Requirements for strains in the production of entomopathogenic preparations

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Abstract: *Entomopathogenic fungi Beauveria bassiana (Vuill) play an important role in controlling insect pests. In the investigations 37 strains have been used through isolation from natural population of B.bassiana fungus which in its turn was isolated from infested and dead samples of sucking pests of greenhouses. These strains of entomophatogenic fungus were grown under sterile condition in Petri plate and test-tubes with nutrient media. Abundant and fast formation of entomopathogen fungus conidia was observed mainly in the strains inoculated potato peptone nutrient medium. It was identified that conidia titer that was formed in B.bassiana fungus in nutrient media showed 32,4·106 cfu/ml in beer wort, in Chapek 15,7·106 cfu/ml and in potato peptone 68,9·106 cfu/ml.*

Key Words: *Entomopathogenic fungi, Beauveria bassiana, sucking pests, inoculation, nutrient medium, conidia titer.*

1. INTRODUCTION:

Among entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. species differs from insects with its range of pathogenicity peculiarities. Therefore, the preparations that are made on the basis of fungi against pests, are mainly produced from *Beauveria* fungus species strains [2].

One of prior tasks of preparation production with high effectiveness is an implementation of strain production that is isolated from natural population of fungi, forming a lot of spores and fast-growing [1].

Beauveria family representatives' virulence doesn't always coincide with their conidia forming rate, however, there have been views about the use of multi-spore bearing strains [2, 3].

2. MATERIALS AND METHODS:

In the investigations 37 strains have been used through isolation from natural population of *B.bassiana* fungus which in its turn was isolated from infested and dead samples of sucking pests of greenhouses.

These strains of entomophatogenic fungus were grown under sterile condition in Petri plate and test-tubes with nutrient media such as *agar – Chapek, beer wort* and *Potato-peptone-glucose agar*. pH value of these nutrient media was around 4,6-6,7.

The growth of fungus mycelium, conidium nodes and formation of new conidia were performed by observing with microscope the samples taken from agar nutrient media to the sterile glass object.

The rate of speed conidia forming of fungus was calculated with Goryayev camera. Its virulence was noted according to its influence on imago of greenhouse whitefly.

3. RESULTS AND DISCUSSION:

For the selection of entomophatogenic strains, their intensive growth, conidia forming rate, their development and virulence have been considered as a main measure. Besides, the changes of morphologic-cultural traits were studied while growing strains in various nutrient media.

In the results of observations it was noted that their conidia started formation after 24 hours from inoculating time in nutrient media. From the second day germinated conidia length made 25 *mkm* and conidium nodes formation also was observed.

While observing the conidia nodes with microscope from the third day 1-2 young conidia were formed and their formation rate increased towards the next days. In the result of abundant formation of several whorls of branches conidia nodes and fast development of conidia in them, the form of fungus colonies in nutrient media became strewed instead of dense by the 15th day.

After inoculating strains of *B.bassiana* fungus in nutrient media, the growth of their mycelia and formation of conidia rate were observed during 25 days.

Abundant and fast formation of entomopathogenic fungus conidia was observed mainly in the strains inoculated Potato-peptone-glucose agar nutrient media. In initial period the intergrowth rate difference of strains of these nutrient media wasn't almost noted by the end of 25 days period.

It was identified that conidia titer that was formed in *B.bassiana* fungus in nutrient media showed $32,4 \cdot 10^6$ cfu/ml (cfu- colony-forming units) in *beer wort*, in *Chapek agar* - $15,7 \cdot 10^6$ cfu/ml and in *Potato-peptone-glucose agar* - $68,9 \cdot 10^6$ cfu/ml (Table 1).

Table 1

***Beauveria bassiana* fungus growth in nutrient media and formation of conidia**

№	Nutrient media	Conidia titer, cfu/ml (colony forming unit/ml)			Average	Colonies diameter, mm			Average
		Repetitions				Repetitions			
		1	2	3		1	2	3	
1	Agar beer wort	$33,7\ 10^6$	$29,2\ 10^6$	$34,3\ 10^6$	$32,4\ 10^6$	78,4	73,9	72,3	74,6
2	Agar Chapek	$13,5\ 10^6$	$17,3\ 10^6$	$16,5\ 10^6$	$15,7\ 10^6$	66,5	71,8	75,4	71,2
3	Potato Peptone Glucose agar	$62,8\ 10^6$	$73,3\ 10^6$	$70,6\ 10^6$	$68,9\ 10^6$	73,9	77,1	79,5	76,8

The diameter of colonies made by strains of experiment in various nutrient media doesn't differ from each other. Mean diameter of colonies in beer wort nutrient media consisted 74,6 mm, in Chapek 71,2 mm, in Potato-peptone-glucose agar 76,8 mm. Furthermore, the strains grown in these nutrient media differ from each other by their cultural traits, that is, the colonies made by them are specific for each nutrient media. In Potato-peptone-glucose agar nutrient media fungus strains grew fast, and in initial stage of development was formed conidia layer. In Chapek agar nutrient media the growth of strain mycelium occurred slowly and the density of layer made by conidia occurred less. In nutrient media prepared from beer wort the formation of conidia layer of strains occurred intensively, but the rate of conidia formation went more slowly than in Potato-peptone-glucose agar nutrient media.

The strains separated from *B.bassiana* fungus nature presented the highest results on all indications in Potato-peptone-glucose agar nutrient media among other nutrient media which were prepared for experiment.

4. CONCLUSION:

Considering the results obtained from the experiments it can be concluded that in order to produce entomophatogenic biological preparations on the base of *B.bassiana* fungus for the selection of strains the conidia titer which were formed by them should be taken as a main measure. Furthermore, growth rate of strains in agar nutrient media, fast formation of conidia made by them, existence of dense conidia layer in colonies and virulence peculiarities are also to be taken as measure. Besides, creases shouldn't be there in the colony formed by strains.

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