Disinfectants efficiency on microorganisms - active gray rot causative agents within the process of sugar beet storage

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Abstract

Introduction. The rotting of roots in a heap owing to emergence of microbial processes sometimes is the main cause of loss in beet mass and sucrose, as well as a sharp decline in the quality of raw materials and intermediates.

Materials and methods. The objects of research were selected pure cultures of bacteria of the slime-forming bacterias of *Leuconostos* genus and mycelial fungi which are active agents for gray rot of sugar beet; roots of selection German hybrid "Oryx"; a new generation of disinfectants.

Results and Discussion. Determination of gray rot causative agents activity carried out at different terms and temperatures of roots storage, previously affected by a certain type of gray rot causative agent. Thus, the fungus *Botrytis cinerea Pers* is a very active gray rot causative agent. Increasing of storage temperature by 15 ... 20 °C promotes the development of Mucorales and the most common types of *Mucor mucedo* and *Rhizopus nigricans*, which in a short time can turn the beet into unprocessable product. The sugar beet samples infected with *Geotrichum candidum* and *Torula beticola*, during storage at a temperature of 0 ... 5 ° C for 45 days revealed the presence of external mycelium, but there was almost no development of gray rot causative agent. "Sanitarin", "Javel-Kleyd", "Biodez" and "Hembar" showed the high efficiency on mycelial fungi of different genus. The "Nobak-enzyme" should also be noted which compared to "Nobak" has showed high antimicrobial effect to a wider range of microorganisms. "Betastab" showed high efficacy in slime-forming bacteria, including the *Leuconostoc* genus, at the same time, at these values it is not effective on Micromycetes. Disinfectant "Kamoran" is active on different groups of microorganisms, including Micromycetes and slime-forming bacteria.

Conclusions. The most of the researched means have stable fungicidal and fungistatic effect against a broad spectrum of Micromycetes, these agents are also effective in inhibiting the development of slime-forming bacteria.

Introduction

Improving technological properties and increased resistance of sugar beet to various diseases during growing season and storage is an urgent problem of sugar-beet industry, including complex issues of selection of resistant varieties of beets, compliance with modern agricultural technologies of growing, harvesting and transportation regulations, warehousing and storage technology, the use of fungicidal agents for processing roots that are placed in the heap, and so on. The practice of storing beets indicates that rotting of roots in a heap sometimes is the main cause of loss in beet mass and sucrose, as well as a sharp decline in the quality of raw materials [1, 2, 3, 4, 5, 6, 7].

Ensuring the quality of sugar beet during storage is an important task, because during processing of sugar beets affected by gray rot or mucous bacteriosis, the technological parameters of juices and products are being significantly deteriorated, and there are associated negative consequences - namely, gas formation in the diffusion machine, foaming during saturation, significant difficulties in filtering juices, slow massecuite boiling [1, 8, 9, 10]. As a result of the above-mentioned technological problems sugar plant capacity is reduced, sucrose losses are increased due to decomposition to some organic acids, including through the course of microbiological processes, which leads to a decrease in overall sugar yield and deterioration of its quality.

Having a rotten mass in addition to direct loss of sucrose, leads to a deterioration of a number of technological parameters during beets processing. The results of our reaearch show that in the case of significant microbiological processes, beets become unsuitable for processing, which is also consistent with the findings of other researchers [2, 11, 12, 13, 14].

The emergence of microbiological processes during storage of beet in heaps is caused by long-term storage of root crops in the field after excavation; poor conditions of gray field, entering into heap of the beet with reduced resistance to microbiological lesions (mechanically damaged, immature, affected with microbiological diseases during the growing season, sleepy, frozen), accumulation of large amounts of top within some places of heap, fragments of roots and weeds, which rot in the first place, which promotes gray rot spreading to other root vegetables, etc [6, 12, 15, 16].

Thus, to achieve high economic performance and the production of white sugar, according to DSTU (National Standards of Ukraine), one should pay great attention to ensuring adequate technological quality of sugar beets which enter the processing, including the microbiological contamination of roots. Accordingly, our research objectives was to investigate the effectiveness of a new generation of disinfectants on inhibiting the activity of microorganisms which are active agents for gray rot of sugar beet.

Materials and methods

We found that the contaminating microflora of sugar beet includes a number of Micromycetes of *Fusarium, Botrytis, Mucor, Penicillium, Aspergillus, Trichotecium, Verticillium* genus [17]. In addition, under adverse conditions of roots storage in the heap, active lesions of sugar beet with slimy bacteriosis is possible due to bacteria of the *Leuconostoc* genus, and due to the combined action of ammonifying bacteria *Bacillus subtilis and B. megatherium* and others. The research of the effectiveness of these disinfectants was performed on pure museum or isolated cultures of microorganisms from the heaps of Nabutivsky sugar plant.

As for the objects of research, we selected pure cultures of bacteria of the slime-forming bacterias of *Leuconostos* genus and mycelial fungi of the following genus: *Botrytis, Rhizopus, Mucor, Fusarium, Aspergillus, Penicillium, Geotrichum, Gliocladium, Torula*.

The research applied the use of roots of selection German hybrid "Oryx", which were grown in a research farm. Indicators of technological quality of the roots amounted to an average of: sucrose content in beet - 16.2 ... 16.4%, purity of beet juice 85,9 ... 86,3%, juice ratio - 93.0 ... 93.4%, root mass 640 ... 750g.

According to modern requirements [2] applicable to disinfectants, the chemicals used as an active substance should be characterized by a wide spectrum of biocidal action and maintain its activity for a long time, should not make a negative impact on the quality of products, as well as belong to class III-IV of moderately hazardous substances under the parameters of acute toxicity. Considering the above factors, the research applied selection of the following disinfectants: based on dichloroisocyanuric acid sodium salt - "Sanitarin", "Javel-Kleyd"; on polyhexamethyleneguanidine (PHMG) - "Biodez", "Hembar"; on cytrocide - "Nobak", "Nobak-enzyme"; monenzyne sodium - "Kamoran", natural hydroxy acids - "Betastab."

Selection of active agents. Method of selection of active agents is the study of destructed tissue on the verge of healthy one. To do this, the roots were cut under sterile conditions through the rotted portion, then a small amount of tissue on the verge of healthy and rotten pieces of the root was selected using scalpel and put under sterile conditions into a test tube of Czapek molten medium, beetroot agar, MIA(meat infusion agar). After being stirred, the contents of the tubes were poured into Petri dishes.

According to the methodology, after allocation of mycelial fungi with lesions located on the surface of the root, a part of the external mycelium was selected with thin fried microbiological needle and placed into a test tube with the appropriate nutrient medium. After beng stirred, the medium with particles of mycelium and spores was poured into sterile Petri dishes. The cultivation of mycelial fungi in both methods was carried out in thermostats at temperatures of 27 °C and within duration of up to 7 days. From germinated colonies the cultures were inoculated into test tubes with nutrient medium. Pure cultures of fungi were plated on nutrient dense medium for learning morphological characteristics of isolated cultures of microorganisms.

Determination of the activity degree of selected agents. The activity of certain fungi species on gray rot development of sugar beet was determined by means of the following method [18]. For the experiment there were selected unaffected roots of sugar beet of approximately the same size. Roots were preliminary disinfected with a solution of potassium permanganate of pink colour. To make an insert of pure culture of a certain type of Micromycetes there were formed three swath scars on top of each root using a sterile scalpel and the same amount of spore material was inserted into them. For averaging the results of the experiment each type of microorganism had six infected roots. Control experiments were conducted accordingly on the six roots with scars without making spore material.

Within the research there were used both the pure cultures of microorganisms and bacterial cultures and their associative group, isolated with rotten roots.

Infected according to the above method roots were placed in a moist chamber (in desiccators or in sterile plastic bags), where they were kept within 25 ... 45 days at a certain temperature. We used two temperature ranges: 0 ... 5, and 15 ... 20 °C. After the expiration of the retention period the phytopathological survey of roots was conducted. Initially, all the roots were examined externally, to determine the nature of mycelium growth and external picture of destruction, then roots were cut across the scar and the degree of beet tissue rotting was determined.

Determination of the effectiveness of disinfectants activity. To determine the sensitivity of microorganisms to antiseptic preparations, the "holes in the thick agar" method was used. Cultivation of microorganisms was performed on the following nutrient medium:

a - MIA(meat infusion agar) + saccharose and beetroot agar with inclusion of pure culture of Leuconostoc mesenteroides;

b - Czapek medium with inclusion of pure cultures of Micromycetes - Rhizopus nigricans, Mucor mucedo, Aspergillus niger, Penicillium, Botrytis cinerea Pers, Fuzarium culmorum, Gliocladium roseum.

Nutrient medium with corresponding culture of microorganisms were poured into sterile Petri dishes. After solidification of nutrient medium the holes were made within each 1.8 ... 2.2 cm from the edge of the dish, using a sterile drill. The appropriate disinfectant solutions of various concentrations were inserted into the holes.

Conclusions about the effectiveness of disinfectants at a certain solution concentration were made according to the availability of areas of stunted growth of microorganisms. No areas of stunted growth indicates that the studied culture is insensitive to the action of the product at the specified concentration. With the zone diameter of 15 mm we believe that microorganisms have a small degree of sensitivity to the corresponding concentration of the product, with the zone diameter of 15 to 25 mm the average degree of sensitivity is indicated. Availability of the zone with diameter greater than 25 mm indicates a high degree of sensitivity of microorganisms to the concentration of the antimicrobial agent.

Results and discussion

Determination of gray rot causative agents activity. Since the species composition of the microflora of sugar beet is represented by more than 100 types of gray rot causative agents, activity of which depends on a combination of physiological and morphological properties, as well as environmental conditions, it is important to analyze the microflora in sugar beet heaps in order to detect the most active causative agents of gray rot.

Within the research conducted on Nabutivsky sugar plant it was found that the roots selected from the heap were affected by associative group of mycelial fungi, which leads to their rapid rotting. In particular, besides the types of mycelial fungi that were found in the analysis of beets included into the heap, infected roots after storage revealed micromyceta of *Botrytis cinerea, Mucor mucedo, Rhizopus nigricans, genera Penicillium, Aspergillus, Trichotecium, Verticillium, Gliocladium, Fusarium, Trichoderma, Torula and bacteria Bacillus subtilis, Leuconostoc. mesenteroides, L. Dextranicum* species.

Gray rot development is a complex process due to a number of factors, and is a consequence of life of a wide range of microorganisms, and the degree of root damage is largely dependent on the activity of the gray rot causative agent. So the study of activity of the most active representatives of mycelial fungi on the intensity of gray rot is of scientific and practical interest because usually most of the bacteria have no ability to penetrate the intact surface of plant organisms and are secondary infection after Micromycetes affect [17, 18].

As for an object of research, there were used pure cultures of microorganisms *Botrytis cinerea, Mucor racemosus, Rhizopus nigricans, Fusarium angustum, Penicillium rugulosum, Fusarium oxysporum, Geotrichum candidum, Torula beticola, Fusarium sulmorum.*

Table 1 and 2 show the results of research at different terms and temperatures of roots storage, previously affected by a certain type of gray rot causative agent.

Comparative analysis of Tables 1 and 2 shows the fact that among the selected Micromycetes cultures there take place gray rot causative agents, capable to destroy the root tissue and less active species, which can destroy the root tissue in much less active manner.

Table 1

Analysis of the activity of gray rot causative agent within sugar beet storage for 25 days at a temperature of 15-20 °C

Type of microorganism	Content of rotten tissue, %	
	after 10 days	after 25 days
Botrytis cinerea	20	49,3
Mucor racemosus + *Rhizopus nigricans*	26	58,2
Fusarium angustum	14	30,4
Penicillium rugulosum	10	17,5
Fusarium oxysporum	4,5	9
Geotrichum candidum	0,9	1,3
Torula beticola	1,7	3

Thus, the fungus Botrytis cinerea Pers is a very active gray rot causative agent, which is consistent with the results of other researchers [1, 17, 18]. Increasing of storage temperature by 15 ... 20 °C promotes the development of Mucorales and the most common types of Mucor mucedo and Rhizopus nigricans, which in a short time can turn the beet into unprocessable product. According to [12, 17], at the temperature conditions above 15 ... 20 °C these fungi far outweigh Botrytis cinerea Pers by degree of tissue destroying activity.

Table 2

The activity of gray rot causative agent within sugar beet storage for 45 days at a temperature of 0-5 °C

Type of microorganism	Content of rotten tissue, %	
	after 25 days	after 45 days
Botrytis cinerea	6,9	16,4
Mucor mucedo, Rhizopus nigricans	4,1	9,5
Fusarium angustum	1,1	2,64
Penicillium rugulosum	0,3	0,75
Fusarium oxysporum	0,5	0,9
Torula beticola	-	-
Geotrichum candidum	-	-

The sugar beet samples infected with Geotrichum candidum and Torula beticola, during storage at a temperature of 0 ... 5 ° C for 45 days revealed the presence of external mycelium, but there was almost no development of gray rot causative agent.

Determination of the effectiveness of disinfectants activity. Since the active gray rot causative agents include filamentous fungi, a research was conducted to determine the effectiveness of antimicrobial action of the above disinfectants on Micromycetes species: Rhizopus nigricans, Mucor mucedo, Botrytis cinerea, Fuzarium culmorum, Gliocladium roseum, Aspergillus niger, Penicillium rugulosum. Moreover, given the extremely high difficulty of processing of sugar beet affected by mucous bacteriosis, the research applied the use of such culture as Leuconostoc mesenteroides species. Results of the research on the effectiveness of the above-mentioned disinfectants for certain types of microorganisms are given in Table 3.

Table 3

Results of the research on the effectiveness of antimicrobial action of some disinfectants on pure cultures of microorganisms by "holes in the thick agar" method

Disinfectants expenditure, g	The diameter of the activity zone of antimicrobial agents on microorganisms, mm							
	Rhizopus nigricans	*Mucor mucedo*	*Penicillium rugulosum*	*Botrytis cinerea Pens*	*Fuzarium culmorum*	*Gliocladium roseum*	*Aspergillus niger*	*Leuconostoc mesenteroide*
Sanitarin								
0,0002	27	38	32	14	39	38	10	14
0,0004	38	No growth	40	24	No growth	No growth	14	20
0,0006	No growth			36	No growth	No growth	19	28
0,0008	No growth						25	32
Javel-kleyd								
0,0002	24	35	28	12	37	32	Solid growth	12
0,0004	32	40	34	22	No growth	40	8	20
0,0006	38	No growth	38	32	No growth	No growth	16	26
0,0008	No growth						22	30
Biodez								
0,0005	11	12	8	12	19	24	Solid growth	12
0,001	25	22	16	24	26	30	7	16
0,002	29	28	30	30	32	38	13	23
0,003	37	38	35	36	37	No growth	22	28
Hembar								
0,0005	14	14	10	15	20	25	4	13
0,001	26	24	17	26	28	30	8	18
0,002	32	30	32	33	34	39	14	24
0,003	No growth	40	38	40	No growth	No growth	23	29
Nobak								
0,00025	Solid growth							19
0,0005	Depressed growth the areaof the cup					–	Solid growth	24
Nobak-enzyme								
0,00025	28	26	22	22	23	–	20	22
0,0005	No growth					–	32	34
Betastab								
0,0025	Solid growth							32
0,005	Solid growth							38
Kamoran								
0,001	20	15	8	Depressed growth	25	–	12	24
0,002	23	16	14	16	24	–	14	36
0,004	28	22	16	16	28	–	17	40

Analysis of research results (Table 3) demonstrates the high efficiency of "Sanitarin", "Javel-Kleyd", "Biodez" and "Hembar" on mycelial fungi of different genus. The "Nobak-enzyme" should also be noted which compared to "Nobak" has showed high antimicrobial effect to a wider range of microorganisms.

As for the "Betastab", which is an environmentally safe product, derived from hydroxy acids of hops, it shows high efficacy in slime-forming bacteria, including the Leuconostoc genus, at the same time, at these values it is not effective on Micromycetes. Disinfectant "Kamoran" is active on different groups of microorganisms, including Micromycetes and slime-forming bacteria.

Fig. 1-4 graphically illustrates the results of research in order to identify the most effective disinfectants.

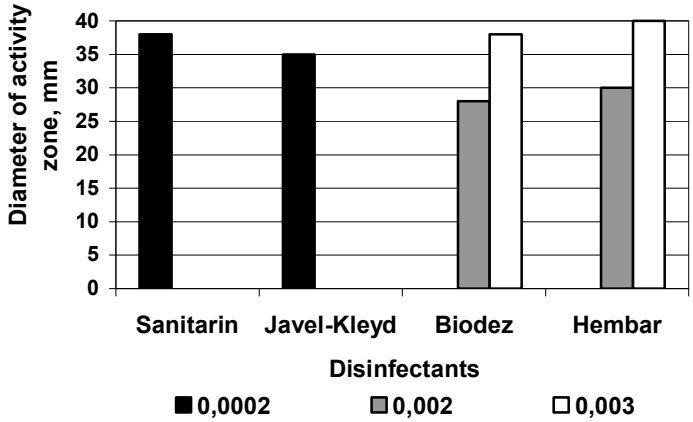

Figure. 1. The diameter of the zone of stunted growth of Micromycetes of Mucor mucedo species using disinfectants "Sanitarin", "Javel-Kleyd" at expenditures of 0,0002 g, "Biodez", "Hembar" – 0,002 and 0,003g

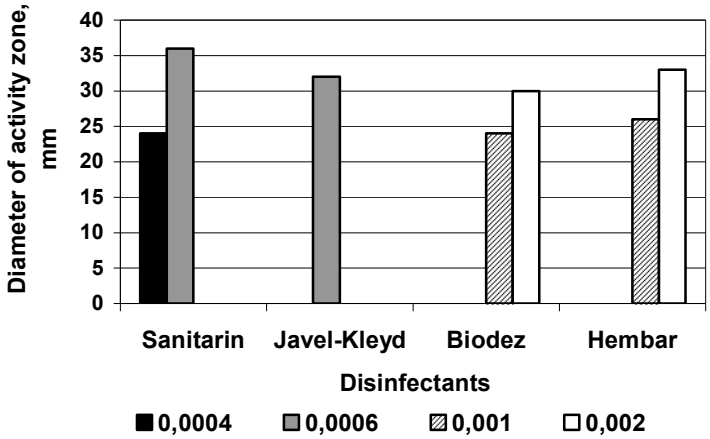

Figure. 2. The diameter of the zone of stunted growth of Micromycetes of *Botrytis cinerea Pers* species using disinfectants "Sanitarin", "Javel-Kleyd" at expenditures of 0,0004 g and 0,0006, "Biodez", "Hembar" – 0,001 and 0,002g

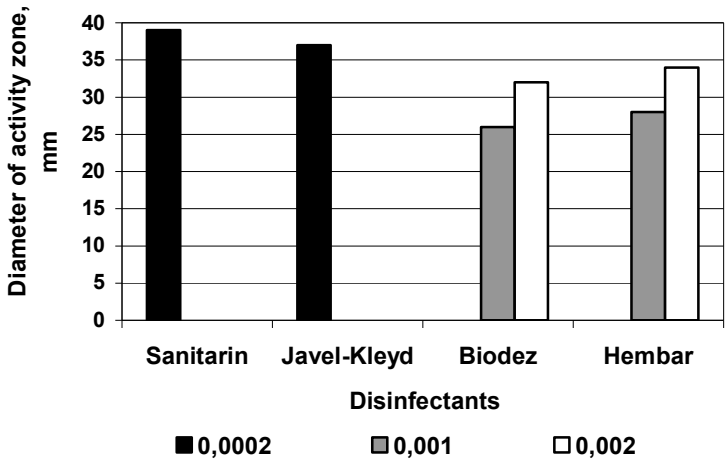

Figure. 3. The diameter of the zone of stunted growth of Micromycetes of *Fuzarium culmorum* species using disinfectants "Sanitarin", "Javel-Kleyd" at expenditures of 0,0002 g, "Biodez", "Hembar" – 0,001 and 0,002g

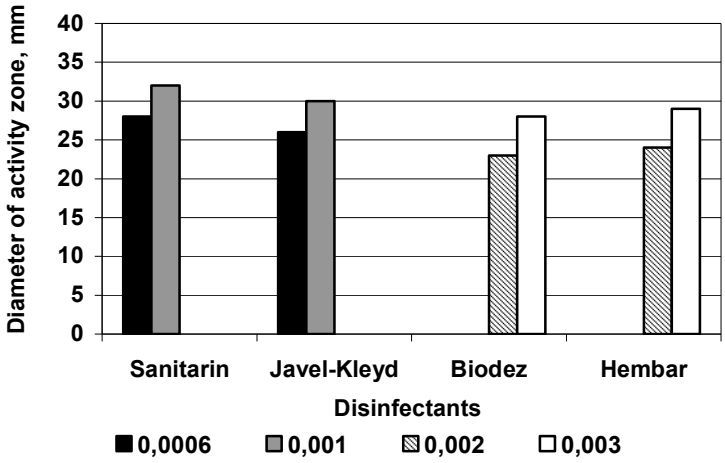

Figure. 4. The diameter of the zone of stunted growth of slime-forming microorganism culture of *Leuconostoc mesenteroides* species using disinfectants "Sanitarin", "Javel-Kleyd" at expenditures of 0,0006 and 0,0001 g, "Biodez", "Hembar" – 0,002 and 0,003g

Analysis of experimental research results on Mucor mucedo species (Fig. 1) has shown the high efficiency of "Sanitarin" and "Javel-Kleyd" at expenditures of 0,0002 g, as well as "Biodez", "Hembar" at expenditures of 0,002 and 0,003 g.

These products have slightly lower efficiency on Botrytis sinerea Pers species (Fig. 2). However, according to the results of research shown in Table 2, with increasing expenditures of the product based on dichloroisocyanuric acid sodium to 0.001 g the complete environment sterility is being achieved.

One of the most active gray rot causative agents are the Fuzarium genus. The results of the above products on Micromycetes of Fuzarium culmorum genus are presented in Fig. 3 and indicate the high efficiency of their actions at the following expenditures: "Sanitarin", "Javel-Kleyd" – 0,0002 g, "Biodez", "Hembar" – 0,002 g

The high efficiency of the presented products on slime-forming bacteria (Table 3, Fig. 4) should be noted. Thus, at the following disinfectant expenditures: "Sanitarin" 0,0004... 0,0006 g, the zone of stunting growth of slime-forming bacteria Leuconostoc mesenteroides is 20 ... 28 mm, which demonstrates the high efficiency of the product. The products based on PHMG are also effective on slime-forming bacteria. At the following disinfectant expenditures: "Hembar" and "Biodez" 0,002...0,003 g, the zone of stunting growth is 23...29 mm.

According to the analysis of experimental studies, we can conclude that products "Sanitarin", "Javel-Kleyd", "Hembar", "Biodez", "Nobak-enzyme", "Kamoran" have stable fungicidal and fungistatic effect against a broad spectrum of Micromycetes. In addition, the marked products are effective in inhibiting the development of slime-forming bacteria.

Given the results of the research, there is a proved need of the further study on the effectiveness of products based on chlorine ("Sanitarin", "Javel-Kleyd") and based on PHMG ("Hembar", "Biodez") for processing of sugar beet before entering into heap storage.

In order to establish the range of necessary expenditures for further processing of roots there was conducted an additional research to determine the effectiveness of their action on certain types of bacteria and yeast that characterize contaminating microflora of sugar beet.

The following bacteria were selected as objects of research: Bacillus subtilis, B. megatherium (gram positive spore-forming) ammonifying bacteria Pseydomonas aerygenosa, yeast Sacharomyces cerevisea, Rhodotorula glutinis, Endomyces lactis. Cultivation of microorganisms was performed on the following nutrient mediums:

a - MIA (meat infusion agar) and beetroot agar with inclusion of pure cultures of microorganisms of Bacillus subtilis, B. megatherium;

b - wort-agar with inclusion of pure cultures of microorganisms Sacharomyces cerevisea, Rhodotorula glutinis, Endomyces lactis.

Analysis of the research results (Table 4) shows the high efficiency of selected products on bacterial microflora of sugar beet production. The nature of the toxic action of chlorine–based chemicals is associated with oxidative processes in the cytoplasm of microbial cells, leading to its death [2]. Thus, in the case of using disinfectant "Sanitarin" in the range of active ingredient expenditure of 0,0002...0,0004 g there was observed the loss of vegetative forms of spore-forming mesophilic bacteria B. subtilis, B. megatherium, and the yeast Rhodotorula glutinis, Endomyces lactis.

Thus, according to the results of experimental research, ther should be noted the high efficiency of selected products -"Sanitarin", "Javel-Kleyd", "Hembar", "Biodez" on a wide range of microorganisms.

As the products proposed for the research purpose are two groups of active substances, such as dichloroisocyanuric acid sodium and PHMG, the approximate values of reasonable expenditures for 100g of disinfectant working solution (Table 5) have been determined for their subsequent use for processing of sugar beet.

Table 4

Results of the research on the effectiveness of antimicrobial action of some disinfectants on pure cultures of microorganisms by "holes in the thick agar" method

Disinfectants expenditure, g	*B.subtilis*	*B.megatherium*	*Psevdomonas*	*Sacharomyces cerevisea*	*Rhodotorula glutinis*	*Endomyces lactis*
Sanitarin						
0,0002	25	24	28	17	32	34
0,0004	33	32	40	22	No growth	No growth
Javel-Kleyd						
0,0002	20	20	25	15	28	26
0,0004	32	32	36	19	35	33
0,0006	No growth	No growth	No growth	26	No growth	No growth
Biodez						
0,001	18	18	34	20	22	–
0,002	26	26	38	28	30	32
0,004	30	32	No growth	33	35	36
Hembar						
0,002	22	30	36	36	40	34
0,004	29	36	42	39	в.р	38

Table 5

Expenditures of disinfectants to inhibit activity of certain microorganisms

Culture of microorganism	"Sanitarin" product expenditure		"Biodez" product expenditure	
	g	In 100 g of the working solution	g	In 100 g of the working solution
Rhizopus nigricans	0,0002	0,02	0,001	0,1
Mucor mucedo	0,0001	0,01	0,002	0,2
Penicillium rugulosum	0,0002	0,02	0,002	0,2
Botrytis cinerea Pers	0,0004	0,04	0,002	0,2
Fuzarium culmorum	0,0001	0,01	0,001	0,1
Gliocladium roseum	0,00005	0,005	0,001	0,1
Aspergillus niger	0,0008	0,08	0,004	0,4
Leuc. mesenteroides	0,0006	0,06	0,0025	0,25
B. subtilis	0,0002	0,02	0,002	0,2
B. megatherium	0,0002	0,02	0,002	0,2

Conclusions

Thus, the research results have shown that the products"Sanitarin", "Javel-Kleyd", "Hembar", "Biodez", "Nobak-enzyme", "Kamoran" have stable fungicidal and fungistatic effect against a broad spectrum of Micromycetes which are gray rot causative agents and lead to poor technological quality of sugar beet. In addition, these agents are also effective in inhibiting the development of slime-forming bacteria.

According to results of the experimental research, we can conclude on the feasibility of the aforementioned means for sugar beet processing for the purpose of disinfection and prevention of gray rot. Whilst, the range of working solutions concentrations for root processing is as follows: for products based on active chlorine "Sanitarin", "Javel-Kleyd" - 0.02 ... 0.006%, based on PHMG - 0,1 ... 0,2%.

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