THE USE OF OZONE TECHNOLOGIES IN GRAIN STORAGE

V.I. Golota[1], G.V. Taran[1], A.A. Zamuriev[1], P.O. Opalev[1], S.G. Pugach[1], S.N. Mankovskyi[1], V.P. Petrenkova[2], I.N. Nyska[2]

[1]National Science Center "Kharkov Institute of Physics and Technology", Kharkov, Ukraine;
[2]Plant Production Institute named A. V.Ya. Yuryev of NAAS, Kharkov, Ukraine

The results of experimental studies of toxicogenic fungi activity inhibition at various ozone concentration and doses by ozone-air mixture, which were carried out at the developed and manufactured experimental stand are given. The ozone inhibitory effect on the growth of fungi infection *Aspergillus flavus* and *Penicillium nordicum* is defined. The ozone-air mixture ability to limit the mold fungi development on the wheat and barley grains during their storage is shown.

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INTRODUCTION

The grain production in Ukraine is the traditional direction in agriculture. "Seed of Ukraine" program is provided to reach the level of annual output about 80 million Tons. [1]. Meanwhile because of high diseases and pests progression the potential harvest losses can reach 15…30%, and in the years of epifitotiyny progression they can make 50% [2]. Except real harvest loss the phytopathogens can produce toxins, therefore grain is not suitable for eating and animal feeding. Therefore the optimization of seeding phytosanitary state is the considerable reserve for increase in grain production and for saving its quality.

Grain which is put on storage in elevators must satisfy safety and qualities requirements according to DSTU 3768-2010 "Wheat. Technical conditions" [3]. It has to be free from toxins which were created as a result of pathogenic microorganisms activity because these food raw materials ares used for grain, torments, beer manufacture and etc. The problem of mycotoxins still remains worldwide relevant. As toxins are low-molecular substances, they can spread quickly to the cells of plant affected parts and can kill even those cells which are far from the place of microorganism effect [4].

The annual loss from the mold fungi growth on the agricultural products and the industrial raw materials in the world exceeds 30 billion US dollars [5]. The significant threat is posed by known now fungi sorts of mold microorganisms which met in grain raw materials such as *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*. Therefore the limiting of fungi infection development on grain during its storage on elevators is one of the effective methods of toxins content minimization in food parties of grain weight.

There are many ways in agriculture to restrict the infection growth on grain, in particular, mechanical, physical, chemical, biological, physical, chemical and others. By the experimental works of certain authors, it is proved poor efficiency of these methods, the significant spending of time for their realization, and that some of them are dangerous for environment, people and animals [6]. Chemical methods are limited to grain processing by oxidizers solutions and strongacids or alkalis that leads to mycotoxins destruction, but at the same time the most part of useful product elements are destroyed too. The analysis of these methods of fungi infection growth restriction on grain during its storage on elevators gives a reason to admit the importance of such questions as research and development of modern, efficient, low-cost, safe methods of grain masses detoxicating, as an example the using of ozone-air mixtures. Ozone is the strong oxidizer which is used in many technical applications for fight with harmful microbes and volatiles.

Within implementation of the MycoKey project according to the European Horizon 2020 program the research problem of ozone-air mixture influence on a pathogenic fungi infection of such sorts as *Aspergillus* and *Penicillium* was set. Therefore, the purpose of the carried out researches was the definition of ozone-air mixture influence on growth of fungi infection of sorts *Aspergillus* and *Penicillium* in pure culture and on wheat and barley grains, that was infected in vitro.

For purpose achievement such tasks were carried out: the stand, intended for fungi mycelium treatment on pure culture, placed in Petri dishes, and on small parties of the infected grain (to 3 kg), was developed and manufactured; the patogenicity of fungi strains, sorts *Aspergillus flavus* and *Penicillium nordicum*, which were received from our colleagues from Valencia's (Spain) University was defined; the optimal concentration in ozone-air mixture and ozone dose for fungi mycelium growth restriction in pure culture and on wheat and barley grains infected in vitro by spores of *Aspergillus flavus* and *Penicillium nordicum* was determined.

METOD AND EXPERIMENT

The studies were carried out in the laboratory conditions of plant immunity to diseases and pests of the Plant Production Institute nd. A.V.Ya. Yuryev of NAAS and at the department of nonequilibrium low-temperature plasma chemistry in NSC "Kharkov Institute of Physics and Technology" NAS of Ukraine.

The methods of pure culture for fungi selection and subculture [7], the seeds analysis for detection of external infection [8], a microscopy for definition of fungi species were used. The options of pure fungi culture treatment by ozone-air mixture used such ozone concentration: 0.04, 0.1, 0.5 g O_3/m^3 with exposure time 4, 24, 48, 72, 168 hours. The infected grain was processed by ozone-air mixture with concentration 0.1 g O_3/m^3 and exposure time 8, 24, 48 and 72 h. Three-time iteration was carried out. The grain masses which were used in researches belong to a harvest of 2016.

The grain and pure fungi culture was treated at the "Ozone-agro-1L" stand. The Ozone-agro 1L stand con-

sists of following function boxes: an air compressor with efficiency up to 50 l/min and with a maximal pressure up to 12.7 kPa, company Secoh sangyo (Japan), a gas loss meter (SM-4 GU3 type), the laboratory ozone generator "Ozone-agro-1L", ozone concentration meters, company Teledyne instruments (USA), model 454H with the ozone concentration measurement range 0.1…100 g/m^3 and Ozone Solutions (USA) the ES model – 600 with the ozone concentration measurement range 0.01…0.1 g/m^3, the laboratory camera for samples placement and ozone destructor, Figs. 1, 2.

The barrier-free ozonizer on a streamer discharge of atmospheric pressure [9] which was made durimg implementation of the MycoKey project according to the European Horizon 2020 [10] program was used for ozone synthesis. It has obvious advantages over barrier ozonizers for application in agriculture. First of all it is their low sensitivity to the water vapor content in the reacting gas, no need for high-precision accuracy of electrode system assembly, no need for water cooling of electrodes, low resistance to gas mixture hesitation pumping through the ozone generator module. They can stably produce ozone from ordinary atmospheric air for a long period (without use of special air preparation system) with ozone-air mixture parameters necessary for grain treatment.

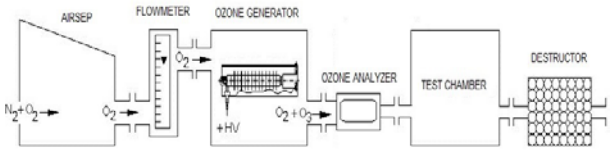

Fig. 1. Function boxes block diagram of the laboratory stand "Ozone-agro 1L"

The laboratory stand "Ozone-agro 1L" is on Fig. 2.

Fig. 2. The laboratory stand "Ozone-agro 1L"

Grain, infected with fungi infection, sorts *Aspergillus flavus* and *Penicillium nordicum*, for treatment in the experimental camera was placed on a lattice surface, under which the camera was fed by the ozone-air mixture, that uniformly spread throughout the volume.

RESULTS AND DISCUSSION

As a result of carried out studies about definition of ozone-air mixture influence on microorganisms vital activity in pure three and seven-day culture the efficiency of ozone treatment with the corresponding exposure time that led to a lethal fungi mycelium distraction was defined. That is at the repeated fungi subculture on a medium (in 7 days after ozone treatment) the total loss of their ability to sprouting is noted (Table 1).

Table 1

Ozone-air mixture influence on pathogenic microorganisms vital activity in pure culture

Micro-organism	Fungi age, days	Treatment mode		Fungi morphological features		
		concen-tration, g O_3/m^3	exposure time, year	straight after ozone treatment	in 7 days after ozone treatment	in 7 days after subculture
Aspergillus flavus	3	0.1	4	mycelium turned white and dried up	turned green	sprouted
		0.1	24		turned green	sprouted
		0.1	48		white, dry	not sprouted
		0.1	72		white, dry	not sprouted
		0.5	4		turned green	sprouted
Aspergillus flavus	7	0.04	72	mycelium turned white and dried up	white, dry	not sprouted
		0.1	24		white, dry	not sprouted
		0.1	168		white, dry	not sprouted
		0.5	168		white, dry	not sprouted
Penicillium nordicum	3	0.1	24	mycelium turned white and dried up	turned green	sprouted
		0.1	48		white, dry	not sprouted
		0.1	72		white, dry	not sprouted
Penicillium nordicum	7	0.04	72	mycelium turned white and dried up	white, dry	not sprouted
		0.1	5		turned green	sprouted
		0.1	72		white, dry	not sprouted

Ozone lethal effect on three-day culture *Aspergillus flavus* is defined in option with ozone concentration in mixture 0.1 g/m^3 during 48 and 72 hours. Smaller exposure time of ozone influence (4 and 24 hours) and also a low dose (0.5 g O_3/m^3 during 4 hours) were ineffective because didn't depress fungi germination ability after its subculture on a medium in 7 days after treatment. The selected options of seven-day *Aspergillus flavus* culture treatment by ozone-air mixture had a lethal outcome on fungi, as its germination at repeated subculture wasn't noted. As for *Penicillium nordicum* vital activity after impact on three-day pure culture by ozone-air mixture the lethal concentration 0.1 g O_3/m^3 with exposure time 48 and 72 hours is defined. The seven-day culture of this fungi was depressed by ozone concentration 0.04 and 0.1 g/m^3 with exposure time 72 hours [11].

As a result of researches of mold fungi growth limiting on wheat and barley grain raw materials the effective conditions of treatment by ozone-air mixture, which depressed development of the pathogenic microorganisms cultivated on simulated medium and put on grain in vitro was determined.

Treatment of wheat and barley samples, which were infected in vitro with a fungi infection *Aspergillus flavus* was carried out by ozone-air mixture with ozone concentration 0.1 g/m^3 in the model of an elevator silo, with ozone-air mixture feeding from center to periphery. The mixture consumption made 0.5 m^3/hour. Samples selection was carried out after 2, 4 and 6 days of treatment. Samples were placed in the thermostat and kept at the temperature 25°C and humidity 90%. The registration of grain affection was carried out on 3 and 7 days.

Data on barley affection are presented in the Fig. 3, and wheat – in the Fig. 4.

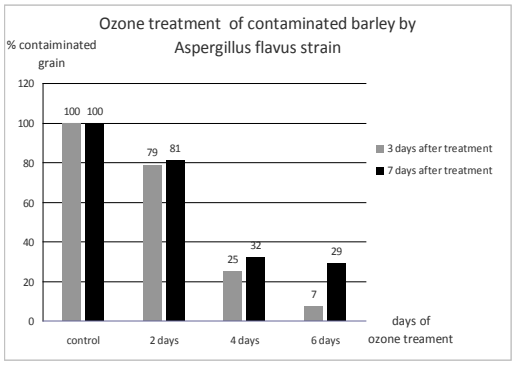

Fig. 3. Ozone-air mixture influence on barley grain affection by fungi infection Aspergillus flavus on 3 and 7 day after embedding into the thermostat

From the schedule in the Fig. 3 the conclusion can be made that barley affection decreases by 3-4 times with treatment duration more than 3 days and slightly depends on growth time of fungi infection *Aspergillus flavus*. However, the grain seeding significantly depends on its growth time while treating during more than 5 days (7 and 29%).

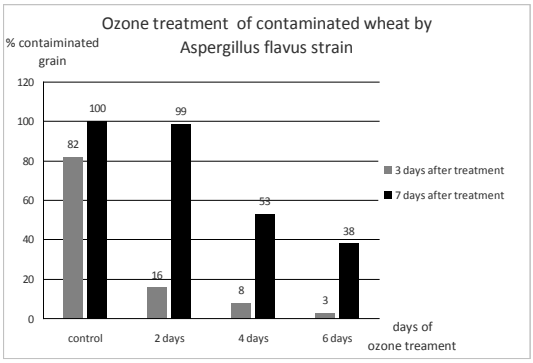

Fig. 4. Ozone-air mixture influence on wheat grain affection by fungi infection Aspergillus flavus on 3 and 7 days after embedding into the thermostat

From the schedule in the figure 4 the conclusion can be made that wheat affection significantly depends on growth time of fungi infection and, for example, makes less than 10% from monitoring after 4 days' treatment and 3 days' growth, and more than 50% – after 7 days of fungi infection *Aspergillus flavus* growth.

For barley samples treatment, which were infected with fungi infection *Penicillium nordicum* in vitro the ozone-air mixture was fed from the center to the periphery with ozone concentration 0.1 g/m^3 in the model of elevator silo. The mixture consumption made 0.5 m$_3$/h. Samples selection was carried out after 2, 4 and 6 days of treatment.

Experiment procedure is the same, as for research of fungi infection *Aspergillus flavus* affection. Data on barley affection are presented in the Fig. 5.

From the schedule in the Fig. 5 the conclusion can be made that at smaller initial activity of fungi infection in control samples (37 and 64%), the barley affection by fungi infection *Penicillium nordicum* decreased in 3-4 times after 3 and 7 days germination.

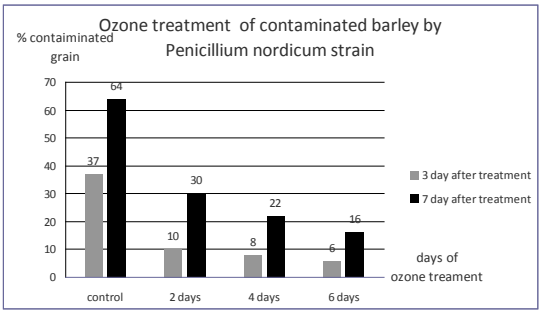

Fig. 5. Ozone-air mixture influence on wheat grain affection by fungi infection Penicillium nordicum on 3 and 7 day after embedding into the thermostat

For determination of fungi infection *Aspergillus flavus* activity after wheat and barley seed samples treatment by ozone-air mixture the experiment on flushing subculture on a medium was carried out. For this purpose, from the barley and wheat seeds infected with the *Aspergillus flavus* pathogen and treated by ozone was made a spores flushing and received suspensions injection on pure nutrient medium (KGA). On the 3rd day the measurements of colonies growth were carried out. The results of fungi infection colonies count in 7 days after subculture are given in Table 2.

Table 2

Development of fungi infection Aspergillus flavus colonies after flushing from the wheat and barley seed samples treated by ozone-air mixture

Treatment options	fungi colonies diameter, mm	
	barley	wheat
monitoring	30	24
2 days	11.5	10.6
4 days	12.3	10.8
6 days	12	13

From Table 2 one can see that diameter of fungi colonies, treated by ozone-air mixture, is twice smaller in comparison with untreated.

CONCLUSIONS

The laboratory stand for research of possibility to suppress the toxicogenic fungi activity by ozone-air mixture during wheat and barley seed storage is developed and manufactured. Ozone concentration at the ozone generator output can be regulated from 0.05 g/m³ to 1 g/m³.

The efficient inhibition of ozone-air mixtures on pathogenic organisms *Aspergillus flavus* and *Penicillium nordicum* growth in pure culture at cultivation on the simulated medium (KGA) is defined.

The lethal effect on the three-day *Aspergillus flavus* culture was provided by barley and wheat seed samples treatment by ozone-air mixture with ozone concentration 0.1 g/m^3 during 48 hours and 72 hours, on seven-day – 0.04 g O_3/m^3 with exposure time 72 hours and 0.1 g O_3/m^3, 24 hours. The vital activity of three-day *Penicillium nordicum* culture was suppressed by ozone-air mixture with ozone concentration 0.04 g/m^3 during 72 hours and 0.1 g O_3/m^3 – 48 hours, and seven-day 0.04 and 0.1 g O_3/m^3 with the same explosure time 24 hours.

On grain samples infected by fungi infection, both *Aspergillus flavus* and *Penicillium nordicum*, the efficient influence on inhibition of infection growth (by 3-4 times) is defined by ozone-air mixture treatment with ozone concentration 0.1 g/m^3 and the exposure time not less than 72 hours.

Thus, the ozone-air mixture ability to suppress the mold fungi growth on wheat and barley grain confirms the opportunity to use this technology for grain storage on elevators.

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ИСПОЛЬЗОВАНИЕ ОЗОНОВЫХ ТЕХНОЛОГИЙ ПРИ ХРАНЕНИИ ЗЕРНА

В.И. Голота, Г.В. Таран, А.А. Замуриев, П.О. Опалев, С.Г. Пугач, С.Н. Маньковский, В.П. Петренкова, И.Н. Ниска

Приведены результаты экспериментальных исследований подавления озоно-воздушной смесью активности токсигенных грибов при различных концентрациях и дозах озона, которые проведены на разработанном и изготовленном экспериментальном стенде. Определено ингибирующее воздействие озона на развитие грибной инфекции *Aspergillus flavus* и *Penicillium nordicum*. Показана способность озоно-воздушной смеси подавлять развитие плесневых грибов на зерне пшеницы и ячменя во время их хранения.

ВИКОРИСТАННЯ ОЗОНОВИХ ТЕХНОЛОГІЙ ПРИ ЗБЕРІГАННІ ЗЕРНА

В.І. Голота, Г.В. Таран, О.О. Замурієв, П.О. Опалєв, С.Г. Пугач, С.М. Маньковський, В.П. Петренкова, І.М. Ниска

Наведено результати експериментальних досліджень пригнічення озоно-повітряною сумішшю активності токсигенних грибів при різних концентраціях і дозах озону, які проведені на розробленому і виготовленому експериментальному стенді. Визначено інгібіруючий вплив озону на розвиток грибкової інфекції *Aspergillus flavus* і *Penicillium nordicum*. Показана здатність озоно-повітряної суміші пригнічувати розвиток плісеневих грибів на зерні пшениці і ячменю під час їх зберігання.