelt wheat in ecological agriculture system brings in attention the biological measures designated to limit the fungal infections. In this way the choice of the variety an of the bio-products used for seeds treatment is very important. According with the literature (Podolska et al., 2015; Gaweda et al., 2019) the variety Rokosz can be successfully cultivated in ecological cropping system due to its nutritional qualities, due to the content in gluten and to the resistance to the attack of the pathogens and pests.

CONCLUSIONS

The contamination index with *Fusarium* spp. of the spelt wheat seeds was different from a variant to other. The lowest contamination index was registered in V2 where the seeds were sterilized previously with alcohol. In V2 non-treated and sterilized the fungal contamination index reached to 73%. In V3 treated with the bio-product Micover WP the contamination index was 80%. The difference between the treated variant (V3) and non-treated (V1) was insignificant from statistical point of view and significant in the case of the variant sterilized with alcohol. The high contamination index with *Fusarium* spp. of the treated variant can be determined by the seed coating that hasn't allowed the product the adhere at the seed epiderma. There are necessary more laboratory and field researches to demonstrate the efficiency of the inefficiency of a certain bio-fungicide.

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