Selection of methods for sterilization of *C. purpurea* sclerotia for isolation of rye biosecurity agents

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**Abstract.** Winter rye is a traditionally cultivated grain crop that is susceptible to ergot, a serious disease of the Poaceae family, caused by *C. purpurea*. Along with agrotechnical, organizational, and economic methods, biological control of ergot in grain crops is one of the ways to combat this disease. To isolate *C. purpurea* hyperparasites in a pure culture, the optimal sterilization options were selected, which were to exclude the infection of ergot sclerotia with saprophytic microflora. Before sterilization, *C. purpurea* were additionally washed in running water with the addition of SAS for an hour. 70% and 96% ethanol, bleaching agent “Belizma” (Whiteness) were used as sterilizing agents. The exposure time varied from 1 to 12 minutes. Sclerotia were cultivated on potato sucrose agar at 24 °C. The options for sterilizing sclerotia for 10 and 12 minutes in the “Belizma” bleaching agent were recognized as the best.

1. **Introduction**

Winter rye is the most important unpretentious cereal crop. It has an important role in the agriculture of countries of northern and central Europe, the main production of winter rye is concentrated in Russia, Poland, Germany, Belarus, which account for more than 70% of the total world harvest of its grain. More than a third of all crops are concentrated in Russia, where 1/4 of the gross grain harvest of this crop in the world is produced [1]. However, rye does not always provide grain stability because of some disturbance of agrotechnical measures, as well as disease damage [2].

Common on the crops of winter rye, ergot of cereals is caused by the micromycete *Claviceps purpurea*, the first phytopathogenic causative agent of the human disease. The disease caused by poisoning with toxic substances of ergot (ergotism) has had a serious impact on the history of peoples and entire states, as well as the development of agriculture. Information about epidemics and deaths of people from this disease has been known since 945. In the 16th century, it was established that the cause of the disease is fungal sclerotia that gets into grain products. Alkaloids with a strong nerve effect were found in sclerotia [3-5].

It should be noted that in recent years in a number of regions of Russia there has been an increase in the infestation of grain crops with ergot.

To date, there are no varieties of grain crops resistant to ergot in production. The main role in the fight against the pathogen, no doubt, belongs to agrotechnical, organizational, and economic measures, the correct and strict observance of which allows you to successfully fight it. With regard to chemical control measures, seed dressers do not provide complete protection against ergot. their biological
efficiency is low, and the proportion of sclerotia in the seed is very small [6, 7]. Therefore, the task of integrated protection of cereals, in particular rye, from ergot, using both fungicides and biological products based on C. purpurea antagonists, is still urgent. Fungi of the genera Fusarium, Trichoderma, Clonostachys, and others can act as bioagents for plant protection against ergot [8-13].

When isolating potential agents of bioprotection of rye from ergot into a pure culture, it is necessary to exclude superficial contamination of sclerotia by saprophytic microflora. In this regard, the aim of this study was to evaluate the effectiveness of using various methods of sterilization of C. purpurea sclerotia.

2. Materials and results
The research was carried out in the laboratory of physiology and biotechnology of the Krasnoyarsk Research Institute of Agriculture. 70% and 96% ethanol, bleaching agent "Belizna" were used as sterilizing agents. The concentration of sodium hypochlorite in it according to TU 2382-106-70864601-2007 is from 5 to 15%. To remove surface contamination, the collected sclerotia were washed in the running water for an hour with the addition of SAS. Then the ergot horns (Secale Cornutum) were immersed in sterilizing substances with different exposures, followed by washing in 3 portions of sterile distilled water for 5 minutes each (table 1).

| №  | Option            | Exposure, min |
|----|-------------------|---------------|
| 1  | 70% ethanol       | 1             |
| 2  | 96% ethanol       | 1             |
| 3  | "Belizna"         | 1             |
| 4  | "Belizna"         | 2             |
| 5  | "Belizna"         | 3             |
| 6  | "Belizna"         | 4             |
| 7  | "Belizna"         | 6             |
| 8  | "Belizna"         | 8             |
| 9  | "Belizna"         | 10            |
| 10 | "Belizna"         | 12            |

Then sclerotia were spread through the flame of an alcohol lamp in Petri dishes on the surface of potato sucrose agar (PSA) with the addition of Triton X-100 detergent and antibiotic solution to the medium. On the 3rd day of cultivation at a temperature of 24C, the microflora began to be analyzed.

The most effective options for isolating C. purpurea hyperparasites into a pure culture were those with sterilization of sclerotia for 10 and 12 minutes in the Whiteness bleaching agent (Figure 1). Colonies of micromycetes on the surface of the PSA did not merge, they were isolated. There was practically no bacterial growth. Figure 1a shows a vivid example of hyperparasitic activity of a fungus of the genus Fusarium against C. purpurea. Its active growth was noted on ergot sclerotia. This isolate, among the same active micromycetes, was screened out for more specific identification and further studies of its properties.

The persistence of the viability of the rye ergot pathogen after exposure to "Belizna" in variants 9 and 10 can be judged by the growth of colonies of its conidial stage Spacelia segetum around sclerotia (figure 1b).
Figure 1. Infection of sclerotia with C. purpurea (after surface sterilization with “Belizna”, 7 days on PSA): a - 10 min, b - 12 min.

When the ergot horns were exposed in "Belizna" for 1 to 8 minutes (options 3-8), the surface sterilization was incomplete. Bacterial growth, colonies of representatives of saprophytic mycoflora were observed in Petri dishes (figure 2).

Figure 2. Infection of sclerotia with C. purpurea (after surface sterilization with Belize, 4 days on PSA): a - 1 min, b - 6 min.

In phytoexamination, ethyl alcohol is often used for surface treatment of seeds [14-15]. Sterilization of sclerotia in C2H5OH of different concentrations had no effect. In the variant with 96% antiseptic, strong bacterial growth was noted, and 70% ethanol did not ensure the absence of so-called storage molds on sclerotia (figure 3). Additional experiments on increasing the exposure time in
ethanol made it possible to draw a conclusion about the aggressive effect of the substance on the dense outer membrane and the inner contents of the sclerotia.

Figure 3. Infection of sclerotia with C. purpurea (after surface sterilization with ethanol for 1 min, 4 days on PSA): a - 70% ethanol, b - 96% ethanol.

3. Conclusion
In the course of the experiment, it was found that variants with 10 and 12 minutes exposure in the “Belizna” bleaching agent are optimal for sterilization of C. purpurea sclerotia as sources of potential agents for biological protection of rye from ergot.

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