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THE SUPPRESSION OF POTATO PATHOGENS DEVELOPMENT DURING STORAGE PERIOD AS INFLUENCED BY AIR MEDIUM, CONTACTING WITH NATURAL POTASSIUM SALTS

SUMMARY

One of the urgent problems of agricultural production is preservation of the grown yield, minimizing storage losses, which can reach 30-50 percent. The ways to solve this problem are observed in given article. The analysis of existing methods of products storage based on modern technologies is fulfilled. Using of unique properties of natural potassium salts is admitted to be one of promising technologies for potato and root crops storage. The research program has been launched in Perm Federal Research Center of UB RAS to develop new technologies for storage and fertilizing seed potato based on the properties of natural potassium salts and waste products from K-Mg ores.

Natural potassium-magnesium salts have a number of specific properties affecting the air medium. One of these properties is the ability to produce light air ions due to air molecules contact with potassium and sodium cations. Such environment has the ability to inhibit the growth of many microorganisms' types, including pathogens, which is used in world practice to treat some chronic human diseases (speleotherapy). Preliminary studies conducted in used drifts of K-Mg ores deposits showed the possibility of storing potato and vegetables in underground storages. The next task is to simulate the conditions of underground drifts in typical potato storages. The most promising variations are lining the storage facilities with sylvinite tiles and aerosol treatment as a result of passing an air stream through a layer of sylvinite and the subsequent infiltration of the aerosol through the potato mass. The identification of potato pathogens in samples from the harvest of 2016 and 2017 was carried out after the main storage period. The most common diseases were fusarium, phomosis, as well as bacterial wet rot, late blight presence was insignificant. The number of infected tubers was 10.2 percent of the studied quantity.

The primary modeling of storage conditions under the influence of air ions in a special climatic chamber was carried out. The influence of saline saturated

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atmosphere on the growth of phytopathogens isolated cultures with growth inhibition assessment was determined. For this purpose phytopathogens cultures planted on potato agar bouillon were placed in sealed chambers with volume 5.28 liters. According to the results of fulfilled studies, the number of microorganisms colonies decreased by 13-63 percent compared with the control.

A system of equations has been developed that can be used to determine the optimal concentration of potassium aerosol in potato storages to achieve the best preservation of products. After fulfillment of research work in typical storages equipped with plenum ventilation system, a technology for potato storage, based on the creation of favorable air environment saturated with light negative air ions and saline aerosol, will be developed.

Keywords: potato storage, K-Mg ores, potassium salts, air ions, saline aerosol, potato pathogens

INTRODUCTION

Russia has the huge potential of agricultural products cultivation. In this regard the question of safety of the grown-up harvest, loss minimization at storage which can reach up to 30-50 percent is very relevant (Olsen, 2014,Makarova et.al., 2017).

Currently, there are known methods of storing crops in clamps, containers, bins, in storages with natural ventilation or equipped with a ventilation system, systems for maintaining and monitoring temperature and humidity (Kopylov et.al., 2012, Ponomarev, 2014) These methods do not provide high safety products laid down for long-term storage. In some Russian and foreign works, various methods have been proposed and observed for potato tubers influence in order to increase their preservation: magnetic field, electric current, and ionization (Lu,1986, Brynjolfsson,1989, Rezaee et.al., 2013 Nikitenko et.al. 2016). The researches, as a rule, applied γ-rays as ionogen and the range of absorbed radiation rates from 0.1 to 2.0 kGy. The literature sources also present a number of studies on improving potato preservation through low-temperature storage and the use of germination inhibitors (Macqueen, 1985, Cools et. al., 2014, Foukaraki et.al., 2016). According to (Douglas et. al., 2018, Kaznak, 2018) treatment with such inhibitors causes undesirable side effects, in particular the accumulation of residual chemicals. The use of biological preparations prepared from plants, for example, peppermint, avoids these undesirable effects and prevents the premature potato germination, but appeared to be ineffective method of inhibiting the potato pathogens development during storage (Sanli, 2019). Deep cooling application for potato storing at ultralow temperatures is often associated with significant economic costs and an increased risk of yield damage from hypothermia (Chourasia, 2001).

The use of bactericidal properties of the air contacting with natural potassium salts is considered to be the promising direction in potato storage. One of the largest deposits of potassium, magnesium and sodium salts, located in Russia - Verhnekamskoe, contains a third of the world's reserves, which are

mainly used for the potassium fertilizers production. However, the unique physical and chemical properties of natural potassium, sodium and magnesium salts, as well as their mechanical and filtration characteristics, the presence of huge worked out spaces (drifts) supported for a long time by hard pillars, can significantly expand the use of both natural salts and man-made underground cavities formed during mining. Natural potassium-magnesium salts have a number of specific properties affecting the air medium. One of these properties is the ability to produce light air ions due to exposure to air molecules with potassium and sodium cations. Such environment has the ability to inhibit the growth of many types of microorganisms, including pathogens, which is used in some cases in world practice to treat a number of chronic human diseases (speleotherapy) and produce rare foods (Faynburg et.al., 2008, Krasnoshtein et.al., 2008) . This feature is mainly due to the presence in the salts of the potassium isotope K-40, possessing radioactivity (beta and gamma radiation). Ionizing radiation, interacting with air molecules, "knocks out" electrons from them. A positive charge remains on the molecule, while the electron is captured by the electron-acceptor molecule and charges it negatively.

This process leads to the formation of a large number of light negative and positive air ions. Herewith, the radiation influence of natural potassium to humans does not exceed the corresponding allowable limits regulated by national Sanitary Rules (SR 2.6.1.758-99) which is due to the fact that potassium isotope content in the total mass of potassium is about 0.012 percent. Distinctive features of potassium salts thermodynamic properties are their high heat capacity and low thermal conductivity, which lead to rapid stabilization of microclimatic parameters of the atmosphere in closed space.

Thus, potassium salts have a huge potential for expanding their application. One of these options is the formation in the vegetable storages of air medium with bactericidal properties. In the 1990s, a series of experiments were conducted at the Mining Institute of the Ural Branch of the Russian Academy of Sciences, which later became part of the Perm Federal Research Center, for storing potato in the used drifts of potassium mines (Krasnoshtein et.al.,1997). Positive results were obtained, but the mechanisms of action were not studied in details. It is assumed that the main factors that positively affect the storage of potato are: an increased concentration of light aero ions and the presence in the atmosphere of a salt aerosol with bactericidal properties.

In this regard, the purpose of this research is to study the impact of these factors on the microclimate of the storage, the ability to inhibit the development of the main pathogens during storage of potato, the preservation of potato tubers during storage, and the possibility of calculated determination of the quantitative parameters of these factors.

MATERIAL AND METHODS

The equation of charged particles kinetics was used to calculate the concentration of aeroions in the air atmosphere, taking into account beta radiation

levels in potassium mines (Boyarchuk, 1999). The initial phytopathogens determination in the infected material was carried out according to morphological characteristics and microscopy observation data. Phytopathogen cultures were isolated by direct seeding on selective media from potato tubers with characteristic signs of diseases - late blight, phomosis, wet rot, etc. To isolate pathogens, wet rot (*Pseudomonas solanacearum*, *Corynebacterium sepedonicum* and others), fusarium infection (*Fusarium* spp.), phomosis (*Phoma exiqua*, var. *Foveata*) rhizoctoniosis (*Rhizoctonia solani*) and common scab (*Streptomyces* spp) were used two main media - potato agar and Chapek's medium.

Species identification of bacterial isolates was carried out by polyphase taxonomy methods in accordance with the Bergey species guide and the Bergey's Manual (Whitman et.al., 2012). The exact identification of bacteria - phytopathogens was carried out by DNA diagnostic methods: using Polymerase chain reaction (PCR) analysis of genes and subsequent sequencing of 16S rDNA genes.

For PCR analysis of 16S RNA genes of bacterial potato plant pathogens and gene sequencing, primers 27F AGAGTTTGATCCTGGCTCAG and 1391R GACGGGCGGTGWGTRCA were used. To identify phytopathogenic fungi, morphological characters were determined, including features of conidiogenesis. To detect the studied fungi and streptomycetes — phytopathogens in the mixed material, the well-known PCR systems were used (Hussain et.al.,2014, A'Hara, 2015, Xu, 2016, Khan et.al.,2017, AlHusnain, 2019).

For study the influence of salt saturated atmosphere on the growth of isolated cultures of phytopathogens and assessment of growth repression degree, phytopathogenic cultures planted on potato agar were placed in airtight chambers with linear dimensions of 200x240x110 mm (V=5.28 liters). A salt-saturated atmosphere was created in the chamber by blowing air through a column filter with dimensions of 1 = 200 mm, d = 24 mm, filled with sieved fractionated salt with a particle size in the range of 0.5-1 mm.

The distinctive feature of potassium salt, causing the interest in its use for the storage of agricultural products, is the presence in it of the natural radioactive isotope potassium-40 with β-radioactivity. Abundance ratio of potassium-40 in nature is 0.0117 percent, and the activity of one gram of isotopically pure potassium-40 is 2.65·105 Bq. Under the influence of β-radiation, the air contacting with potassium salt is ionized.

According to (Krasnoshtein et.al.,2008), β-radiation levels in potasium mines are: $4 \cdot 10^{-3}$ part/m^2·min - in rock salt used drifts; $2.8 \cdot 10^{-3}$- $3.5 \cdot 10^{-3}$ part / m^2 ·min - in sylvinite used drifts. The main reason for beta- radiation of potassium salt is the radioactivity of one of the potassium isotopes - potassium-40. Using the equation of charged particles kinetics it is possible to calculate the concentration of aero ions in the air atmosphere, taking into account the levels of β-radiation in potash mines.

Besides the generation of aeroions from the surface of salt particles, there are processes of attachment, recombination, detachment of electrons, destruction

of negative aeroions and interactions with neutral oxygen and nitrogen molecules, which are not taken into account in this analysis, since it is assumed that the lifetime of aeroions is longer than the time period during which an elementary volume of air will interact with the stored agricultural products and leave the storage. According to the data of (Kowalski, 2000), the average life span of light aeroions in a natural setting ranges from 46 to 60 s. The decrease in the concentration of aeroions in the framework of this model occurs only due to transport along with the main air flow.

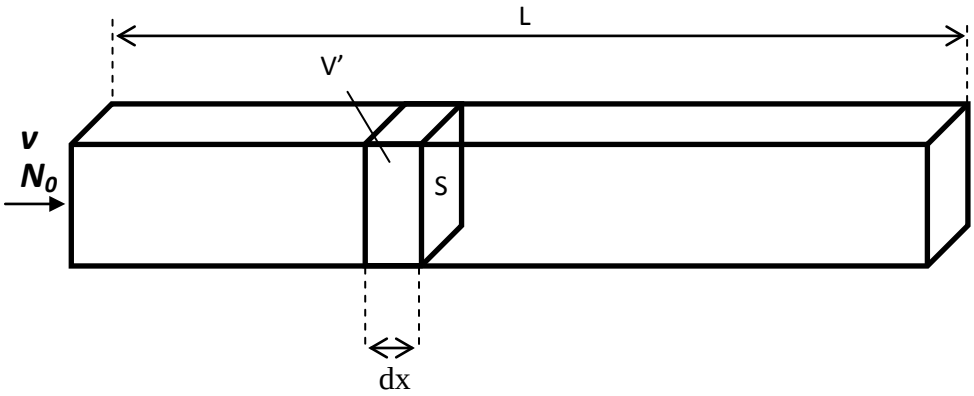

Figure 1. The model of agricultural product storage

For a storage of length L (Fig. 1), the average concentration of aeroions in the entire storage is calculated according the equation:

$$\widetilde{N} = \widetilde{N}_0 + \frac{3\rho C q_n}{v R_p}\frac{L}{2}$$

where:
$\tilde{N}$ - the average concentration of aeroions in the specific storage volume V,
$\tilde{N}_0$ - concentration of aeroions $\tilde{n}$ at the entrance to the storage;
C - concentration of salt dust in the air volume (kg / m^3);
ρ - density of the particle, kg / m^3;
q_n– component of the air ion flux vector normal to the surface of the dust particle, (β-radiation level),
part/ $м^2$ min;
R_p - the radius of the dust particle, m;
v - constant average speed of the air stream (m/min)

The maximum concentration value $\tilde{\tilde{N}}$ is reached at the end of the air path.

The influence of negative air ions on the content of microorganisms in the atmosphere is modeled by the equation:

$$\frac{\widetilde{N}_b(t)}{\widetilde{N}_b^{(0)}} = \exp\left(-Z \cdot E_{eff} \cdot t\right),$$

where: $\tilde{N}_b(t)$ – average concentration of microorganisms in storage at time t;
$\tilde{N}_b^{(0)}$- initial average concentration of microorganisms in the storage;
Z - susceptibility of bacteria to radiation, m^2/J.
E_{eff} - average beta radiation intensity, W/m^2;

The constant Z is the indicator showing the sensibility of a specific microorganism to radiation influence and, therefore, is a very important parameter in predicting the concentration of microorganisms in any storage. The value of Z should be determined experimentally for each particular type of microorganism and type of radiation (Kowalski, 2000, Peccia et.al., 2000). The constant E_{eff} depends on the concentration of aero ions in the atmosphere of the storage, on the energy of a single electron Q_e during β – decomposition of potassium-40, and on the average air velocity v.

$$E_{eff} = c_1 \cdot Q_e \cdot \widetilde{N} \cdot v.$$

The given system of equations allows calculating the dynamics of the average concentration of microorganisms in the storage over time at various concentrations of potassium aerosol (Nakaryakov, 2017, Shalimov et. al., 2018). However, before proceeding to the design of such storage facilities, it is necessary to fulfill a number of laboratory experiments that confirm in practice the possibility of exposure to pathogenic bacteria by air medium contacting with potassium salts. The results of one of these experiments are presented in this paper.

RESULTS AND DISCUSSION

To simulate such a process, isolates of phytopathogenic microorganisms were isolated by direct seeding on selective media from potato tubers with signs of characteristic diseases - late blight, phomosis, wet rot, etc. Samples of infected potato tubers of the 2016 harvest were taken from the farm “Truzhenik Ltd” (Perm Region). According to the analysis of potato phytopathogens distribution in the 2017 yield, the number of infected tubers was 10.2 percent of the studied quantity. The total number of tubers in the combined sample was 8000 sp, including: dry rot *(Fusarium* spp) 5.12 percent; wet rot (*Pseudomonas solanacearum* and others) 3.15 percent; dry rot (*Phoma exiqua*) 1.86 percent; ring rotation (*Clavibacter michiganensis* ssp. *sepedonicus*) 0.05 percent; late blight (*Phytophthora infestans*) 0.02 percent; common scab (*Streptomyces* spp) 0.0 percent 2; other 0.01 percent.

Thus, the prevailing diseases of potato tubers of the 2017 harvest were fungal infections - fusarium and fomosis, as well as bacterial wet rot. Late blight was observed in a less proportion, usually the spread of this infection reaches high values in wet seasons.

To isolate cultures of model phytopathogens, three samples of tubers with signs of late blight, wet bacterial rot, and brown rot were selected. Phytopathogenic cultures identified as representatives of the species *Phytophthora infestans* and *Pectobacterium carotovorum* were isolated.

The primary modeling of atmospheric parameters and storage conditions in a special climatic chamber was carried out. The influence of salt saturated atmosphere on the growth of isolated cultures of phytopathogens and assessment of growth repression degree were determined. For this purpose, phytopathogenic cultures planted on potato agar were placed in airtight chambers. A saturated atmosphere was created in the chamber by blowing air through a column filter. The growth of cultures was studied on Petri dishes with salt in three versions: 1) the air flow volume through the filter with salt is 1.0 litre / min; 2) the air flow volume through the filter with salt of 0.1 litre/ min; 3) sealed chamber without ventilation (with salt in open Petri dish).

The effect of air humidity in sealed chamber on the stability of pathogens has been studied. The experiments were carried out at three relative humidity values: 16, 50, 85 percent in a salt saturated medium and in an air medium passed through a sterilizing membrane filter. The growth repression of bacteria and fungi was evaluated by the number of colonies seeded on Petri dishes with potato agar with a dilution 10^{-6} (Table 1).

Table 1. The number of colony forming units (CFU) of potato pathogen cultures *Pectobacterium carotovorum* and Phytophthora infestans cultured in a salt saturated atmosphere

	Pectobacterium carotovorum			*Phytophthora infestans*		
Humidity %	Atmosphere without salt CFU/ dish	Salt saturated, atmosphere CFU/ dish	Growth repression, %	Atmosphere without salt CFU/ dish	Salt saturated, atmosphere CFU/ dish	Growth repression, %
16	255,0±6,4	194,7±4,8	23,7	187,3±4,7	113,3±4,6	39,5
50	326,0±10,6	208,0±7,1	36,2	308,7±7,3	159,7±4,8	48,3
85	383,7,0±12,1	212,7±5,4	44,6	426,0±16,5	212,7±5,4	63,1

The development of bacterial cultures of *Pectobacterium carotovorum* was maximum at a humidity of 85 percent. At the same time the saturation of medium with salt led to the suppression the growth of the phytopathogen culture by 23.7 percent at a humidity of 16 percent, by 36.2 percent with humidity of 50 percent and by 44.6 percent with humidity 85 percent. The absolute number of colonies was minimal in salt saturated medium with 16 percent humidity. The study of the influence of salt saturated atmosphere on *Phytophthora infestans* showed even greater dependence on the humidity of the environment and a greater degree of growth repression in atmosphere saturated with salt air ions. The growth inhibition of this phytopathogen was noted as 39.5 percent at 16 percent moisture

content, 48.3 percent at 50 percent humidity and 63.1 percent at 85 percent humidity.

The growth repression of phytopathogens was enhanced by 3-5 percent under the influence of convection, with little advantage of treatment with the air flow volume 1.0 litre/min but the difference between treatments was insignificant (Table 2).

Table 2. The effect of convection on the number of colony forming units of *Pectobacterium carotovorum* cultures when cultured in a saturated atmosphere

lum °	Atmosphere without salt CFU/ dish	Salt saturated, atmosphere CFU/ dish			Growth repression, %		
		No convection	Convection 0.1 litre / min	Convection 1.0 litre / min	No convect.	Convection 0.1 litre / min	Convection 1.0 litre / min
16	255,0	194,7±4,8	187,3±2,2	182,3±2,9	23,7	26,5	28,9
50	326,0	208±6,0	197,7±3,5	192,7±4,7	36,2	39,6	40,9
85	383,7	212,7±5,4	198,7±3,7	194,3±5,2	44,6	48,2	49,3

It was also discovered that with rise the air temperature from 10 to 20, and further to 30 °C, the influence of salt saturated atmosphere increases proportionally (Table 3).

Table 3. Growth repression of *Pectobacterium carotovorum* culture in a saturated atmosphere at different temperatures in version with air flow volume 0.1 litre/min, CFU / dish

Humidity, %	Atmosphere without salt CFU/ dish	The quantity of colony forming units, CFU/ dish			Growth repression, %		
		10 ºC	20 ºC	30 ºC	10 ºC	20 ºC	30 ºC
16	255,0	192,3±5,9	187,3±2,2	116,3±4,4	24,6	26,5	54,4
50	326,0	204±4,6	197,7±3,5	120,7±6,2	37,4	39,6	63,0

Such increase in the repression effect with temperature rise can be associated both with an increase in the permeability of the cell membrane for aeroions and with greater sensitivity of cells in conditions of more intensive metabolic processes. However, the practical application of temperature dependence studies should be coordinated with the physiological norms of storage temperatures for potato tubers in storage facilities, preventing their germination and moisture loss. It is well known fact, that the optimal temperatures for the preservation of the vendible and physiological value of healthy potatoes are +15 °C or less, but not lower than + 1 °C. In all treatments, the salt saturated atmosphere exerted suppression effect on phytopathogens (Fig. 2), that may be explained both by cytostatic and cytotoxic factors.

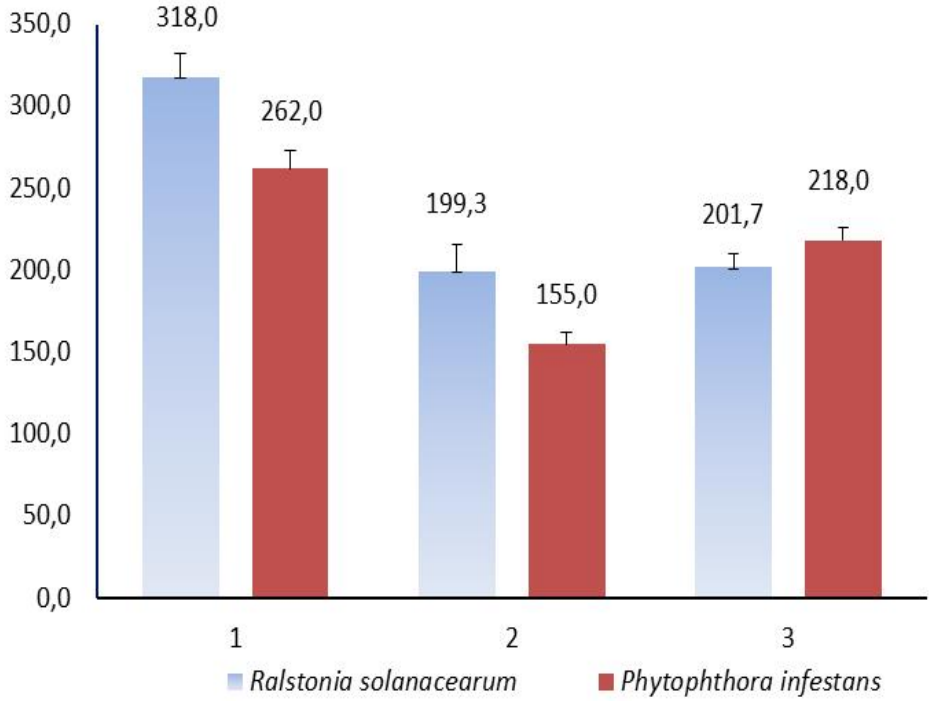

Figure 2. The number of colony forming units (CFU / cup) cultivated in a salt saturated atmosphere and growing in an atmosphere without salt.

Treatments: 1 - control, without salt; 2 - saline atmosphere; 3 - cups transferred to the atmosphere without salt after 10 hours of incubation in salt saturated atmosphere.

The usage of bacterial culture as a model phytopathogen, a cytotoxic (bactericidal) effect prevailed. Using a mushroom culture, both cytotoxic and cytostatic effects were observed. In samples removed to normal atmosphere after 10 hours of exposure in a saturated atmosphere, the number of colonies increased by 24 percent.

The effect of changes in humidity, convection, and the temperature of the air medium contacting with salts on the development and transmission rate of infections is studied. In this study 200 µl of suspension of *Pectobacterium carotovorum* or *Phytophthora infestans* with a cell density / CFU of 10^{-6} were applied and evenly distributed on the surface of potato tubers cuts treated with 70 percent ethanol.

It was shown that in salt saturated atmosphere, the effectiveness of experimental infection by transferring suspensions of *Pectobacterium carotovorum* and *Phytophthora infestans* to a potato cut reduced from 14-22 to 2-7 percent and from 16-29 to 6-11 percent, respectively.

CONCLUSIONS

The calculation results, confirmed by preliminary experiments, provide the information about the influence of the air medium contacting with potassium salts on the growth and development of major fungal and bacterial infections that accompanying potato storage process.

It was shown that storage of potato tubers in the air medium contacting with potassium salts and saturated with light negative aeroions and salt aerosol promotes growth repression of main phytopathogens and better preservation of potato during storage period, the number of microorganisms colonies decreased by 13-63 percent compared with the control.

The obtained data can be used to develop new methods for storing potatoes, root crops and vegetables, to design a new type of vegetable storages equipped with systems for maintaining optimal microclimatic parameters of the air medium, including fungi and bactericidal properties. These systems will allow to reduce significantly product loss during storage period..

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