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Mycotoxin accumulation and the possibilities of biological control of wine production quality

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Abstract. Against the background of climate change and the increasing impact of phytopathogenic agents of mycotic origin on the vine favors the appearance and toxicity of mycotoxins in wine. Ochratoxin produced by some species of the genera Aspergillus and Penicillium becomes particularly dangerous, which manifests nephrotoxic, immunotoxic, teratogenic, hepatotoxic and carcinogenic effects on animals and humans. This publication includes information on the particularities of synthesis and contamination of different grape varieties and types of wine. The evolution of mycotoxigenic phytopathogenic agents and the increasing risk of mycotoxin contamination of wine products determine the need to develop and establish means and ways to reduce the risks of mycotoxin contamination. Determining the action of microbiological means to combat phytopathogenic agents of mycotic origin (Trichodermin, Gliocladin), applying measures to inhibit mycotoxin-producing fungi and developing ecologically harmless and effective methods to combat phytopathogenic agents represent the foundation of contamination prevention and decontamination systems in wine production.

1. Introduction

Climate change complicates the phytosanitary status of agricultural crops, especially vines, increases the impact on wine production and the risk of mycotoxin contamination [1, 2]. The worsening of phytosanitary problems against the background of climate change lies in the modification of the epidemiological particularities of phytopathogenic agents and the increase in the prevalence of harmful organisms by manifesting various difficulties in carrying out the technological processes of production of agricultural crops, especially phytotechnical raw material. There is evidence of an increased risk of mycotoxin contamination in wheat (trichothecene) and maize (aflatoxins) as a result of climate change [3, 4].

The expansion of the application of modern biotechnologies with the use of useful microorganisms, including toxigenic fungi, which act strongly in very small quantities, increases the seriousness of the problems related to the synergism of these toxins from the moment of their accumulation in agricultural production and contamination of higher organisms. The increase in the carbon dioxide content in the atmosphere increases the risk of contamination of wine with ochratoxin [5] expanding the range of populations of Aspergillus niger and other toxigenic fungi.

Starting from the particularities of mycotoxins, as toxic substances produced by several species of microscopic fungi, we find that they are chemically stable and do not undergo degradation to heat treatments [6]. The large number of mycotoxins pose considerable risks in threatening human and animal health, and among the most dangerous are aflatoxins, the monomethyl ether of alternariol, penicilic acid, ochratoxin A, citrinin, patulin, fumonisins, zearalenone and nivalenol. With entry into the food chain, poisoning occurs from the consumption of contaminated food or products obtained from animals fed with spoiled products. Their toxic action often manifests itself in nephrotoxicity, hepatotoxicity, cytotoxicity, teratogenicity and immunotoxicity and aggravation of human and animal health problems and worsening environmental conditions.

Ochratoxin A is a mycotoxin produced by some species of the genera Penicillium and Aspergillus, among which P. verrucosum is the most important species, and A.

carbonarius is the main cause of wine contamination [7]. Ochratoxin A has various toxic effects on the organ systems of animal organisms and humans, especially on the kidneys, although nephrotoxic, immunotoxic, teratogenic, hepatotoxic and carcinogenic effects on animals are known [8, 9].

Chemically, ochratoxin A is a stable molecule that can persist in food and feed due to its resistance to summary food processing treatments such as heating or fermentation [10].

Against the background of climate change, the increase in atmospheric temperature, the increase in the frequency of storms and the volume of precipitation affect not only productivity, but also food safety, as well as favoring the spread of pathogens, including mycotoxins [11].

Temperature is one of the most important factors influencing the contamination of wine with mycotoxins, which could lead to both a higher frequency of wine contamination and a higher concentration of mycotoxins in wine [12]. Humidity also leads to the creation of a favorable environment for contamination of wine production in areas previously considered to be at low risk [13].

The control of plant pathogens is based on the use of chemicals, which is constantly restricted by global legislation due to their harmful effects on the environment and human and animal health. Biological control agents [14] can be used as a control solution, which is an alternative way of decontamination and control of the appearance of mycotoxins.

The biosynthesis of mycotoxins as a result of the metabolic processes of microscopic fungi is largely determined by temperature and humidity, and the optimal temperature level is 25-30°C depending on the strain. However, the optimal temperature for ochratoxin production within the same strains was different [15]. The indicator is determined by the characteristics of the variety and its genetic peculiarities, and the compact form of the bunches is more strongly exposed to contamination. Currently, the positive correlation between the content of accumulated mycotoxins and the concentration of some soluble components, the content of pectin and anthocyanins, as well as the early stage of ripening, when the pH and sucrose content as lower has been established [16].

Another factor that can generate the production of mycotoxins is carbon dioxide, establishing that higher concentrations of CO_2 stimulate their production. The primary winemaking process also influences the contamination of wine with mycotoxins. It has been established that white wines are pressed immediately after harvesting, while grapes for red wines are first crushed, and the must is macerated for a few days, during which time the synthesis of mycotoxins and their passage from grapes to must is recorded [17].

Starting from the increase in needs in wine production and the worsening of the impact caused by fungal phytosanitary agents and mycotoxins, in particular, the need for an in-depth investigation of this phenomenon and the development of means, including biological means, to reduce the impact caused by mycotoxins becomes current and opportune. In confirmation of world trends, scientists have created a biosensor based on graphene chains and nucleic acids to determine mycotoxins in wine, which demonstrates the high sensitivity and superior operating speed of the device.

2. Research materials and methods

Sampling for analysis was carried out in accordance with mycotoxin sampling methods [13].

For the detection of mycotoxin-producing fungi in grape pomace, real-time polymerase chain reaction was used [18].

The degree of action of biological agents on mycotoxigenic entities was determined under laboratory conditions. Testing under laboratory conditions, on the experiment and production batches of microbiological means of plant protection was carried out in randomized trials [19] with statistical processing of the results.

3. Results and discussions

3.1. Reducing the risks of mycotoxin contamination

The problem of contamination of grapes with mycotoxins begins in the vineyard, where the first preventive measures must be taken [20]. Mycotoxins penetrate deep into the depths of plants attacked by phytopathogenic fungi and do not cover only their surface, which determines the need to comply with the respective technological requirements for the production and storage of agricultural raw materials.

Starting from the relationships between toxigenic fungi and the conditions of biosynthesis of mycotoxins in the vineyard, the critical period needs to be extended from the early entry of the grapes into the vineyard to the beginning of winemaking. Of major importance is the correct application of irrigation procedures to avoid breaking the skin of the berries, which is especially important in the later stages of winemaking, and the harvest must be planned correctly. The time between harvesting and winemaking must be as short as possible: no more than 4 hours for manual harvesting and a maximum of 2 hours for mechanized harvesting [21].

Based on the relationships between mycotoxigenic fungi and harmful insects, it is very important to control Lobesia botrana, which can significantly contribute to reducing the risk of contamination [21], knowing that grapes infested with Lobesia botrana contain higher amounts of toxins than uninfected ones. The use of biological control agents of L botrana in the field for two years ensured an 80% reduction in the mycotoxin content of the grapes.

In order to reduce the health risks associated with mycotoxins, it becomes necessary:

- avoiding planting vines in places with excess moisture;
- observance of the timing of dry pruning and application of fertilizers;
- strictly performing operations in green;
- gathering and destroying the attacked weeds and bunches left after harvesting;
- permanent verification of the frequency of the attack and the intensity of the development of mycotoxin-producing pathogens;
- avoiding damage to the raw material before and during the collection process, during storage or processing, as damaged grains are more susceptible to infection with toxic fungi;
- compliance with the rules for storing bunches and avoiding long-term storage, as well as measures for obtaining the must and its fermentation.

3.2. Biological means of controlling the occurrence of mycotoxins in wine

Reducing the impact of mycotoxins becomes possible by applying biological methods, i.e. microorganisms involved in food fermentation. Biological methods consist of using non-toxic bacteria, yeasts and even fungi and other microorganisms as natural components [22]. Unlike the chemical method of plant protection, biological methods have many advantages, such as low costs and minimal side effects of environmental protection and food safety. Biological methods can be classified into three groups based on the mechanisms of action: inhibition of mycotoxigenic fungi, adsorption of mycotoxins and their degradation or detoxification [23].

Biological agents and their associated enzymes allow for a specific, environmentally friendly and effective approach with minimal impact on the sensory and nutritional quality of food compared to chemical methods used to reduce mycotoxin concentration [24]. Yeasts, especially Saccharomyces species and lactic acid bacteria are mainly used as part of the natural microbial population in spontaneous fermentation and as starter cultures in the food and beverage industry [24].

Carbohydrates and protein components of the cell wall play an important role in binding mycotoxins; the binding efficiency depends on the strain of the microorganism, the amount of mycotoxin, the environmental conditions (pH) and the stability of the microorganism-mycotoxin complex [26]. Many microorganisms, including bacteria, yeasts, filamentous fungi, and protozoa, can biologically eliminate, reduce, or degrade mycotoxins. These potential microorganisms represent a unique group of biocontrol agents for the biodegradation, removal or binding of ochratoxin due to their role in winemaking without affecting the organoleptic properties of the wine.

The use of microbiological products is a technological element in integrated plant protection systems, which contributes to reducing environmental pollution and producing organic wine products, competitive on both local and international markets.

In the Phytopathology and Biotechnology Laboratory of the Institute of Genetics, Physiology and Plant Protection, the biological agent was identified and the biological characteristics of the microscopic fungus *Gliocladium virens*, strain 3X, were determined, on the basis of which the biological product was developed and the biotechnology for the production and application of the Gliocladin-SC preparation was proposed for approval for combating gray rot. The use of microbiological products is a technological element in integrated plant protection systems, which contributes to the reduction of environmental pollution and the production of ecological viticultural products, competitive both on the local and international markets.

The researched variants were placed on randomized experimental plots in 3 repetitions. Depending on the climatic conditions against gray rot in the investigated variants, 5 treatments were carried out in the phases most favorable to the attack: after flowering, berry growth, cluster compaction, the beginning of fallow, before harvesting. To determine the comparative biological effectiveness, the chemical standard - the well-known Chorus 75 WG botryticide with a consumption rate of 0,75 kg/ha - was included in the scheme of experiments. The biological effectiveness was determined compared to the data obtained in the version of the untreated control.

The determination of the biological effectiveness of the product Gliocladin - SC with a titer of $2x10^8$ part/ml for combating the gray rot of the grapevine under production conditions was carried out according to the following scheme.

From the analysis of the obtained data, it is found that in the variant with the use of the biological product Gliocladin - SC in the minimum dose of 5,0 l/ha, the frequency of attack by the gray rot pathogen reached the level of 26,97% with a disease development intensity of 7,73%, which emphasizes the absence of the prospect of using the minimum dose in the fight against gray rot (table).

With the increase in the dose of use of the biological product, the intensity of the development of the disease decreased significantly. Thus, in the variants with the use of the preparation Gliocladin - SC in doses of 7-10 liters/ha, the frequency of the attack was 19,66% - 14,55%, registering a respective development of the disease of 3,84-5,24%, respectively research variants. In the untreated control the frequency of attack by gray rot reached the level of 41,75% with a disease development intensity of 12,22%.

Table 1. Biological effectiveness of the biological product Gliocladin - SC in combating gray rot in grapevines.

VARIANTS	Frequency (F, %)	Intensity (I, %)	Efficiency (E, %)
Gliocladin - SC 5 l/ha	26,97	7,73	36,73
Gliocladin- SC 7 l/ha	19,66	5,24	57,16
Gliocladin – SC 10 l/ha	14,55	3,84	68,55
Chorus 75 WG	6,65	1,77	85,45
Control	41,75	12,22	-

Depending on the researched consumption norms and the climatic conditions that were created during the test period, it also depended on the level of reduction of the degree of attack by gray rot on the vines. The lowest result was obtained in the version with the application of the biological product Gliocladin - SC at a dose of 5.0 l/ha, in which a biological efficacy of 36.73% was recorded. As the rate of consumption increases, the degree of attack of gray rot obviously decreases. In the version with the use of the biological product Gliocladin - SC with a norm of 7,0 l/ha, the biological efficacy reached the level of 57.16%. The highest level of reduction in the degree of attack by gray rot was detected in the version with the application of the respective product with the dose of 10,0 l/ha, in which the biological efficacy was 68.55%.

Against the background of the accumulation of biotechnological achievements in agriculture, in order to reduce the impact of toxigenic fungi against mycotoxins, results are recorded and research is developed in the following directions: genetic selection of plants resistant to contamination with toxigenic fungi; application of genome editing technologies on obtaining plant varieties with properties of inhibition of mycotoxin biosynthesis; production and application of seedlings with endophytic bacteria, antagonists of toxigenic fungi; prior infection of plants with microorganisms useful for competition with toxigenic species [23].

The development and implementation of complex measures to combat phytopathogens is the cornerstone of plant biological protection systems, which considerably reduce mycotoxin-related risk factors in conventional and organic farming [21].

3.3. Reduction of toxicity and possibility of decontamination of Patulin wine products

The increased impact of toxigenic fungi in food is largely driven by the accumulation and contamination of alphatoxins, ochratoxins and patulin. The problem is getting worse especially with the increase in the volume of non-alcoholic products. At the analysis of the dependence of patulin content in bunches of different varieties affected by Botrytis cinerea It has been established that when infecting the main varieties up to 50%, the level of patulin reaches 20-30 mcg/dm3. The role of the main mold-producing genera of fungi has been established, establishing that Botrytis cinerea, Penicillium, Mucor, Alternaria and Aspergillus synthesizes up to 40 mcg/dm3 of patulin, which has guided winegrowers and winemakers to develop and apply integrated pest control systems for phytopathogens with emphasis on the role of mycotoxic microbiological agents. Measures have been developed to decontaminate juice and wine from biological toxins [21].

Knowing the special role of patulin, measures have been argued to decontaminate it in the raw material with different degrees of damage with phytopathogenic agents, recommending the application of a spectrum of means: the application of sorbents in the form of natural and modified bentonites, as well as colloidal solutions of silicon dioxide, the use of synthetic sorbents (soluble and insoluble forms of polyvinylpyrrolidone, hydrolytic ferment preparations, treatment at cold temperature (0ºC -2ºC) for 12-16 hours and pasteurization at 70ºC for 15 min. Although some means ensure a poor reduction in patulin content in wine production by 32.0 to 37.5 %, the application of sorbents at low temperatures, as well as the use of enzyme preparations, removes 50 to 78 % of the patulin contained in the control samples. Complete removal of patulin was recorded by complex application of soluble and insoluble polyvinylpyrrolidone with bentonite and subsequent cold decantation [27].

3.4. Inhibition of mycotoxigenic fungi

Mycotoxin production can be inhibited in different ways, depending on the type of microorganisms used [28]. Biological control can be used directly against toxigenic fungi. Based on their antagonistic effect, it has been shown that contamination in the field was reduced by inoculation with a strain of Aspergillus flavus that does not produce aphlatoxins [29, 30].

Ample evidence of the beneficial effect of microbiological entities and their additional probiotic properties gives them significant potential for their application in food and feed production technologies, and they are also effective candidates as mycotoxin biotransforming bacteria [31]. Starting from the biological peculiarities of useful microorganisms applied in the control of a wide range of agricultural crops, it constitutes a consistent basis for their verification, development and application to reduce the damage caused by toxicogenic fungi.

The study of the role and capacities of microorganism species in inhibiting toxicogenic fungi demonstrated that Lactobacillus acidophilus, L. rhamnosus, L. sanfranciscens and L. plantarum have the greatest ability to remove ochratoxin from the fermentation environment. Thermally inactivated cells of the genus Lactobacillus were found to reduce ochratoxin content (46.2-59.8%) more effectively than live cells (16.9-35.1%). Due to cell surface changes, mycotoxin binding is permanent when the cells of useful microorganisms are dead (heat or acid treated), while live bacteria can release some of the mycotoxin content over time [32].

Significant results have been recorded in the research of antagonistic fungi of the genus Trichoderma (Trichoderma viride, T. polysporum, T. harzianum, T. hamatus, T. koningii), which produce extracellular enzymes and metabolize various substrates organically and manifest depolluting qualities of petroleum hydrocarbons, being used for the ecological recycling of polluted cenoses.

Several species of Trichoderma have demonstrated antagonistic properties against a significant range of phytopathogens, being used as environmentally harmless means of biological protection of plants. The antagonism of these species is determined by several factors and is also manifested in the fight against phytopathogenic agents, including toxigenic fungi [12].

The higher adsorption of ochratoxin by dead cells compared to viable cells can be explained by changes in the bacterial cell wall or by the hydrophobic nature of the bacterial cell wall [33]. Thermal or acid treatment of cells leads to protein denaturation and increased permeability of the outer layers of the cell wall [34]. As a result, a greater number of active sites are formed, which are responsible for the absorption of different compounds, which opens up obvious possibilities for biological reduction of the impact of mycotoxins on human, animal and environmental health.

4. Conclusions

Against the background of the expansion of the area of spread and resistance of fungal toxicogens and the need to apply ecologically harmless means of protecting wine crops, it becomes evident the need to deepen studies and develop strategies and systems for applying the means to reduce the impact of mycotoxins on humans, animals and the environment.

Toxicogenic crops and the products of their activity, especially mycotoxins spread to vines, show high resistance to environmental factors and increase their action under climate change conditions.

The reduction of the impact of mycotoxins on vines and wine products is recorded by the application of environmentally harmless technological processes and means aimed at the application of effective biological control of toxigenic mushroom strains, as well as the rapid elimination of mycotoxins from wine. This analysis shows that biological control is an important and effective tool for controlling the presence of mycotoxins in wine.

Various biological methods of decontamination and detoxification have been proposed and applied through technology transfer to reduce or avoid contamination of must and wine with mycotoxins.

Addressing the problems of toxicogenic entities requires further research into biological agents, which can prevent the emergence of mycotoxins or their safe removal from wine.

Reducing the impact of vine pathogens requires the development and application of integrated control systems with the application of effective agrotechnical, prophylactic and therapeutic means based on the achievements of modern agricultural biotechnologies.

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