ECOLOGICAL METHOD TO PREVENT FUNGAL DISEASES IN CEREALS WITH OZONATED WATER

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Abstract. The aims of the present work were to determine effects of ozonated water in reducing the contamination of wheat with fungi in the fields and antifungal efficacy against *Fusarium spp. in vitro.* The organic winter wheat (*Triticum aestivum L.*) during shoots, flowering, maturity and harvesting growth stages was sprayed with 2.5-3.0 mg/L ozonated water.

In the field conditions on wheat fungal colony-forming units (CFU/g) count compared with the control group was lower: 64.2% (shoots growth stages); 76.3% (flowering growth stages); 78.5% (maturity); 65.4% (harvesting). The experimental results *in vitro* showed that 3.0-5.0 mg/L and 6.0 mg/L ozonated water had effect against to spores of *Fusarium* spp respectively 69-86% and 98 %.

Keywords: ozonated water, fungi, wheat, antifungal effect

Introduction. Ozonated water (OW) is an alternative for chemical cereal fields spraying and fungal diseases prevention. It has been determined that toxins produced by *Fusarium, Aspergillus, Penicillium, Alternaria*, *Stachybotrys* and other genera of fungi are especially dangerous. Various information sources declare that mycotoxins possess carcinogenic, mutagenic, teratogenic, immunotoxic, haematopoietic, hepatotoxic, nephrotoxic effects (Raju et al., 2002). Using of the ozonated water in the cereal fields is ecological and safe method. Ozone is an environmentally friendly oxidant, since it decomposes to O_2 without producing self-derived byproducts in the oxidation reactions (Guo, 2012). This ensures animal health and well-being. It can be applicable to all kind of cereals. Research in Belgium showed that the fog of ozonated water generates a slightly moister environment than ozone gas and it can improve the contact of ozone with the malting barley (*Hordeum vulgare L.*) grain‘s surface. The treatments were performed using specific concentrations of dissolved ozone into water (7.0, 7.6 and 9.8 mg/L respectively). The maximal concentration (9.8 mg/L) pointed to a disinfection effect of about 80% for *Fusarium* spp (Spanoghe, 2016). In Iran the best result was obtained when wheat samples were treated with 2.5 mg/L (the highest concentration) of ozonated water and fungal spores concentrations were 10 and 10^2 CFU/g for inhibition of fungal growth, respectively (P<0.05) (Mohammadi, 2015). An ozone-air mixture using for active ventilation-drying of grain (with an ozone concentration of 0.7 mg/L) reduced of mycological pollution depending on moisture content (w) in grain, from 2.2 times (w=15.2%) to 3 times (w=22.0%). A direct link was detect between grain moisture and ozone absorption and more moist grain has greater absorption (Raila, 2006).

There is a lack of studies about ozonated water effect on cereal grains in the field conditions. In Lithuania, the weather conditions are also favourable for the occurrence of Fusarium species, where the frequency of identify cation of these fungi is as high as 93.5%. The most frequently isolated *Fusarium* species are *F. culmorum*, *F. Avenaceum*, *F. Poae*, *F. Sporotrichioides* and others (Lugauskas, 2004).

The aims of the present study were to determine effects of ozonated water in reducing the contamination of wheat with fungi in the fields and antifungal efficacy against *Fusarium spp. in vitro.*

Methods. The winter wheat (*Triticum aestivum L.*) in the organic farm was sprayed with 2.5-3.0 mg/L ozonated water during shoots, flowering, maturity and harvesting growth stages. In wheat field was sprayed 1.43-1.86 L/are every time, speed of the tractor (with ozonator) was 3.3-5.0 km/h. Water temperature 8-10 ^oC in the ozonator. Climatic conditions were measured during sprays.

Total count of fungi was defined according to Lithuanian standard LST ISO 7954:1998E. Microbiology. Fungi counting. General directions. The analysis of each sample was performed in three replications. Genus of fungi were identified by Lugauskas and other descriptions. Pure Fusarium spp. Cultures were isolated, identified and used *in vitro* study.

In vitro antifungal activity of ozonated water was tested in the “Ozono centras” laboratory. The broth dilution method was used to determine effectiveness of different ozone concentration in water to reduce *Fusarium spp*. according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001). Sabour’s agar with chloramphenicol (50 mg/L) (OXOID, UK) was used in the present study.

Statistical analyses were carried out using SPSS software (version 17.0 for Windows, SPSS Inc., Chicago, IL, USA) and “Microsoft Office Excel 2010” calculating the mean of values (X), standard error (SE). The P- value of 0.05 was set as a limit for statistically significant difference in the studies.

Results. Air temperature during sprays of ozonated water was 18-28^oC, wind speed 2-5 m/s, mostly fair weather. Relative air-humidity (spray moment) was 39-65%.

Results of fungal colony-forming units on wheat in the field conditions are presented in table 1.

Table 1. **The fungal colony-forming units on the winter wheat in the field conditions**

Groups	Growing stages			
	Shoots	Flowering	Maturity	Harvesting*
Non-treated (NT) (logCFU/g)	2.25±1.16[a]	2.59±2.03[a]	2.74±1.74[a]	3.09±1.84[a]
Treated with ozonated water (OWT) (logCFU/g)	1.80±0.76[b]	1.97±1.97[a]	2.07±1.50[b]	2.91±1.47[b]

* Samples collection after harvesting, without spraying

** Different indexes a and b in superscript indicate statistically significant difference (P<0.05) among not treated and treated samples collected in the same days.

The best results were achieved in wheat maturity growth stage and the least effect – in shoots growth stage. Fungal colony-forming units count in the field conditions was lower compared with the control group: 64.2% (shoots growth stages); 76.3% (flowering growth stages); 78.5% (maturity growth stages); 65.4% (harvesting). The best results were achieved in wheat maturity growth stage (78.5%) and the least effect – in shoots growth stage (64,2%).

During in the present study fungal CFU increased in both groups (NT and OWT). Main fungi genus: *Alternaria spp. (17.25%), Fusarium spp. (14.00%), Penicillium spp., Mucor spp.* and others. *Fusarium* spp.genus was used *in vitro* study, results are presented in figure 1.

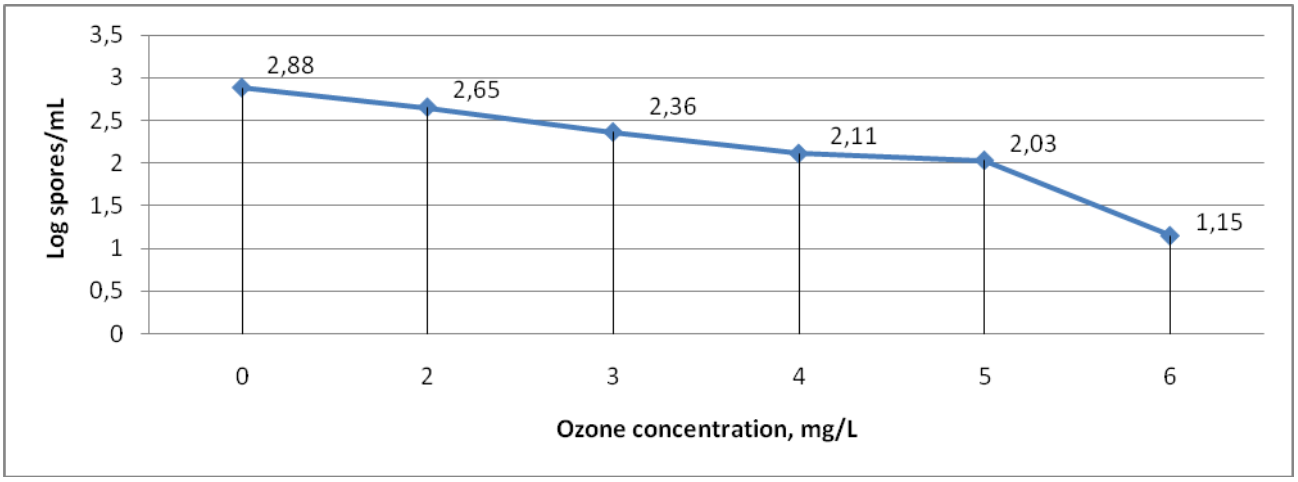

Fig 1. **Ozonated water different concentrations antifungal efficacy against *Fusarium spp***

In the current study ozonated water's effectiveness was: 2.0 mg/L – 40.0%; 3.0 mg/L – 69.3%; 4.0 mg/L – 82.9%; 5.0 mg/L – 85.6%; 6.0 mg/L – 98.1% in compare with control group (0.0 mg/L, distiled water). Strong negative correlation was found (r=-0.97; P<0.05) between concentration and growing spores.

Discussion

In the present study CFU strongly increased in the second half of summer (both OWT and NT). First half of summer was very dry and the second half had high rainfall. Warm and rainy weather during flowering stage has the most conducive to the occurence of fungi infection in cereal grain.

There are slight possibility that ozone can cause malformation, chlorosis or necrosis (like it is done by tropospheric ozone pollution). (www.ars.usda.gov, seen 2016-09-28) We do not have information how ozonated water impacted the cereals (structure, nutritional value). In current study was not found no negative visual effect on growing wheat and on grain after harvesting. Some studies about ozonated water impact to wheat structure, nutritional value and other negative changes are needed.

In Belgium 80% *Fusarium* spp. disinfection by fog of ozonated water effect was reached with 9.8 mg/L and in the present study - with 4-5 mg/L. Differences may be caused by different study conditions.

Conclusion

In field conditions, during harvesting fungal CFU count on wheat was reduced 65.4% by ozonated water (2.5-3.0 mg/L). The results *in vitro* showed that 3.0-5.0 mg/L and 6.0 mg/L ozonated water had effect against *Fusarium* spp respectively 69-86% and 98 %.

Need to continue studies for more information how higher ozone concentration in water (4-6 mg/L) would affect wheat.

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