Conditions for surface cultivation on liquid nutrient media of the producer strain of microbiopreparation M-24 *Penicillium* sp., the antagonist of the sunflower Phoma rot pathogen

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**Abstract.** In recent years (2019-2021), research has been carried out at the biomethod laboratory of the V.S. Pustovoit All-Russian Research Institute of Oil Crops on the development of environmentally safe microbiopreparations of a multifunctional type of action with a prolonged shelf life against the pathogen of sunflower Phoma rot caused by the fungus *Phoma macdonaldii* Boerema, a teleomorph is *Plenodomus lindquistii* Gruyter, Aveskamp & Verkley. To develop the elements of the technological regulations for the production of a microbiopreparations in the preparative form of a “wettable powder” when growing the producer strain M-24 *Penicillium* sp. on the liquid nutrient media, there were determined the following optimal conditions for cultivating the strain: temperature is 25 °C, pH is 6; to use starch as a source of carbon nutrition is advisable; the optimal source of nitrogen nutrition is corn extract.

1. **Introduction**

The sunflower Phoma rot is caused by the fungus *Phoma macdonaldii* Boerema [1], the teleomorph is *Leptosphaeria lindquistii* Frezzi [2], the current name is *Plenodomus lindquistii* Gruyter, Aveskamp & Verkley [3]. The disease is spread in many countries of the world: Hungary, Romania, Bulgaria, Italy, France, Argentina, USA, Serbia, Canada, Australia, and China, on the territory of the former Yugoslavia [4-8]. In Russia, over the past 15 years, Phoma rot on sunflower has become an economically significant crop disease [9].

Currently, agriculture in the world and in Russia is aimed at environmentally safe plant protection including the microbiological method [10-12]. At the beginning of the development of a microbiological method, after the identification of promising producer strains of microbiopreparations, the conditions of their cultivation are studied [13, 14].

At the biomethod laboratory of the V.S. Pustovoit All-Russian Research Institute of Oil Crops, one of the few in Russia, microbiological products of oil crops protection from diseases are developed and studied [15]. Particular attention is paid to the development of the microbiopreparations in a preparative form of a “wettable powder” (WP) with a prolonged shelf life during surface cultivation on the liquid nutrient media, which is especially important for fungal producer strains.

During the development of a microbiological method of decreasing harmfulness of Phoma rot on sunflower, a gradual screening of antagonist strains from the laboratory’s collection was carried out, as a result of which the most promising producer strains of microbiopreparations were identified [16].
The purpose of this work was to select conditions for surface cultivation of the fungal producer strain M-24 *Penicillium* sp. to develop technological regulations for the production of a microbiopreparations in a WP preparative form.

2. Materials and methods
The physiological characteristics of the active strain were studied on a liquid Czapek medium [17]. Surface cultivation was carried out for 10 days in Erlenmeyer flasks (250 ml) with medium volume of 100 ml by sowing the same agar block with the mycelium of the antagonist strain, replacing the sources of carbon and nitrogen nutrition. The sources of carbon nutrition were starch, glucose, sucrose, mannitol; the sources of nitrogen nutrition were thiourea, carbonate and ammonium chloride, corn extract, urea. The optimum temperature for cultivating the strain was studied on Rudakov’s medium [18] at temperatures of 20, 25, 30 and 35 °C. The selection of the optimal medium acidity was carried out by growing the strain on Rudakov’s medium at the optimum temperature with the addition of citric acid or alkali. The medium pH was set within 3.0, 6.0, 8.0 and 10.0.

We recorded the fungus growth on liquid nutrient media according to the scale that we developed: 0 – no growth, or only the fouling of a sowing block; 1 – growth of mycelium in separate colonies; 2 – mycelial film is confluent, plicate, thick; 3 – mycelial film is confluent, plicate, thick with partial setting deep into the medium.

At the end of cultivation, the dry residue of the fungal weight after drying was determined at a temperature of 105 °C to constant weight. The number of replications in each experiment was three.

3. Results and discussion
To develop technological regulations for the production of a microbiopreparations in a WP preparative form there were determined the optimal conditions for cultivation of the producer strain M-24 *Penicillium* sp. during the surface cultivation on liquid nutrient media.

The effect of temperature and medium reactions on the growth of the producer strain of the microbiopreparation M-24 *Penicillium* sp. during the surface cultivation on liquid nutrient media

The mycelium growth of the fungus M-24 *Penicillium* sp. on liquid nutrient media was evaluated by points according to the scale that we developed (figure 1).

![Figure 1](image_url)

**Figure 1.** The growth of the strain M-24 *Penicillium* sp. on liquid nutrient media by points (orig.).

The optimum temperature and medium reaction for the cultivation of the strain M-24 *Penicillium* sp. were determined on Rudakov’s medium (table 1).

The most favorable conditions for the growth of mycelium and sporulation is the cultivation of the fungus at a temperature of 25 °C, at which the maximum mycelial film (3 points) and the weight of dry mycelium (1.63 g per 100 ml of nutrient medium) were noted.
The strain M-24 Penicillium sp. depended on the medium reactions. The surface cultivation of the fungus on the Rudakov’s medium at 6 pH provided the maximum growth of mycelium with sporulation and the largest dry weight. The determination of optimal nutrients for surface cultivation of the strain M-24 Penicillium sp. on liquid nutrient media

The presence of starch in the nutrient medium ensured the maximum mycelium growth (3 points) and an increase in its dry weight during the surface cultivation of the fungus M-24 Penicillium sp. on Czapek medium at a temperature of 25 °C with the addition of various carbon sources (table 2).

The corn extract caused to the biggest growth of the fungus mycelium (3 points) and the maximum dry weight (0.47 g) in comparison with other variants.

Thus, the optimal conditions for the cultivation of strain M-24 Penicillium sp. are the temperature of 25 °C, pH of 6; to use starch as a source of carbon nutrition is advisable; the optimal source of nitrogen nutrition is corn extract.

4. Conclusion
To develop the elements of the technological regulations for the production of a microbiopreparation in the preparative form of a “wettable powder” during the surface cultivation of the producer strain M-24 Penicillium sp. on liquid nutrient media, there were determined the following optimal conditions for

| Table 1. The effect of temperature and medium reactions on the growth of the producer strain of the microbiopreparation M-24 Penicillium sp. during the surface cultivation on Rudakov’s medium (Krasnodar, V.S. Pustovoit All-Russian Research Institute of Oil Crops, 2019). |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Temperature, °C             | Temperature, °C             | Temperature, °C             | Temperature, °C             |
|                            | 20                          | 25                          | 30                          | 35                          |
| Mycelium growth, point      | 3/1.54 ± 0.02               | 3/1.63 ± 0.16               | 3/1.44 ± 0.03               | 0/0.33 ± 0.26               |
| The dry mycelium weight, g  | 3/1.58 ± 0.1                | 3/1.78 ± 0.13               | 2/1.33 ± 0.08               | 2/1.39 ± 0.08               |

*Mycelium growth, point.
*The weight of dry mycelium per 100 ml of nutrient medium.

| Carbon source       | Starch              | Glucose             | Sucrose             | Mannitol            |
|---------------------|---------------------|---------------------|---------------------|---------------------|
|                     | 3/0.43 ± 0.11       | 2/0.26 ± 0.05       | 1/0.16 ± 0.02       | 1/0.06 ± 0.03       |

*The weight of dry mycelium per 100 ml of nutrient medium.

| Nitrogen source     | Thiourea            | NH₄NO₃             | NH₄Cl              | Corn extract        | Urea               |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                     | 1/0.06 ± 0.03       | 2/0.23 ± 0.02       | 2/0.24 ± 0.05       | 3/0.47 ± 0.06       | 2/0.30 ± 0.07       |

*The weight of dry mycelium per 100 ml of nutrient medium.
cultivating the strain: temperature of 25 °C, pH of 6; starch as a source of carbon nutrition; corn extract as the optimal source of nitrogen nutrition.

**Acknowledgements**

This research was carried out with the financial support of a grant from the Russian Federation for Basic research and the Administration of the Krasnodar region r_Nastavnik No 19-416-235003.

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