

DISINFECTION OF WINTER WHEAT GRAIN BY OZONE AND NEGATIVE CORONA DISCHARGE FIELD

Valentina Avdeeva, Sergey Antonov, Anatoly Molchanov, Igor Devedorkin

Stavropol State Agrarian University, Russia

Avdeeva_VN@mail.ru, antonov_serg@mail.ru, molchanov_41@mail.ru, devedorkin@mail.ru

Abstract. Today, an urgent problem of agricultural production is decline of food and fodder grain quality. One of the reasons of decline of grain quality is its contamination with mushroom infection, and as a result, formation of mycotoxins - poisonous products of mold mushroom metabolism. The grain keeps quality if the condition of its storage should be favorable for insects and microorganisms. Besides preventive measures it is necessary to carry out processing of grain for the purpose of prevention and decrease of grain molding. The existing grain disinfecting methods, such as chemical, thermal, biological and other, have some advantages and disadvantages. All methods have high disinfecting effect, but some are ecologically dangerous, others are power-intensive, labor-consuming, and expensive. Recently electrophysical methods of grain crop disinfecting attract great attention of scientific different directions because they are various in means and opportunities. In the Stavropol State Agrarian University researches on the influence of electrophysical factors on decrease in contamination of wheat grain by mushroom infection were conducted for several years. In particular, dynamics of development of mushroom colonies *Aspergillus* and *Fusarium* was determined. As a result of long experiments the optimum modes of disinfecting of grain are revealed by ozonized air and the field of the negative crown discharge (FNCD). The results of this experiment showed that the III mode of grain processing is optimum: FNCD + ozone, intensity of the field $3.6 \cdot 10^5 \text{ V} \cdot \text{m}^{-1}$, the processing time of grain is 70 seconds, a dose of ozone is $28.8 \text{ g} \cdot \text{s} \cdot \text{m}^{-3}$. Grain after processing in the III mode can be stored for 150-180 days without danger of development of mushroom colonies *Fusarium* and in 120 days colonies of mushrooms *Aspergillus* are suppressed.

Keywords: fungal infection, disinfection, wheat grain, ozone, negative corona discharge field.

Introduction

Currently, there are annual losses of 1.0-2.0 % of cereal dry substances worldwide as a result of the active vital activity of harmful insects, bacteria, pathogenic and mold fungi. In modern Russia, this figure is even greater, since the main territory of the country is located in areas with high humidity, where the harvested yield is favorable environment for the development of pathogenic fungi and pests. It has been found that even in relatively dry years a large amount of grain with the moisture content 20.0 - 22.0 % and higher is brought to the barn-floor. In such cases biochemical processes actively take place in the bunker of the combine, the intensity of breathing increases, which is accompanied by the release of a large amount of heat, which leads to loss of the grain mass and deterioration of its quality.

The mycotoxins produced by the fungi *Fusarium* are widespread in the world as a whole and in Russia in particular. Fungi of this genus most often affect cereal crops and are capable of producing a number of mycotoxins of the trichothecin group, of which deoxynivalenol is released by its toxic properties and high detection rate [1]. Also, aflatoxins produced by the fungi of the genus *Aspergillus* (*Asp. flavus* and *Asp. Parasiticus*), which have a pronounced carcinogenic effect, and which affect the liver, pose a serious danger. Many other mold fungi can also produce toxins. At the present time more than 100 mycotoxins resistant to temperatures, acids or reducing agents used during processing of grain have been found and studied. Therefore, the most reliable way to protect food from them is to exclude molding of grain.

The spread of fungal infection in grain in natural conditions before harvesting can be prevented to a certain extent by appropriate technological methods. Preventing the formation of mycotoxins after harvesting in grain put for storage is more difficult. In order for the grain to retain its qualities, the storage conditions must be unfavorable for both insects and microorganisms. When storing grain mass in large volumes, there is a problem of moisture migration, while the formation of condensate stimulates the development of insects and microorganisms and the formation of "hot spots". Proven ways to control the existing problem of grain crops are grain tumbling and ventilation of granaries, which help prevent unwanted moisture spread. However, the prophylactic procedure can be considered effective only if it is able to suppress the growth of fungi completely, which is almost impossible.

Along with the preventive measures, grain processing is carried out by physical methods, chemical and biological preparations used to prevent and reduce mold molding. The chemical method is based on the use of modern fungicides that inhibit pathogens and do not exert a destructive effect on beneficial microbiota. The existing methods of grain disinfection: chemical, thermal, biological, etc. have their advantages and disadvantages. Along with the high disinfection effect, some are environmentally hazardous, others are energy-intensive, labor-consuming, expensive.

In this connection, scientific and practical interest is the search for effective, environmentally safe methods of action on the toxin producing fungi affecting crops. Electrophysical methods of disinfection have recently attracted great attention of scientists in different directions, since they are more diverse in means and possibilities. Such methods include disinfection by negative corona discharge field (NCDF), ultrahigh frequencies, infrared and ultraviolet radiation, radiation treatment by gamma radiation. Also, a promising way of processing grain to reduce the contamination of the processed material to an acceptable level is electro-ozonization. The use of ozonized air in agricultural production when disinfecting grain, livestock rooms, eggs before incubation were studied by Borodin I. F., Oskin S. V., Trotskaya T.P., Ksyonz N. V., Tkachyov R. V. and other scientists. Based on the works of these scientists in the field of ozonic technologies, in the educational - scientific research laboratory of the Stavropol State Agrarian University researches on the influence of ozone and the field of the negative crown category on decrease in contamination of grain of wheat by a mushroom infection were conducted for a number of years.

Materials and methods

In the Stavropol State Agrarian University for a number of years, studies were conducted on the effect of physical factors on the reduction in the contamination of wheat grains by fungal infection. As a result of long-term experiments, we identified the optimal regimes for disinfection of grain by ozonized air and the field of negative corona discharge. In particular, the dose of ozone, capable of destroying the pathogenic mycobiota and, at the same time, not triggering the activation of grain germination processes, is $28.8 \text{ g} \cdot \text{s} \cdot \text{m}^{-3}$. When the field was treated with a negative corona discharge, the optimal NCDF strength was $3.6 \cdot 10^5 \text{ V} \cdot \text{m}^{-1}$; the processing time is 70 seconds. It turned out to be the best exposure after 14 days treatment.

Laboratory studies allowed to determine the regimes of grain processing by ozone, but did not answer the question: how long can the cultured culture be stored without fear of repeated infection with harmful fungi? In connection with this, we conducted experiments with detection of the aftereffect of treating winter wheat with ozone in combination with NCDF for fungal infection during storage of the grain for six months with monthly microbiological testing of the development of pathogenic fungi [4]. Wheat grain processing by a field of the negative corona discharge was realized on small-size installation for receiving a field of corona discharge. Grain from the load bunker arrived on a tape of the conveyor which moved in interelectrode space of laboratory installation. The time spent of a biological object in interelectrode space can be changed over a wide range.

Wheat grain disinfecting by ozone was performed by the original technique developed in the Stavropol State Agrarian University. Processing was carried out by means of an ozonizer "Thunderstorm" with productivity up to $60 \text{ g} \cdot \text{h}^{-1}$. The grain processing entity ozone is that ozone for a certain time is forced in the location where the processed material is located. Efficiency of processing is defined by the ozone dose which depends on the concentration of ozone and on the time spent of the processed material in the ozone-air flow. The dose of processing is calculated by formula

$$D = c \cdot t,$$

where D – processing dose, $\text{g} \cdot \text{s} \cdot \text{m}^{-3}$;
 c – concentration of ozone, $\text{g} \cdot \text{m}^{-3}$;
 t – time of processing of seeds (exposure), s.

Concentration of ozone was measured by the instrument "Cyclone-5.41" – an optical gas analyzer of ozone.

Also complex disinfecting of grain by ozone and NCDF in the following sequence was performed: NCDF → one day grain exposition → ozone. The processing of wheat grain by a negative

corona discharge field was carried out on a laboratory plant. Ozone treatment was carried out with the help of the "Groza" ozonizer with a capacity of up to $60 \text{ g}\cdot\text{h}^{-1}$. Complex disinfection of grain with ozone and NCDF was also carried out. The treatment was carried out in twelve regimes with different doses of ozone, NCDF intensity and grain processing exposition.

The analysis of the samples of grain of wheat on presence of mushroom infection was carried out in accordance with the GOST by 12044-93 biological method. Monthly grain was laid on germination on potato-glucose agar in Petri dishes at the temperature of $25 \text{ }^{\circ}\text{C}$ in four replicates.

The counting of the colonies of fungi developing on the grain of winter wheat was carried out after 7 days of germination. The number of colonies was calculated per 100 grains of the sample. Statistical processing of the experimental data was carried out by the method of variance analysis for a two-factor experiment on a PC (B.A.Dospekhov, 1985) [1].

Factor x – the modes of processing of grain of winter wheat ozone and NCDF, factor y – the time of an after-effect of wheat grain processing.

Results and discussion

The treatment regimes for winter wheat grain with ozone and NCDF, which had a significant effect on reducing the infection of wheat grains with fungal infection, are presented in Table 1.

Table 1

Modes of winter wheat grain processing with ozone and POCR

Mode and method	Processing parameters	Exposure of the sample after processing, whole day
I, ozone	Dose of ozone: $28.8 \text{ g}\cdot\text{s}\cdot\text{m}^{-3}$	7, 14, 30, 60, 90, 120, 150, 180
II, POCR	Field intensity $3.6\cdot 10^5 \text{ V}\cdot\text{m}^{-1}$; Exposure 70 seconds	7, 14, 30, 60, 90, 120, 150, 180
III, POCR + ozone	Field intensity $3.6\cdot 10^5 \text{ V}\cdot\text{m}^{-1}$; Exposure 70 seconds	7, 14, 30, 60, 90, 120, 150, 180
	Ozone dose $28.8 \text{ g}\cdot\text{s}\cdot\text{m}^{-3}$	

The results of the effects of ozone and POCR treatments on the development of winter wheat pathogens of the river Fusarium during storage are presented in Table 2.

Table 2

Effect of treatment with ozone and POCR on development of pathogens of winter wheat grain of g.Fusarium during storage (colonies per 100 grains)

Modes of processing, x	Time of aftereffect of treatments, whole day, y								Average value
	7	14	30	60	90	120	150	180	
Without treatment	28.0	28.0	20.0	20.0	24.0	25.0	25.0	25.0	24.4
I(ozone)	19.0	1.0	7.0	4.4	2.0	2.0	9.0	9.0	6.7
II (POCR)	7.0	1.0	2.4	3.4	3.0	3.2	0.0	0.0	2.5
III (POKR + ozone)	4.4	0.4	0.4	3.0	4.0	3.0	0.0	0.0	1.9
Average value	14.6	7.6	7.5	7.7	8.3	8.3	8.5	8.5	-
HCP_{xv, 0.95} = 1.7									

From the results obtained in the two-factor experiment, it follows that all treatment regimes had a significant effect on reducing the contamination of wheat grains by the fungi of g. Fusarium in comparison with the control. Between the modes, the difference is also significant. The best result was achieved with complex treatment in the III regime, in which the grain contamination decreased from 24.4 % in the control to 1.9 %, HCP_{h, 0.95} = 0.5. The contamination of the grain treated in I (6.7 %) and II (2.5 %) regimes is higher than that of the grain treated in III regime, and the differences are significant.

The time after the effects of treatments has a certain influence on the dynamics of the development of the fungi colonies of *g.Fusarium*. There is a significant difference between the weekly (14.6 %) and two-week exposure after decontamination (7.6 %). With further storage, no significant changes occurred. Grain after treatment in the III regime can be stored for 150-180 days of safety of development of the fungal colonies of *g.Fusarium*.

The results of ozone and NCDF treatments for the development of winter wheat grain of the *Aspergillus* grain during storage are presented in Table 3.

Table 3

Effect of ozone and POOCR treatments on development of pathogens of winter wheat grain of *g. Aspergillus* during storage (colonies per 100 grains)

Modes of processing, x	Time of aftereffect of treatments, day, y								Average value
	7	14	30	60	90	120	150	180	
Without Treatment	20.0	20.0	25.0	25.0	27.0	29.0	28.0	28.0	25.3
I (ozone)	5.0	4.0	3.0	9.0	1.0	1.0	22.0	22.0	8.1
II (POOCR)	0.4	1.0	1.0	12.0	12.0	12.0	14.0	13.0	8.1
III (POOCR+ozone)	6.0	0.0	1.0	3.0	1.0	1.0	8.0	8.0	3.4
Average Value	7.9	6.0	7.5	12.3	9.8	10.5	18.0	17.8	-
HCP_{xv, 0.95} = 1.7									

On grain that was stored without processing, the amount of molds increased from 20.0 to 28.0 % per 180 days of storage. I regime reduced the infection of grain with *Aspergillus* fungi by 15.0 % after a weekly exposure, after three months the number of fungal colonies decreased to 1.0 %. However, it can be seen from the figure that it is necessary to store grain processed in this mode for not more than 120-140 days, since after 150 days of storage the aftereffect of ozone ceases, the amount of grain populated by mycoflora increases to 22.0 % compared to the control. The effectiveness of grain processing in the II and III regimes was higher in comparison with ozonization with respect to the suppression of the development of *Aspergillus* fungi colonies. The number of fungi in the II regime decreased to 1.0 % after a weekly exposure, at the end of the experiment the presence of mycobiota was 13.0 %. In the III mode, the best result was achieved after a two-week exposure, the harmful microflora was completely suppressed. Subsequently, a slight increase in fungal infection was observed. The difference is significant between the control and all treatment regimes, i.e. all regimens led to suppression of the fungal infection, the III mode was optimal, the after-treatment time was 120 days.

Conclusions

The results of the research showed that the use of ozone in combination with NCDF to protect the stored grain of winter wheat against various types of fungal infection excludes the restoration of infection for a long time. As a result of long-term experiments, optimal regimes of grain disinfection with ozonized air and negative corona discharge (NCDF) field were revealed. The results of this experiment showed that the III mode of grain processing is optimal: NCDF + ozone, field strength $3.6 \cdot 10^5 \text{ V} \cdot \text{m}^{-1}$, grain processing time 70 seconds, ozone dose $28.8 \text{ g} \cdot \text{s} \cdot \text{m}^{-3}$. Grain after treatment in the III regime can be stored for 150-180 days without the danger of developing the colonies of fungi of *g.Fusarium* and in 120 days by the colonies of fungi of *g.Aspergillus* are suppressed.

Now, in the educational and scientific laboratory of the SSAU researches on grain disinfecting for the purpose of improvement of the quantitative and quality indicators continue in various ways.

References

1. Avdeeva V.N. Application of ecological methods of suppression of pathogenic mycoflora of winter wheat grain during storage: diss. cand. agr. sciences. V.N.Avdeeva. - Stavropol, 2009.
2. Starodubtseva G.P., Avdeeva V.N. Effective methods for reducing the toxicity of grain and fodder affected by mycotoxins, Bulletin of the Agroindustrial Complex of Stavropol. 2012. № 7. pp.28-30.
3. Yusupova, G. G. Theoretical and experimental study of an integrated system of disinfection of grain and products of its processing: author. dis. doctor. of agricultural Sciences, G. G. Yusupova. – Moscow 2005,. 38 c.
4. Denning D.W. Invasive aspergillosis. Clin Infect Dis. 1998.- 26, pp. 781-803.
5. Gannibal Ph.B., Klemsdal S.S., Levitin M.M. (2007) AFLP analysis of Russian *Alternaria tenuissima* populations from wheat kernels and other hosts, Europ. J. Plant Pathol. 119 (2), pp. 175-182.
6. Pitt J.I., J.C. Basilio, M.L. Abarca, C. Lopez. Mycotoxins and toxigenic fungi. Med Mycol. 2000.- 38: pp. 41-46.
7. Wood G. E. Mycotoxins in Foods and Feeds in the United State, J. of Animal Science.-1999, Vol.70, pp. 3941-3949.
8. Trenholm H. L., Foster B. C., Chamby L. L. At all – Effect of feeding diets containing *Fusarium* (naturally) contaminated wheat or pure deoxynivalenol (DON) in grow pigs, Canadian Journal of Animal Science. – 1994: Vol.74, № 2: pp. 361-369.