



DOI 10.32900/2312-8402-2024-131-116-127

UDC 658.562:632

STUDY OF THE EFFICIENCY AND COMPARATIVE ANALYSIS OF THE APPLICATION OF TECHNIQUES FOR EVALUATING THE QUALITY OF PHYTOPHAGES AND ENTOMOPHAGES AT DIFFERENT STAGES OF ONTOGENESIS

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*The concept of quality control of insect crops is proposed, which is based on a targeted approach based on the goal and objectives of the implementation of a certain breeding program and is divided into two main directions: control of phytophage production, control of entomophage production. Quality control methods are based on the general biological rule of dependence between the intensity of insect taxis and the overall viability of biomaterial. The effectiveness of methods for assessing the quality of phytophages (on the example of the mother cultures of the grain moth *Sitotroga cerealella*, the wax moth *Galleria mellonella*) and entomophages (laboratory and natural populations of *P. bioculatus*.) of the Center for Mother Cultures of Insects Engineering and Technological Institute "Biotekhnica" of the National Academy of Sciences (ETI "Biotekhnica") at different stages of ontogenesis was investigated using the indicator the intensity of taxis.*

As a result of research conducted on the culture of phytophagous, grain moth, a significant difference with the control was established in the indicators of viability of eggs, larvae, pupae and adults: 25; 6; 33 and 11%, respectively. Studies conducted with another phytophagous representative - the wax firefly - also showed positive results (16; 17; 16; 11%, respectively). The obtained (as a result of preliminary quality control) and selection culture had significantly higher viability indicators at all stages of ontogenesis.

*It was established that the intensity of chemotaxis of larvae of the 1st age of entomophages directly correlates with the indicator of viability of the insect culture. When selecting the starting material for successful culture, it is possible to select by chemotaxis of the larvae after rebirth from the egg. Thus, viability when selecting for Colorado beetle eggs significantly ($p < 0.01$) exceeds this indicator in other selection options - Colorado beetle larvae and wax firefly larvae: 82; 59; 73% respectively. When working with an artificial population of *P. bioculatus*, on wax moth larvae, the highest intensity of taxis was noted in the variant with wax moth larvae compared to Colorado beetle eggs and Colorado beetle larvae - 37; 34; 29% respectively. This indicates a shift in the number of the population in the direction of individuals that successfully feed on unnatural food. Their viability was significantly higher than the viability in other variants of the study with an artificial population - 78; 71; 68% respectively. The proposed method of selection of starting material for cultivation of *P. bioculatus* allows differentiation of*



individuals for further effective reproduction, and the use of such biomaterial in programs of biological protection of plants from pests.

A comparative analysis of methods for assessing the quality of phytophagous and entomophagous cultures at different stages of ontogenesis was carried out using the indicator of the overall viability of insect populations. It is an integrated indicator that takes into account the viability of the population at all stages of its development. All tested objects of the study showed that control and improvement of culture quality by the proposed method lead to an increase in the viability of biomaterial by 18.71; 18.56 and 8.86%, respectively (egg, larva, pupa).

Key words: industrial entomology, crop quality control, entomophages, phytophages, biomaterial, viability, taxis, insect mother cultures, gene pool, stages of ontogenesis.

ДОСЛІДЖЕННЯ ЕФЕКТИВНОСТІ ТА ПОРІВНЯЛЬНИЙ АНАЛІЗ ЗАСТОСУВАННЯ ПРИЙОМІВ ОЦІНКИ ЯКОСТІ ФІТОФАГІВ ТА ЕНТОМОФАГІВ НА РІЗНИХ СТАДІЯХ ОНТОГЕНЕЗУ

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Запропоновано концепцію контролю якості культур комах в основі якої лежить цільовий підхід, що базується на меті та завданнях реалізації певної програми розведення і поділяється на два основні напрямки: контроль виробництва фітофагів, контроль виробництва ентомофагів. Методи контролю якості базуються на загальнобіологічному правилі залежності між інтенсивністю таксисів комах та загальною життєздатністю біоматеріалу.

Досліджено ефективність застосування прийомів оцінки якості фітофагів (на прикладі маточних культур зернової молі *Sitotroga cerealella*, воскової вогнівки *Galleria mellonella*) та ентомофагів (лабораторною та природною популяціями *P. biosulatus*.) центру маточних культур комах ІТІ «Біотехніка» НААН на різних стадіях онтогенезу із застосуванням показника інтенсивності таксисів.

В результаті проведених досліджень на культурі фітофагів, зернової молі, встановлена значима різниця з контролем у показниках життєздатності яєць, личинок, лялечок та імаго: 25; 6; 33 та 11 % відповідно. Дослідження, проведені з іншим представником фітофагів - восковою вогнівкою - також продемонстрували позитивні результати (16; 17; 16; 11% відповідно). Отримана (в результаті попереднього контролю якості) та добору культура мала значимо вищі показники життєздатності на всіх стадіях онтогенезу.

Встановлено, що інтенсивність хемотаксису личинок 1-го віку ентомофагів напряму корелює з показником життєздатності культури комах. При доборі вихідного матеріалу для успішного ведення культури можливо проводити добір за хемотаксисом личинок після відродження з яйця. Так життєздатність при доборі на яйця колорадського жука значимо ($p < 0,01$) перевищує цей показник в



інших варіантах добору - личинки колорадського жука та личинки воскової вогнивки: 82; 59; 73% відповідно. При роботі зі штучною популяцією *P. bioculatus*, на личинках воскової вогнивки, найбільша інтенсивність таксису відмічена у варіанті з личинками воскової вогнивки в порівнянні з яйцями колорадського жука та личинками колорадського жука – 37; 34; 29% відповідно. Це свідчить про зсув чисельності популяції в бік особин, що успішно живляться на неприродньому кормі. Їх життєздатність була значимо вищою за життєздатність в інших варіантах дослідження зі штучною популяцією – 78; 71; 68 % відповідно. Запропонований спосіб добору вихідного матеріалу для культивування *P. bioculatus* дозволяє диференціювати особин для подальшого ефективного розмноження, і використовувати такий біоматеріал у програмах біологічного захисту рослин від шкідників.

Проведено порівняльний аналіз прийомів оцінки якості культур фітофагів та ентомофагів на різних стадіях онтогенезу з використанням показника загальної життєздатності популяцій комах. Він є інтегрованим показником, що враховує життєздатність популяції на всіх стадіях її розвитку. Всі випробувані об'єкти дослідження показали, що контроль та покращення якості культури запропонованим способом приводять до підвищення життєздатності біоматеріалу на 18,71; 18,56 та 8,86% відповідно (яйце, личинка, лялечка).

Ключові слова: промислова ентомологія, контроль якості культур, ентомофаги, фітофаги, біоматеріал, життєздатність, таксис, маточні культури комах, генофонд, стадії онтогенезу.

Introduction. The mass production of insects is gaining momentum in connection with the growing needs of mankind for food products, means of biological plant protection, waste disposal, etc. The process of cultivating insects, like any production, is accompanied by certain means of control. Quality control ensures the effectiveness of methods of optimizing the mass production of insects by correcting production defects, which leads to the preservation of the applied properties of the culture (Zlotin, A. Z., 1989; Markina, T. Yu., 2016). The quality control of insect crops is understood as the variation of culture indicators within the standard regulations of cultivation in technocenosis. The authors proposed to consider changes in the quality of the culture as a significant deviation of the values of the characteristics monitored beyond the multi-year average values of the characteristic (Klimpinya, A. Ye., & Mikhnevich, O. Ch. 1986; Safonova T. V. 2002; Ghimire, M. N., & Phillips, T. W., 2014).

Increasing the effectiveness of control means, in our opinion, should be based on clear, biologically based concepts and methods (Markina T., Lykova I., 2024). The need to develop the concept of applying methods of quality control of insect cultures at various stages of ontogenesis is due to the need to develop high-quality biomaterial during the implementation of industrial entomology programs aimed at the biological protection of plants from pests (Markina, T. Yu., 2016; 2019; Chernova, I.S., 2022). Each of the stages of insect ontogenesis is characterized by certain requirements, both for cultivation parameters and biological parameters of biomaterial (Markina 2019; Barkar, V. P. et al., 2020). This requires a targeted approach and the development of quality control methods for each of the development stages separately. But the application of the population approach allows you to use the influence on certain structural parameters of the population to further obtain high-quality biomaterial (Barkar, V. P. et al., 2020; Zlotin, A. Z., & Chepurnaya N. P., 1994; Lyashenko, Yu. V., 2001).

The purpose of this work was the biological substantiation of the concept of applying methods of quality control of insect cultures in biological plant protection programs, which allows choosing an algorithm for the analysis of biomaterial and experi-



mental confirmation of the effectiveness of the population approach in controlling the quality of biomaterial.

Materials and methods. Research work is carried out at the experimental base of the ITI "Biotechnology" of the National Academy of Sciences in the research department of industrial entomology.

Research methodology included:

- application of standard in technical entomology methods of cultivation of phytophages and entomophages on an artificial nutrient medium in thermostats under optimal hygrothermal conditions for the studied species. The experiments involve testing the selection of the grain moth and wax moth according to the intensity of chemotaxis and phototaxis and the entomophagous *Perillus* according to the intensity of chemotaxis, as an indicator positively correlated with the viability of insects.

- conducting a comparative analysis using the indicator of the overall viability of insect populations as an integrated indicator that takes into account the viability of the insect population at all stages of its development and reflects its relationship with the environment and intra-population interaction. The determination of the indicator of the general viability of the culture was carried out according to the formula:

$$V = V1 \times V2 \times V3 \times 100\%, \text{ where}$$

V – general viability of the population; V1 – viability at the egg stage; V2 – viability at the larval stage; V3 – viability at the pupal stage, which takes into account the viability of the biomaterial at different stages

Experimental studies with phytophages were carried out with the mother cultures of the grain moth *Sitotroga cerealella* and the wax moth *Galleria mellonella* and *Perilus biosulatus*. During the experiment, biomaterial obtained by selecting individuals based on the intensity of chemotaxis was used. All experiments were carried out in triplicate. Biomaterial of the corresponding species, which was not subject to selection, was used as a control.

Studies with entomophages were conducted with laboratory and natural populations of *P. bioculatus*. A series of experiments was conducted, where *P. bioculatus* larvae immediately after hatching (100 individuals) were placed in a container divided into three segments. In each of the segments there was potentially attractive food - eggs and larvae of the Colorado potato beetle and larvae of *Galleria mellonella*. The research was carried out in five repetitions. The intensity of chemotaxis was determined by the number of individuals that reacted to a certain food stimulus within 15 minutes. Subsequently, the material selected in this way was fed with larvae of the Colorado potato beetle (30 individuals in 3 repetitions for each variant of the experiment) and the viability of the obtained biomaterial was determined.

Research results. Based on the results of the analytical work, the concept of using techniques for controlling the quality of insect cultures in biological plant protection programs is proposed, which is based on a targeted approach (see the diagram in Fig. 1). This allows you to choose an algorithm for biomaterial analysis, which includes three main stages:

- I. Control of production conditions;
- II Control of the production process;
- III. Control of the final product of production.

At all stages, the control of the production of entomophages and phytophages, both mother crops and directly the material that participates in the programs of plant protection from pests, is provided..

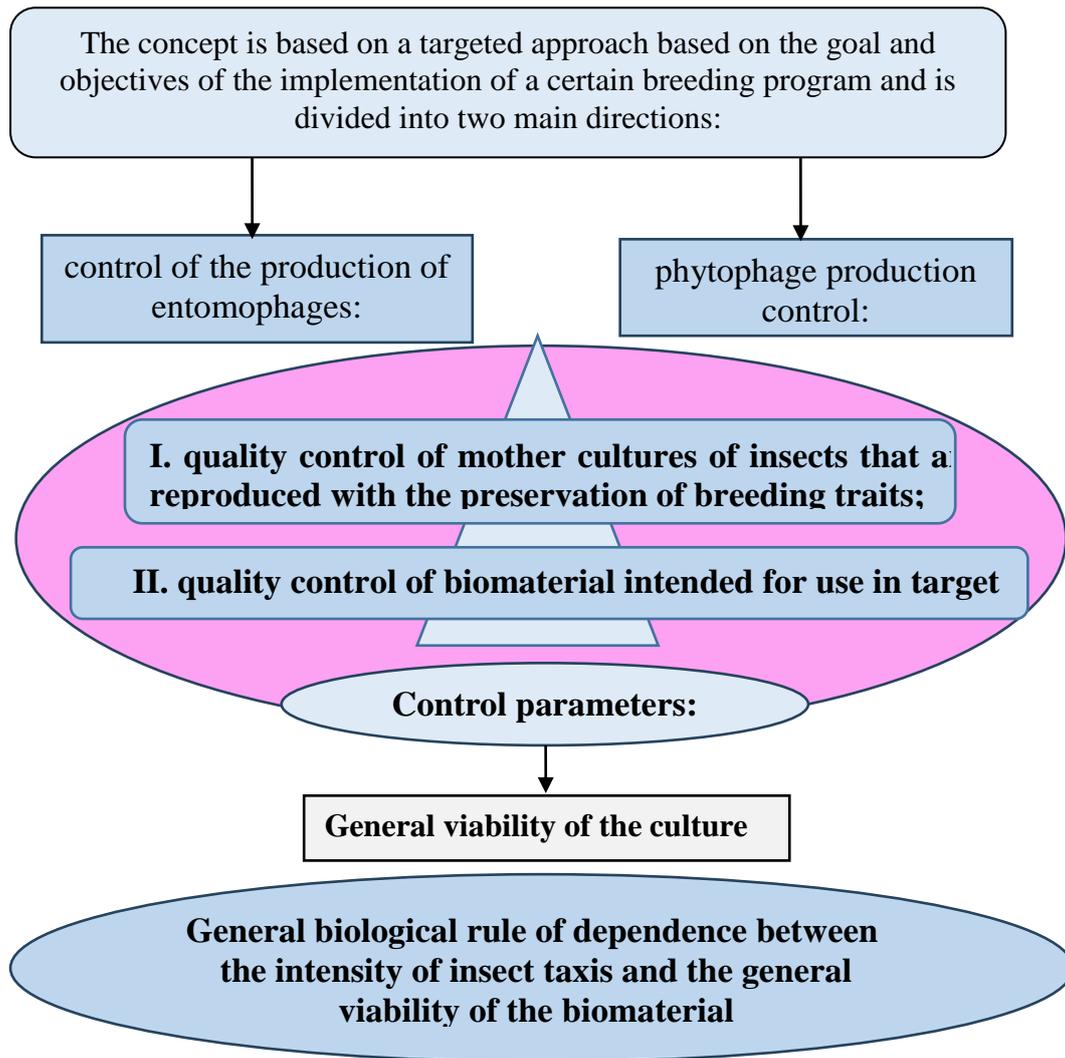


Fig. 1. Scheme of the concept of the application of methods of quality control of coma cultures

The main criterion of quality control is the viability of insect cultures, which is an indicator of survival at certain stages of the life cycle, and the general viability, which reflects the state of the entire culture as a whole (Pristavko, V. P., 1986; Stefanovska, T., et al., 2014).

Currently, the viability of populations is understood as their ability to maintain existence in changing environmental conditions. The viability of insect cultures is the ability to survive to a certain segment of the life cycle or a complete cycle of cultivation, depending on the goals of breeding (Zlotin, A. Z., 1989; Markina, T. Yu., 2016).

In previous studies, the relationship between the general viability of insects and the intensity of their taxis was established.

The proposed concept was based on experimental studies with insect cultures that were subject to selection based on the intensity of chemotaxis. In this way, the most viable biomaterial was selected a priori at the larval stage (at the time of hatching). Subsequently, this material was fed under standard cultivation conditions to determine the effect of this selection method on the biological indicators of various stages of insect ontogenesis.

As a result of research conducted with phytophages on the culture of the grain



moth *Sitotroga cerealella*, a significant difference was established in the indicators of egg viability, larval viability, pupal viability and imago viability. As a result of selection based on the intensity of chemotaxis, the resulting culture had indicators that met the requirements for this biomaterial. Studies conducted with a wax fire have shown positive results in the same way. The culture obtained as a result of preliminary quality control and selection had significantly higher indicators of viability at all stages of ontogenesis. Which testifies in favor of the proposed method of quality control. The established regularity between the intensity of insect taxis and their viability makes it possible to obtain the highest quality entomological biomaterial already at the egg stage using the express method (Table 1)

Table 1

Vitality parameters of phytophages at different stages of ontogenesis

Variant of experience	viability of eggs, %	viability of larvae, %	viability of caterpillars, %	viability of adults, %
<i>Sitotroga cerealella</i> control	62,3±4,2	79,5±0,2	54,9±2,4	84,3±1,2
<i>Sitotroga cerealella</i>	87,3±4,2*	85,5±1,2*	87,9±1,4*	95,3±0,9*
<i>Galleria mellonella</i> ; control	68,2±3,1	62,5±1,8	73,3±2,3	78,6±1,4
<i>Galleria mellonella</i>	84,2±2,1	79,5±1,5	89,5±2,1	89,3±1,2

Note: * Difference is significant relative to control

The proposed concept was tested on a laboratory culture of the predatory entomophagous bug *Perillus bioculatus*. When selecting the starting material for successful cultivation of this species, it is possible to select by chemotaxis of the larvae after re-birth from the egg. Thus, viability when selecting for Colorado beetle eggs significantly ($p<0.01$) exceeds this indicator in other selection options. (Table 2).

Table 2

The influence of the selection of larvae according to the intensity of chemotaxis on the viability of the *Perillus bioculatus* population

Type of population	Type of food	Intensity of chemotaxis, (number of individuals within 15 min.)	Viability of larvae, %
natural populations (Odessa)	Larvae of Colorado potato beetle.	37,3 ± 1,4	73,6 ± 1,8*
	Eggs of Colorado potato beetle	52,0 ± 1,6	82,1 ± 1,6*
	Wax firefly larvae	11,1 ± 1,4*	59,8 ± 1,1*
laboratory populations ETI "Bio-tekhnica"	Larvae of Colorado potato beetle.	29,1 ± 1,1	68,3 ± 1,4
	Eggs of Colorado potato beetle	34,2 ± 0,5	70,6 ± 1,2
	Wax firefly larvae	37,1 ± 0,9	78,3 ± 1,2

Note: * $p<0.01$



Interesting data were obtained when working with an artificial population of *P. bioculatus*, which had been kept in the laboratory on wax moth larvae for some time. In this case, the highest intensity of taxis was noted in the variant with wax firefly larvae. This indicates a shift in the number of the population in the direction of individuals that successfully feed on non-natural food. Their viability was significantly higher than viability in other variants of the study with an artificial population.

Further research made it possible to evaluate the effectiveness of the proposed method of population control on indicators of biomaterial viability at all stages of ontogenesis (Table 3).

Table 3

Parameters of viability of an entomophagus at different stages of ontogenesis

Variant of experience	viability of eggs, %	viability of larvae, %	viability of caterpillars, %	viability of adults, %
<i>Perillus bioculatus</i> control	42,1± 2,4	59,8± 1,7		69,3± 1,6
<i>Perillus bioculatus</i>	57,5±2,4	82,1 ± 1,6	-	76,6±2,8

Note: * Difference is significant relative to control

Experiments with the entomophagous *Perillus biosulatus* showed an increase in the indicators of the culture, which was subject to the action of selection. The higher the intensity of taxis, the higher the biological indicators of the biomaterial.

To conduct a comparative analysis of culture indicators before and after the use of our proposed approach, we used an indicator of the overall viability of the culture, which is successfully used in programs for controlling the condition of the mulberry silkworm, trichogramma and other insects (Zlotin, A. Z., & Chepurnaya N. P. 1994; Chepurnaya, N. P., 1995). This is an integrated indicator that takes into account the viability of the insect population at all stages of its development and reflects its relationship with the environment and intra-population interaction. The results of the analysis of the general viability between generations showed that the control and improvement of the quality of the culture by our proposed method lead to an increase in the viability of the biomaterial by 18.7; 18.6 and 8.86%, respectively (Table 4), this indicates the perspective of the proposed method of controlling the quality of biomaterial based on the determination of the intensity of insect taxis.

Table 4

Characterization of the general viability of insect cultures of the center of mother cultures of insects of ETI "Biotekhnica"

Generation	<i>Sitotroga cerealella</i>	<i>Galleria mellonella</i>	<i>Perillus bioculatus</i>
general viability, % A_0	52,42± 1,8	41,21 ± 2,4	27,3 ± 3,5
general viability % A_1	71,13 ± 2,2*	59,77 ± 1,6*	36,16 ± 2,1*

Note: * The difference is significant in relation to A_0

Discussion.

Quality control of insect cultures in programs of technical entomology is one of the most important stages of breeding. Cultivation regulations provide for technical control of the composition, reliability and timely release of products. Control of the



production process involves control of the physical and chemical conditions of the technocenosis and their maintenance within the optimum limits. Control of the final product of production involves compliance of insect culture parameters with the requirements of the standard, or target parameters established by production needs.

In mass breeding of insects, a distinction is made between culture quality indicators and product quality indicators (Ghimire, M. N., & Phillips, T. W., 2014). Culture quality indicators are the criteria used to describe the system, in our case the technocenosis. Product quality indicators are criteria by which industrial monitoring can be carried out. Product quality indicators must meet the following requirements:

- 1) to characterize targeted changes in culture;
- 2) be integral, i.e. immediately inform about the quality of the entire culture;
- 3) technological simplicity of taking into account indicators.

The basis of the proposed concept of application of methods of control of the final product of production is a targeted approach that takes into account the goal of breeding biomaterial, which is always within two main directions:

- control of the production of entomophages, which involves control of parameters of viability and searching activity of insects;
- control of the production of phytophages, which involves control of the viability and productivity of the biomaterial (Fig. 1)

In addition, each of the directions contains separate subdivisions that provide for the application of certain control methods taking into account production requirements.

- 1) quality control of mother cultures of insects reproduced with the preservation of breeding traits;
- 2) quality control of biomaterial intended for use in targeted programs of biomethods of plant protection.

We chose the viability of artificial insect populations as the main quality control parameter. The viability of the population is largely related to the adaptive capabilities of the species, its adaptive reserve and is controlled by genetic mechanisms (Zlotin, A. Z., 1989).

With regard to insect cultures under the conditions of technocenosis, the adaptive reserve of an artificial population should be understood as its ability to maintain a normal level of everyday viability (viability of individuals, birth rate and fecundity), as well as the presence of genetic variability sufficient for the optimized culture to maintain the specified, inherited traits, and the nature of the amplitude of fluctuations of the main biological parameters of the population was included within the limits of certain predictable intervals.

As for the intervals of fluctuations of the main biological indicators, under the conditions of a stable regime of cultivation, they should not reliably exceed the values of extreme deviations in the plus and minus direction for a number of years of observations with the survival of individuals close to the multi-year average. Survival is a particular case of the manifestation of viability of a particular ecological situation (Zlotin, A. Z., 1989).

Due to the fact that the gene pool of each species experiences the effect of natural selection in nature, the alleles of traits that contribute to the survival and reproduction of individuals become more widespread in the population from generation to generation, while genes that are less effective in this sense, will become increasingly rare, as their carriers will leave fewer offspring.

Among the factors that determine the viability of insect cultures, the leading role belongs to the genetic nature of cultures (Lysenko, V. P., & Chernova, I. S. 2018; Chernova, I. S., & Lysenko, V. P., 2018). First of all, the degree of heterogeneity of



cultures is directly related to their viability (the latter largely depends on the number of cultures). The following factors also have a significant impact on the viability of cultures:

- genetic and temporal drift of the system.
- absence of natural selection, as cultivation is carried out in the zone of ecological optimum (Chepurnaya, N. P., 1995). A change in the nature and direction of selection in technocenosis leads to the absence of stabilizing selection for the viability of artificial populations.
- violation of the nature of the spatial, ethological, age and genetic structure of populations (the first three are faster due to a decrease in the level of vitality of cultures^{iv} and lack of "environmental resistance" (Matushkina, N. O., 2020).
- the presence of a genetic load in the culture that is inherited by a latent infection, etc.

The level of viability of insect cultures of a given (and the following subsidiary) generation is affected by the ecological conditions of cultivation (the level of knowledge of the experimenter - the errors of the experimenter) through the channels of decreasing the level of viability of the culture. A certain role in the viability of insect cultures belongs to the unpredictability (unpredictability) of the result, when the process is stochastic in nature (Zlotin, A. Z., & Chepurnaya N. P., 1994; Zlotin, A. Z., 1989).

The concept of applying quality control methods proposed by us is based on the previously established dependence between the intensity of insect taxis and the overall viability of the biomaterial.

Studies with phytophages have shown the effectiveness of using the chemotaxis intensity selection method to determine the degree of culture viability. The research results prove the possibility of predicting further indicators at all stages of ontogenesis by conducting control at the stage of the larva that has just emerged from the egg.

As a result of research conducted with entomophages, it was established that the intensity of chemotaxis of 1st instar larvae is directly correlated with the viability index of the insect culture. The results confirm the data for other insect species such as mulberry silkworm, unpaired silkworm, pear saturnia (Markina, T. Yu., 2019).

The obtained data indicate that artificial populations of entomophages produced on non-property feed require certain attention and constant quality control to increase the efficiency of their use in agrocenoses, this is also confirmed by other researchers (Molchanova, O. D., & Kopko I. A., 2014).

The proposed method of selection of starting material for cultivation of *P. bioculatus* allows differentiation of individuals for further effective reproduction, and the use of such biomaterial in programs of biological protection of plants from pests. The method is low-cost, technologically simple and can be used at any time of the year in selection work, as well as for monitoring the state of natural insect populations in areas of high anthropogenic load.

Conclusions.

1. Based on the results of the analysis of literary sources and own research, the concept of the application of methods of quality control of insect cultures at different stages of ontogenesis was developed.

^{iv} Utility model patent. 43312. Ukraine. IPC (2009): A01K 67/00. Drozda V.F., Kocherga M.O., Melnychuk S.D. Method of assessing the quality of laboratory cultures of entomophages. Application No. u200902737; statement 24.03.2009; published 10.08.2009. Bul. No. 15.



2. The effectiveness of methods for assessing the quality of phytophagous and entomophagous cultures of the center of insect mother cultures at different stages of ontogenesis is shown.

3. A pattern has been established between the intensity of insect taxis and their viability, which makes it possible to obtain the highest quality entomological biomaterial already at the egg stage using the express method.

4. Experiments with the entomophagous *Perilus biosulatus* showed an increase in the indicators of the culture, which was subject to selection based on the chemotaxis indicator. The higher the intensity of taxis, the higher the biological indicators of the biomaterial.

5. The results of the evaluation of the quality of the culture according to the formula of general viability showed the perspective of our proposed method of control based on the intensity of insect taxis. All tested research objects showed that control and improvement of the quality of the culture in this way lead to an increase in the viability of the biomaterial by 18.71; 18.56 and 8.86%, respectively.

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