Breeding of entomopathogenic fungi in different substrates

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Abstract. The issue of entomopathogenic fungi's prodigious rate of reproduction plagues both academic research and clinical practice. Several production technologies and preparations based on them are currently available. These medications appear to be liquid, powdery, dry, and grainy. The selection of nutritional medium and substrates, as well as the identification of ideal breeding conditions, are crucial steps in the manufacturing of pesticide formulations. Making preparations based on fungus is successful when solid nutritional substrates are used. Because in solid nutritional medium, fungus develop more spores. This article presents the results of experiments on the selection of nutrient substrates EMV-71 for the cultivation of strains of *P. varioti* EMR-57 and *B. bassiana*, which have been found to be virulent against spider mites and aphids, for use against sucking pests of vegetable crops in greenhouses. Barley grain has been found to be the most effective food substrate for the cultivation of entomopathogenic fungal strains and obtaining large numbers of spores from them.
Keywords. Entomopathogen, fungus, substrate, insect, strain, pest, aphid, spore, *P. varioti*, *B. bassiana*.

1 Introduction

The use of entomopathogenic fungi, which do not harm the environmental biocenosis, has a special place against sucking pests of vegetable crops in the greenhouse [1, 2]. Entomopathogenic fungi, which are considered pathogens of insects, have the ability to grow in various nutrient media and substrates [3-5].

The study and practice of entomopathogenic fungi is always faced with the problem of their large number of reproduction [5-8]. Currently, there are a number of production technologies of preparations based on them [2, 9, 10]. These preparations have a liquid, powdery, dry and grainy appearance. In the production of preparations used against pests, it is very important to choose the nutrient medium and substrates, to find the correct breeding conditions. The use of solid nutrient substrates is effective in creating preparations based on fungi. Because fungi produce more spores in solid nutrient media [5, 8, 11-13]. Such feeds can include grain substrates and agar media. Entomopathogenic fungi multiply on wheat,

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barley, oats, corn and other grains with solid nutrient media. Korol created a method for the production of the "Boverin" drug by growing B bassiana fungus on barley residue and potato broth, which was considered a waste during trichogram reproduction in biolaboratory conditions [3, 4]. Due to the porous nature of this barley residue, air circulates well, and the dead remains of insect larvae and butterflies in it enrich the feed with chitin. This increased the virulence of the entomopathogenic fungus.

Using the same waste, Mikulskaya achieved a 3.3-3.4 times increase in the biomass of the fungus *B. bassiana* by adding 3 g of $NaNO_3$ and NH_4NO_3 per 1 kg, as well as 50-100 g of the dead butterfly of the grain moth [7, 9]. At the same time, the titer of the biopreparation was $3.1 \cdot 10^9$ cfu/mL, and this indicator remained almost unchanged.

Levchenko conducted experiments on the reproduction of entomopathogenic hyphomycetes *B. bassiana*, *B. brongniartii*, and *Metarhizium anisopliae* fungi in wheat groats, rice, and peas [1, 6]. It was found that the composition of nutrient media, breeding conditions affect the virulence, productivity, spore formation, biomass release and other characteristics of entomopathogenic fungi. Studying the biological characteristics of *B. bassiana* fungus when it is grown in different nutrient media is important for the correct selection of nutrient media in the creation of a biopreparation.

2 Materials and methods

For the cultivation of *P. varioti* EMR-57 and *B. bassiana* EMV-71 strains on grain substrates, 2/3 volume of grain substrate was placed in 3-liter jars. Also, their mouths were covered with high-temperature-resistant cellophane films and tightly sealed with rubber rings.

These jars were then placed in an autoclave for sterilization. Sterilization in an autoclave was carried out for 1.5 hours at a temperature of 120 °C and a pressure of 2 atm. Sterilized 3 L jars were cooled to 25 °C and placed in boxes for seed mycelium planting [14, 15].

Strains *P. variotis* EMR-57 and *B. bassiana* EMV-71 grown in tubes containing agar wort medium were introduced into each jar in a pea-sized piece using a bacterial looper in front of an alcohol lamp flame. Then the jars with fungal mycelium were kept in thermostats at a temperature of 24-25 °C. Every 7 days, jars planted with cereal substrate were monitored and their growth was recorded.

At the next stage of our experiment, a suspension consisting of mycelia and conidia of entomopathogenic fungi was prepared by mixing 1 g of the substrate of fungi grown in grains at a temperature of 24-26 °C with 100 ml of distilled water, and their titer of cfu/mL was determined using a Goryaev chamber.

Spores were detected by inoculating suspensions prepared from specimens on the glass surface of a live Chapek agar nutrient medium. The samples were placed in a sterile Petri dish and stored in thermostats with a temperature of 24-26 °C. The number of germinated spores was counted after one day [11, 12].

3 Results and discussion

EMR-57 and EMV-71 strains were planted in jars with cereal substrate prepared in the above methods and their growth was monitored for 7 days. As can be seen from Table 1, the best result was observed in the experimental variant where barley was used. The barley grain substrate prepared in this variant was distinguished by its sterile and high-quality, economical autoclaving process, and grain scattering. The main reason for such effective results can be that the surface of the barley grain is covered with a thick skin. Bacterial

infection was observed in wheat and millet grains in this regime. To ensure the sterility of these grains, the pressure of the autoclave is raised to 2 atmospheres or kept in it for 2 hours. This led to a negative impact on the structure of the substances contained in the grains. As a result, their graininess was not so great, and the growth of mycelium worsened due to the caramelization of sugar.

Table 1. Influence of the type of grain used for the substrate and the autoclave mode on the reproduction of EMR-57 and EMV-71 strains (Laboratory of the Department of Plant Protection, TSAU).

Autoclave mode	Structural morphological feature of grain substrate	Mycelial coverage in 7 days, %			Presence of infection		
		Wheat	Millet	Barley	Wheat	Millet	Barley
2 atm\ 2 hrs	Grain raw materials are crushed, almost non-granular sugars are starchy	10	10	20	sterile	sterile	sterile
2 atm\ 1.5 hrs	Millet, barley is almost non-grainy wheat and wheat groats	80	10	100	sterile	sterile	sterile
2 atm\ 1 hr	In wheat and wheat groats, the grain is almost non-granular	80	10	100	infected	infected	sterile
1.5 atm\ 2 hrs	Raw grains are crushed and not so grainy, the sugar content is caramelized	50	60	80	sterile	sterile	sterile
1 atm\ 1 hr	-	20	25	90	infected	infected	infected

In order to prevent contamination of cereal substrates with microorganisms, where entomopathogenic fungi are propagated, their effective rate was determined by adding antibiotics to the water used for boiling these cereal substrates (Table 2).

If 100 mg and 200 mg of ciprofloxacin antibiotic were added to 10 L of water, grain substrates were contaminated with microorganisms, while in the variant with 300 mg added, it was found that only bacteria caused the contamination. No contamination was observed in the 400 mg and 500 mg added variants.

It was found that 400 mg of the antibiotic ciprofloxacin per 10 L of water for the preparation of grain substrate is the optimal norm for the reproduction of entomopathogenic fungi.

The best results were observed in the variant of entomopathogenic fungal strains propagated in barley. It was noted that the highest spore titer was $6 \cdot 10^7$ cfu/mL in strain EMR-57 and $9.1 \cdot 10^7$ cfu/mL in strain EMV-71. The lowest fungal spore titer was observed in the varieties planted on wheat groats and wheat grain, and this indicator was $1.3 \cdot 10^7$ cfu/mL, $2.3 \cdot 10^7$ cfu/mL and $2.2 \cdot 10^7$ cfu/mL, and $3.4 \cdot 10^7$ cfu/mL.

In all variants, the spore titer of entomopathogenic fungi strains propagated in barley was higher compared to the standard (Boverin zernovoy - BL) variant. But it was noted that the number of dead insects was lower than the standard variant in all variants except the variant of EMR-57 propagated in barley.

Table 2. Rate of consumption of the antibiotic ciprofloxacin added against foreign microorganisms in the grain substrate where entomopathogenic fungi are propagated (Laboratory of the Department of Plant Protection, TSAU).

Experimental variants with added antibiotics, mg per 10 l of water	Number of 0.5 l bottles with barley	Number of bottles contaminated with microorganisms					
		Total number of contaminated bottles		Contaminated with bacteria		Contaminated with fungi	
		#	%	#	%	#	%
100	10	3	30	2	20	1	10
200	10	2	20	1	10	1	10
300	10	1	10	-	-	1	10
400	10	-	-	-	-	-	-
500	10	-	-	-	-	-	-
Control (no antibiotics)	10	40	40	3	30	-	10

During the implementation of these experiments, the effect on the viability of spores of entomopathogenic fungi propagated in different nutrient media was also studied. For this purpose, 2 months after the fungal strains were planted on the nutrient substrates, samples were taken and examined. According to the obtained data, the highest number of live spores of strains EMR-57 of *P. varioti* and EMV-71 of *B. bassiana* was observed in strains grown on barley (EMR-57-94.1%, EMV-71-91.3%). The lowest number of live spores was recorded in wheat groats (EMR-57-64.2%; EMV-71-51.3%). This indicator was EMR-57-83.5%, EMV-71-78.1% in millet, and EMR-57-72.8%, EMV-71-70.0% in wheat. It was found that it was 77.9% in the ethanol.

4 Conclusions

Barley grain was found to be the most efficient nutrient substrate for the cultivation of entomopathogenic fungal strains and obtaining large numbers of spores from them. It was noted that adding 400 mg of the antibiotic ciprofloxacin to 10 L of water in which grain is boiled is the optimal norm for preventing the contamination of the barley grain feed substrate with microorganisms. It was found that the spore titer of entomopathogenic fungi propagated in barley was $6 \cdot 10^7$ cfu/mL in strain EMR-57 of P varioti and $9.1 \cdot 10^7$ cfu/mL in strain EMV-71 of *B. bassiana*.

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