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Principles of mycological safety of the use of forage plants cultivated in Azerbaijan

Anakhanum Yusifova, Sanubar Aslanova*, Basti Asadova

Azerbaijan State Pedagogical University, Faculty of Chemistry and Biology, Baku, Azerbaijan

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Abstract

Azerbaijan's natural climatic conditions support diverse and productive agriculture. Lowland and foothill areas favor irrigated farming, while mountainous regions are suitable for rain-fed agriculture and animal husbandry. The country has an ancient agricultural heritage, with archaeological evidence confirming its historical role in grain cultivation, viticulture, fruit and vegetable growing, and animal husbandry. Despite wars and internal conflicts, Azerbaijani agriculture has continuously developed, cultivating wheat, barley, and various food and fodder crops. Favorable climatic conditions also allow for floriculture and the production of essential oils. Recent studies on the mycobiota of cultivated plants in the Kur-Araz Lowland identified 112 fungal species, clarifying their ecotrophic relationships and impact on agrocenoses. Similarly, research in the Lankaran-Astara region revealed 85 fungal and fungus-like species, mostly anamorphic ascomycetes affecting crops such as wheat, barley, corn, and sugar beet. Common diseases recorded include fusariosis, spotting, rust, wilt, downy mildew, and septoria. Despite these studies, the total number of fungi species in Azerbaijan remains uncertain due to various challenges in fungal systematics. However, literature confirms 214 species of xylotrophic macromycetes, while estimates suggest the overall fungal diversity could reach 10,000 species. Further research is required to comprehensively document Azerbaijan's fungal biodiversity and its impact on agriculture.

***Corresponding Author:** Sanubar Aslanova ✉ aslanova17.02@gmail.com

Introduction

As is known, all living things are constantly in food and energy exchange with the environment in order to continue their life activities and participate in the ecological functions they perform in nature, and this is characteristic of both producers (plants), consumers (animals), and decomposers (fungi and bacteria) in an ecological sense (Beltrán-Peña and *et al*., 2023) However, the dependence of consumers, as well as decomposers, on the environment is a more sensitive feature than that of producers, since both of the latter, namely fungi and animals, are heterotrophic in terms of nutrition, that is, since they cannot carry out the photosynthesis process, their need for organic matter, as well as oxygen, is met by other sources, primarily plants. Since plants play an important role in providing food for most living things, their comprehensive study has always been relevant and continues to maintain this status in full force today (Yusifova *et al.,* 2024).

The increasing world population and the fact that this is happening on a stable Earth against the background of a decrease in the areas used by people for food purposes have faced humanity with a number of problems. These include issues such as the lack of food, energy, raw materials for industry, etc. If we add to these the global problems of a globalizing world, primarily global warming and biodiversity loss, then we can also note that the situation on Earth is not very encouraging. Among the problems mentioned, the problems related to the needs of the Earth's population, as well as other living beings, for food and feed are of particular importance. Thus, in order for people to live and function, they must constantly receive the necessary substances from the environment in order to meet their needs for food and energy. Today, serious problems are felt in this issue, that is, in providing people with quality food products. It would be appropriate to touch on some facts in this regard. According to the UN FAO, 783 million people are currently suffering from hunger, and this number is not likely to increase, but rather a reality.

According to various forecasts, the population of the Earth is expected to reach 9.3 billion in 2050, that is, the population is expected to increase by 1.33 times. In return for this increase, it will be necessary to increase the current production of agricultural products by about 1.5 times in order to meet the demand for food products of the people living on Earth. This forecast is calculated based on the products cultivated on the area of the Earth that people use today for the production of food products. If we add to this the lands that lose their suitability for cultivation due to salinization, desertification, etc. every year as a result of global warming, urbanization, etc. processes, then it will be necessary to increase the productivity per hectare even more (Abdelmagid and *et al.,* 2021; Dong *et al*., 2019).

According to our research, as well as literature data, the fungus causes powdery mildew disease in cereals, primarily barley, which is found on almost all above-ground organs of the plant.

Surface mycelia cause symptoms of the disease caused by conidial spores. Thus, conidia are formed on short, unbranched single or paired conidiophores in the upper cells, the length of which in the latter can be up to 90 µm and a width of up to 7 µm. The conidia formed there are colorless, unicellular and lemon-shaped, sometimes ellipsoidal. Conidia form chains. The fungus is heterothallic and occurs in equal numbers in natural populations. Cleistothecia are formed by the overlapping of their light-colored mycelium like tiles and can be up to 270 µm in diameter.

The appendages are located on the upper half of the cleistothecia, which are thick-walled, colorless, septate, and irregularly branched. The number of sacs located in the cleistothecia can be up to 30, which when fully formed are cylindrical in shape and each produces 8 colorless ascospores (Jabraylzadeh *et al.,* 2024; Nichols *et al.,* 2012; Yusifova *et al.,* 2025).

The disease caused by the fungus can be considered dangerous, since the yield loss due to its effects can reach 10-15%, and sometimes 30-35%.

Previously, studies conducted in the world and a small part of it, Azerbaijan, have determined the distribution of the fungus.

Materials and methods

Sampling and analysis were carried out in the following sequence:

1. Samples taken from fodder plants cultivated in agrocenoses and growing in natural cenoses were passported on site (place of collection, type of plant, organ from which the sample was taken, biological condition of the sampled plant, etc.) and placed in sterilized paper bags, delivered to the laboratory in a short time and prepared for analysis (Chatterjee *et al.,* 2016; Allison *et al.,* 2013; Yusifova *et al.,* 2025).

2. In the study of the removal of fungi that are likely to be present in the sample prepared for analysis into pure culture, the samples taken are either placed directly into standard nutrient media, or the water obtained from washing the area with sterile water, which is suspected of being infected with fungi, is transferred to SQM either directly or by diluting it. During the study, samples are taken, the inoculated nutrient media are placed in a thermostat with a temperature of 28°C and cultivated for 2-5 days. From time to time, the colonies formed in Petri dishes are thinned by transferring visually similar colored ones to new dishes containing nutrient media, and the process is continued until a homogeneous culture is obtained. The purity of the culture is controlled through a microscope, in which a microscope with a magnification of up to 2500 times is used. In order to identify the obtained pure cultures, cultural-morphological (macroscopic and microscopic signs), physiological signs, as well as growth coefficient (GCF) are determined.

During the research, nutrient media such as Saburo agar (SA), agarized malt juice (ASSH), agarized Chapek (AC), potato (KA) and rice (DA) agar were used to extract the fungi into pure culture. Preparation of nutrient media, sterilization and filtration into Petri dishes, as well as inoculation and cultivation with target materials were carried out according to known methods and approaches accepted in mycology.

Identification of fungi was carried out according to known determinants compiled based on their cultural-morphological and physiological characteristics. Clarification of fungal names and determination of their systematic affiliation were carried out according to the information provided on the official website of the International Association of Mycology and other relevant sources.

The frequency of occurrence of fungi and the prevalence of diseases caused by them recorded in the studies were determined according to the formula $P=(n/N)\times100$, where P is the frequency of occurrence (or prevalence) of the disease expressed in %, N is the total number of plants sampled (pcs), a is the number of fungi (or diseased plants) in the samples taken (pcs).

In the studies, the methods and approaches used in the works of various authors were used to determine the antimicrobial activity and chemical composition of some fodder plants.

During the research, the fungi grown in pure culture were also characterized by their growth rates, and in this case, the growth coefficient (GCD) was used, for the calculation of which the formula $GCD = DHS/T$ was used. Here, D is the diameter of the colony (in mm), H is the height of the colony (in mm), S is the density of the colony determined based on the visual image (from 1 to 5), T is the cultivation period (days).

The numerical composition of the fungi was determined based on the formula $A = a\times b\times c/d$, where a is the average number of colonies in the Petri dish, b is the amount of dilution, c is the initial weight of the sample, and d is the dry weight of the sample.

In order to obtain quantitative results in the research, all experiments were performed in at least 4 repetitions and the results obtained were statistically processed. For statistical analysis, the results were processed using the standard squared deviation and the degree of accuracy, and the results were considered accurate according to the formula S/M 0.05 and included in the dissertation.

Results and discussion

Regarding the presence of opportunistic fungi, or more precisely, opportunistic fungi, among the fungi recorded in forage plants in the studies, it should be noted that opportunistic fungi do not exhibit pathogenicity in most cases, but when favorable conditions arise, they can act as pathogens and even lead to fatal consequences. In this regard, among the most widespread diseases in recent times, those caused by opportunistic fungi are currently one of the sources of serious threat to the health of the population. One of the dangerous features of these fungi is that they can infect any part of the human body, spread to all organs with the blood, and are widely distributed in the environment. This last idea was confirmed in studies conducted on forage plants, as it became clear from our studies in this direction that there were also those corresponding to this characteristic among the fungi recorded. Interestingly, fungi with these characteristics belong to the anamorphs and zygomycetes of ascomycetes. As with allergens, in this case, according to the data specified in the literature, various species of the genera *Alternaria* (*A. alternata*), *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger* and *A. ochraceus*), *Cladosporium* (*C. cladosporoides* and *C. herbarum*), *Fusarium* (*F. moniliforme* and *F. oxysporium*), *Paecilomyces variotti*, *Penicillium* (*P. brevicompactum*, *P. chrysogenum*, *P. glabrum* and *P. purpurogenum*), *Trichoderma* (*T. harzianum* and *T. viride*) recorded in Azerbaijan's cultivated and forage plants have been confirmed in studies conducted in various centers to have characteristics typical of opportunistic pathogens. Opportunists are also found among zygomycetes, and some of the fungal species belonging to the genera *Mucor* (*M. circinelloides*, *M. plumbeus*, and *M. rasemosus*) and *Rhizopus* (*Rh. stolonifer*) recorded in our studies are known to have such characteristics. It is interesting that the division of fungi according to the forms of manifestation of ecolotrophic specialization is at first glance conditional, since, as it seems, the same type of fungus simultaneously belongs to toxins, allergens, and opportunistic pathogens (opportunists). In our opinion, this division is not due to the conditional nature of the division, but to the fact that fungi have properties that have a multifaceted effect. This, in turn, once again clearly confirms the need for greater caution in relation to fungi, and it would be appropriate to recall the idea that, willy-nilly, “mushrooms are the offspring of the devil, creatures created to disrupt the harmony of nature and confuse researchers.”

From the above, it became clear that the fungal species involved in the formation of the mycobiota of forage plants cultivated and growing in the territory of Azerbaijan are characterized by a wide diversity in terms of both ecotrophic and ecotrophic specialization, as well as their distribution on individual plants and RT, etc. properties, and they can even cause serious changes in the constituent elements of forage plants. Changes in the composition of forage plants are manifested either by a decrease in the natural constituent elements of that plant, or by the inclusion of specific substances that are not characteristic of them, and the effect of these is manifested in both its biochemical composition and biological value (for example, changes in digestibility).

Both of the latter are issues related to the biochemical composition and digestibility of forage plants, that is, the composition of the forage plant includes organic substances such as protein, hydrocarbon, fat, etc., as well as inorganic substances such as macro- and microelements. The change in their quantity, as well as the ratio of individual compounds, is one of the criteria that determine the quality of forage plants, more precisely, the nutritional value. Therefore, at this stage of the research, one of the tasks was to clarify how the mycobiota, as well as the forage plants themselves, affects the biochemical composition of forage plants.

For this purpose, samples were taken from the organs of the forage plants with different numbers of phytopathogenic fungi, and as a control, visually clean and healthy organs were analyzed for their chemical composition. Considering that this is too large a task to do on the sample of all plants, it was considered appropriate to conduct research in this direction on the basis of samples taken from alfalfa and clover. It became clear from the results obtained that the colonization of fungi in plants leads to a change in the biochemical composition of the studied forage plants, and the level of change is determined by both the number of fungi (Table 1) and the species composition of phytopathogens. First, about the results obtained regarding the number composition. As can be seen from the table, no difference is observed in the biochemical composition of the plant, as well as in the digestibility, when the number composition of mycobiota is ≤103 CFU/g compared to the control. Nevertheless, an increase in the number composition leads to a relative increase in the amount of proteins and fats, and a decrease in other parameters. At first glance, an increase in the amount of protein and fat may be interpreted as an increase in the nutritional value of forage crops, but a decrease in other parameters, primarily digestibility, suggests the opposite.

Table 1. Effect of the number of mycobiota (CFU/g dry weight) of forage crops on their chemical composition (%) and digestibility

	Sop's clover				Three-leaf clover			
	Control	$\leq 10^3$	$\geq 10^3$	10^4 and more	Control	$\leq 10^3$	$\geq 10^3$	10^4 and more
Protein	18,4	18,0	19,3	20,3	16,3	16,0	17,8	19,2
Oil	2,5	2,5	2,7	3,0	2,3	2,2	2,5	2,8
Cellulose	30,4	30,6	32,3	33,4	28,7	28,0	29,7	30,5
Nitrogen-free extractives	37,8	36,9	35,6	34,5	37,6	37,5	36,4	35,3
Ash (mineral substances)	8,6	8,3	8,0	8,1	7,6	7,4	7,3	7,4
Digestibility (in % relative to control)	100	99	94	90	100	100	95	91

It would be appropriate to touch on one point regarding the information given in the table, which is related to the moisture content of the fodder plants in their usable form. Thus, depending on the moisture content, the number composition of the plant's mycobiota changes. The moisture content of the alfalfa traditionally prepared for fodder is less than 20%, and in this case its number composition is less than 103 CFU/g. If the moisture content is higher than this, its storage for use causes an increase in the number composition, and if the moisture content is higher than 20%, the number composition also increases, and if it is higher than 50%, the number composition reaches its maximum. In such a state, the probability of mold growth of the plant increases, and the number composition of fungi inevitably reaches 104 and higher. Therefore, it is important to take this factor into account in this matter.

In order to clarify this issue in fodder plants with fungal disease symptoms, it was considered appropriate to divide the diseased carriers into 3 groups and conduct an assessment for each group. Accordingly, when evaluating the plants, the first group was used for plants with disease carriers caused by anamorphs (I), the second group was used for plants with disease carriers caused by telemorphs (II), and the third group was used for plants with disease carriers caused by basidiomycetes (III). The results obtained show a similar situation, more precisely, the plant with clearly expressed disease symptoms is accompanied by changes in both its constituent elements and digestibility, and in this case, the digestibility of the plant decreases (Table 2). As can be seen, this situation is observed as a result of diseases caused by anamorphs, telemorphs, and basidiomycetes, and the decrease in digestibility is most often accompanied by plants where anamorphs are widespread. The change under the influence of the other two groups is somewhat different, since the digestibility of alfalfa is reduced to a greater extent by the influence of telemorphs, and by the influence of basidiomycetes by the influence of trifoliate grass. In general, it should be noted that both the number of fungi and the diseases caused by them reduce the nutritional value of forage plants, at least due to their digestibility. This was also confirmed by the results of the studies devoted to the study of their separate effects above. When determining the combined effect of these two factors, it became clear that an increase in the number of mycobiota in forage plants with signs of disease leads to a further decrease in their digestibility, and in this case the maximum indicator can rise to 15%, and this is accompanied by a mycobiota count exceeding 104 in plants caused by anamorphs.

Table 2. Effect of the number of mycobiota of forage plants (CFU/g dry weight) on their chemical composition (%) and digestibility

	Sop's clover				Three-leaf clover			
	Control	I	II	III	Control	I	II	III
Protein	18,4	16,8	17,3	17,6	16,3	14,0	14,8	15,2
Oil	2,5	2,5	2,7	3,0	2,3	2,1	2,5	2,9
Cellulose	30,4	34,0	32,3	31,4	28,7	34,0	32,7	33,5
Nitrogen-free extractives	37,8	31,9	34,6	34,5	37,6	30,5	33,4	33,3
Ash (mineral substances)	8,6	7,3	8,0	8,1	7,6	6,4	6,9	7,1
Digestibility (in % relative to control)	100	91	93	94	100	89	95	93

Thus, fungi can be used as an effective criterion for determining the nutritional value of forage plants, both in terms of the diseases they cause and the numbers they inhabit in the plants they inhabit, and in developing principles for their safe use. Therefore, as an initial indicator of the mycological safety of forage plants, the number content should be lower than 103 CFU/g (by dry weight) and its moisture content should be around 15% during storage for use.

Finally, it would be appropriate to touch on an issue related to the recorded fungal species, which is related to the ecotrophic specialization of the recorded fungi. As is known, the basis of the ecotrophic relationships of fungi is the biological state of the source they use to meet their need for organic matter. More precisely, fungi can obtain organic matter either from living organisms, from the dead remains of living organisms, or from both of the above, and therefore it would be more logical to divide them into 3 groups in general, namely biotrophs, saprotrophs, and those that do not have a true biotrophy or saprotrophy character. The latter are sometimes characterized as polytrophs, and sometimes as saprotrophs. In this regard, when characterizing the fungi recorded on fodder plants, it becomes clear that the recorded fungi are characterized by diversity in this aspect as well, and all the fungi that correspond to the above-mentioned division are found among the recorded fungi. From this aspect, when the fungi recorded in the studies are characterized by both large taxa and species, it becomes clear that fungi such as *Absidia ramosa, Mucor circinelloides, Mucor globosum, M. hiemalis, M. racemosus M. mucedo, P. purpurogenum, Trichoderma asperellum, T. atroviride, T. harzianum* and *T. polysporum* belong to true saprotrophs and there is no literature information about their causing any pathology. Endophytes are also found among them. The number of true biotrophs is much greater than that of saprotrophs, as the number of species corresponding to this characteristic is 23 (*Eryshiphe betae, E. cichoracearum, E. communis, E. cruciferarum, E. pisi, E. trifolii, Puccinia artemisiicola, P. coronata, P. graminis, P. helianthi, P. hordei, P. recondita, Tilletia caries, Urocystis nigra, U. cepulae, U. tritici, Uromyces fabae, U. striatus, U. trifolii-repentis, Ustilago cynodontis, U. hordei, U. tritici* and *U. zea*). The number of those that do not have true saprotrophy and biotrophy characteristics is 2.3 times greater than the sum of both. These include

Alternaria alternata, A. solani, A. tenuissima, A. tenuis, Asc. betae, Asc. hordei, Asc. pisi, Asc. sojikota, Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochrceseus, A. ustus, A. versicolor, Blumeria graminis, Botrytis cinerea, Cephalosporium lecanii, Cercospora beticola, C. fabae, C. medicaginis, Cladosporium cladosporioides, C. herbarium, Claviceps purpurea, Cochliobolus heterostrophus, Colletotrichum gloesporioides, C. trifolii, Fusarium avenaceum, F. culmorum, F. gibbosum, F. moniliforme, F. oxysporium F. solani, F. semitectum, F. sporotrichioides, Gibberella fujikuroi, Monilia sitophila, Paecilomyces variotii, Penicillium brevicompactum, P. chrysogenum, P. expansum, P. glabrum, P. martensii, P. notatum, P. thomii, Phoma artemisiae, Ph. betae, Ph. destructiva, Ph. medicaginis, Puccinia cynodontis, Pyrenophora graminea, Ramularia betae, Ramularia medicaginis, Rhisobus stolonifer, Rhizoctonia solani, Sclerotina sclerotiorum, Sc. libertiana, Septoria helianthi, S. nodorum, S. pisi, S. sojina, S. trifoliorum, Septoria tritici, Stemphilium botryosum, Thielaviopsis basicola, T. viride, Trichothecim roseum, Verticillium albo-atrum, V. dahile and *V. nigrescen* belong to the nobs.

As for the larger, more specific divisional distribution of fungi, it became clear from the clarification that among the recorded fungi, saprotrophs mainly belong to the *Mucormycota*, facultatives to the Ascomycota, and true biotrophs to the Basidiomycota divisions. Expressing this numerically, it is clear that 54.5% of saprotrophs (*Absidia ramosa, Mucor circinelloides, Mucor globosum, M. hiemalis, M. racemosus M. mucedo* - 6 species in total) *Mucormycota*, 98.6% of facultatives (*Alternaria alternata, A. solani, A. tenuissima, A. tenuis, Asc. betae, Asc. hordei, Asc. pisi, Asc. sojikota, Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochrceseus, A. ustus, A. versicolor, Blumeria graminis, Botrytis cinerea, Cephalosporium lecanii, Cercospora beticola, C. fabae, C. medicaginis, Cladosporium cladosporioides, C. herbarium, Claviceps purpurea, Cochiliobolus heterostrophus, Colletotrichum gloesporioides, C. trifolii, Fusarium avenaceum, F. culmorum, F. gibbosum, F. moniliforme, F. oxysporium F. solani, F. semitectum, F. sporotrichioides, Gibberella fujikuroi, Monilia sitophila, Paecilomyces variotii, Penicillium brevicompactum, P. chrysogenum, P. expansum, P. glabrum, P. martensii, P. notatum, P. thomii, Phoma artemisiae, Ph. betae, Ph. destructiva, Ph. medicaginis, Puccinia cynodontis, Pyrenophora graminea, Ramularia betae, Ramularia medicaginis, Rhisobus stolonifer, Sclerotina sclerotiorum, Sc. libertiana, Septoria helianthi, S. nodorum, S. pisi, S. sojina, S. trifoliorum, Septoria tritici, Stemphilium botryosum, Thielaviopsis basicola, T. viride, Trichothecim roseum, Verticillium albo-atrum, V. dahile* and *V. nigrescen* - a total of 71 species) belong to the Ascomycota, and 73.9% of the true biotrophs (*Puccinia artemisiicola, P. coronata, P. graminis, P. helianthi, P. hordei, P. recondita, Tilletia caries, Urocystis nigra, U. cepulae, U. tritici, Uromyces fabae, U. striatus, U. trifolii-repentis, Ustilago cynodontis, U. hordei, U. tritici* and *U. zeae* - a total of 17 species) belong to the Basidiomycota divisions. It is interesting that species belonging to the Mucomycota division, biotrophs and facultatives, and to the Basidiomycota division, saprotrophs, were not found during the research.

Conclusion

Thus, it became clear from the studies that the number of mycobiota of cultivated and wild forage plants in Azerbaijan and the presence of signs of any disease are factors that affect their biochemical composition, or rather, cause changes in their quality indicators. Therefore, in order to ensure mycological safety during the use and storage of fodder plants, it is advisable that the permissible limit of the number of fungi in them is less than 103 CFU/g (dry weight), and that the humidity during their storage for use is around 15%, and that there are no signs of diseases caused by anamorph species of phytopathogenic fungi.

It was clear that the fungi *Bipolaris mayds* (Y.Nisik & C.Miyake) Shoemaker [=*Cochliobolus heterostrophus*

(Drechsler)Drechsler)], *Fusarium fujikuroi Nirenberg* [= *Gibberella fujikuroi* (Sawada)Wollenw] and *Diaporthe leptostromiformis* (J.G.Kuhn)Rossman & Udayanga [= *Phomopsis leptostromiforme* (J.G.Kuhn) Bubak], which are among the fungi involved in the formation of the general mycobiota of forage plants, are new to the mycobiota specific to Azerbaijan, and the recorded fungi are not distributed unevenly across individual plant species, which is reflected in their number of species varying between 9-23 species depending on the plant. Of the studied plants, *Artemisia vulg*aris is characterized by the poorest, and *Medicago sativa* is characterized by the richest mycobiota.

Phytopathogenic fungi recorded in the studies were classified into 3 groups (those with serious threat, those with potential threat, and species of unknown threat) according to their impact on plant productivity, and the first group included fungi such as *Alternaria alternata, A. solani, Botrytis cinerea, Fusarium oxysporium, F. moniliforme, F. solani, Trichotecum roseum, Vertisellium albo-atrum,* and *V. dahile*. The prevalence of these fungi on the studied plants varies between 52-88%. The prevalence of fungi (18 species) that are potential threat sources is 12-48%, and the prevalence of diseases caused by fungi belonging to the last group (48 species) is 4-8%.

It has been determined that the number of fungi recorded in fodder plants and their infection with one or another disease cause changes in their biochemical composition and digestibility, which leads to the highest negative change in the number of fungi in plants with diseases caused by anamorphic fungi when the number of fungi is 104 CFU/g (dry weight).

In order to ensure mycological safety when using feed plants, the permissible number of fungi should be less than 103 CFU/g (dry weight), and during their storage for use, it is advisable to have a moisture content of around 15%, and to avoid signs of diseases caused by anamorph species of phytopathogenic fungi.

Agrocenoses used for the cultivation of fodder crops should be analyzed from a mycological aspect before sowing, and it is advisable to cultivate plants in accordance with their phytosanitary condition. Measures aimed at improving the phytosanitary condition of agrocenoses where fungi, which are a source of serious danger, are spread, primarily using biological control methods, are appropriate in terms of preventing a decrease in the productivity of fodder crops.

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