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ИСПОЛЬЗОВАНИЕ ЭНТОМОПАТОГЕННОГО БАКУЛОВИРУСНОГО ПРЕПАРАТА ДЛЯ БИОЛОГИЧЕСКОГО КОНТРОЛЯ ВРЕДИТЕЛЯ В РЕСПУБЛИКЕ МОЛДОВА

USING OF ENTOMOPTOGENIC BACULOVIRUS PESTICIDES FOR BIOLOGICAL CONTROLS OF PEST INSECTS IN THE REPUBLIC OF MOLDOVA

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Во многих странах в последние годы были созданы энтомопатогенные препараты, применяемые на практике с целью уменьшения количества вредителей. Выработка альтернатив пестицидам на основе энтомопатогенных бакуловирусных препаратов находится в фокусе внимания ведущих научных и производственных учреждений во многих странах. На основе биопрепаратов возможно осуществление эффективного, селективного, экологического, экономически выгодного и технологичного управления численностью и вредоносностью опасных вредителей, вызывающих значительные уровни потерь сельскохозяйственной продукции. Они повсеместно распространены в окружающей среде и как известно, вносят важный вклад в регулирование популяций насекомых. Цель исследований является определение эффективности оптимизированного биопрепарата Вирин-АББ-3 против Американской белой бабочки (Hyphantria cunea Drury), как в лесных, так и в сельскохозяйственных биоценозах. Задачи. Определение эффективности и хранения бакуловирусного инсектицида. Материалы и методы изоляция, выявление и определение биологических особенностей бакуловирусных изолятов были выполнены в соответствии с общепринятыми методами. В процессе работы в лабораторных условиях использовались микробиологические методы исследования, а при оценке биологической и эффективности биопестицида в полевых условиях – методические указания по регистрационным испытаниям инсектицидов в сельском хозяйстве. Результаты. В статье представлены результаты исследования по изучению эффективности бакуловирусного инсектицида Вирин-АББ-3, который способствует защите растений от вредителя. Отмечены перспективы создания и применения бакуловиросов на основе природных штаммов микроорганизмов. Норма расхода экологического биоинсектицида равна 0,1-0,2 кг/га сроки хранения препарата составляет 3 года при температуре хранения не выше 22 °С в хорошо проветриваемых помещениях. Применение бакуловирусного инсектицида позволяет получить биологическую эффективность на уровне 91,52%, в сравнении с биологическим эталоном Лепидоцидом 100%.

Ключевые слова: Hyphantria cunea, биологический контроль, бакуловирусный инсектицид, VG, VPN, Республика Молдова.

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Abstract. In many countries in recent years, to use natural entomopathogenic pesticides to control the number of pests. Potential agricultural production loss caused by the activity of pest organisms in the world was always a concern at international level. In many countries were established baculoviral preparations applied in practice in order to reduce the number of pests. In such context, the problem is connected to large application of baculoviral preparations that have become a reality only by elaboration and organization of production of such biological means, work registered after execution of deep biotechnological researches. The aim of the present paper is to discuss the results of two years attempt of biological control of H. cunea populations with a baculoviral product. Materials. For determination of concentration of baculoviral suspensions there were used different methods, especially electronic microscope. The isolation, detection and determination of the biological features of baculovirus isolates were carried out in accordance with generally accepted methods. Results. In the report there are also submitted the results of the joint application of the biological preparation Virin ABB-3. The preparation is based on viruses of nuclear polyhedrosesis and granuoses with cumulative and synergetic action. In such context, the problem is connected to large application of baculoviral preparation that have become a reality only by elaboration and organization of production of such biological means, work registered after execution of deep biotechnological researches. The criterion of stable ecological system and the methods restoration and construction were also analyzed. Testing of the preparation Virin-ABB-3 for control development, II generation, on plants has demonstrated a significant biological efficacy of 91,52% in comparison with that of chemical etalon Lepedocid 100 %. Meanwhile, the experiment in which viruses were used and successfully control of the species H.cunea persistently indicate that the insect viruses play an important role in restoring and constructing stable natural and antropinated ecology system.

Key words: Hyphantria cunea DRURY, baculoviral preparation, VG, VPN, Republic of Moldova.

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The microbial biopesticide market still only represents about 1% of the sales of chemical pesticides (CPL Consultants., 2010; Marrone., 2007). Factors impeding the establishment of strong MPCA markets are complex (Chandler et al., 2011; Marrone., 2007; Ravensberg, 2011) but include the burdensome costs associated with the registration of commercial products that are aimed at relatively small niche markets (Chandler et al., 2011; Ehlers, 2011).

Baculoviruses only infect insects, are ubiquitous in the environment and are known to be important in the regulation of many insect populations. Baculoviruses are host specific, infecting only one or a few closely-related species, helping to make them good candidates for management of crop and forest insect pests with minimal off-target impacts (Hewson et al., 2011). In fact, baculoviruses have been recognized as being amongst the safest of pesticides (Black et al., 1997).

Different biopesticides, such as viruses, (bacteria, fungi, etc.), microorganism products, plant derived products have been developed and used across the world (Mazhabi et al., 2011; Islam and Omar, 2012).

Baculoviruses microorganisms have shown to be a good tool for insect pest control. Numerous natural baculoviruses microorganisms have been used as biopesticides worldwide as they are naturally organisms.

Therefore, the forest pest control in Moldova in the 21st century must follow the sustainable forest protection strategy. The forest pest control technology which is reasonable, based on ecological principle, and harmonious with the environmental protection is very important to implement the strategy.

The present paper gives information of baculovirus preparats, we discuss how baculovirus evolution, host range determination and pathogenesis have contributed to their inherent safety for non-target organisms including humans. The virus also can accumulate themselves in the environment and the host population, and it can control the pest insects for a long term by forming epidemic disease in the pest insect population through the external environment stimulation. The quite stable food chain relation of plant-pest insect- natural enemy can be gradually established. Thus it can reduce the risk of the pest insect continuous outbreak and realize the persistent control of pest insects.

MATERIAL AND METHODS

The researches have been realised on the caterpillars of 2–3 ages of the H. cunea. In the study, we used the Nuclear Polyhedrosis Virus, selected and identified in the laboratory of the insect viruses. For the contamination of the laboratory insects, we used the dosed feeding, which contains 10 polyhedrons for each caterpillar. The monitoring of the insects lot and the estimation of the dead caterpillars has been carried out daily, beginning with the 3rd day of the contamination. The caterpillars H. cunea were kept under laboratory conditions at 27 °C.

For infection of larvae there was necessary a preliminary preparation of viral suspensions, using for that purpose pure or initial suspensions and applying dosed infection of insects according to the Vago C. procedure (1972) and its different modifications (Ciuhrii M., Volosciuc L, 1990,1991).

During the process of identification and determination of biological activity of baculoviruses there was necessary its purification. At initial phases purification of VPN and VG does not differ substantially. Dead larvae were soaked with the help of a mixer, and the biological mass was mixed with sterile filtered bidistilate through an apron screen. For purification of VPN were used several methods, for which we have used the modifications of our institute, consisting of the following phases. Filtered viral suspension is centrifuged within 30 min at 1000 rpm in ŢLN-2 centrifuge. The obtained deposition is washed three times with water. The obtained suspension is centrifuged in the gradient of sucrose concentration (70–20%) and is centrifuged at 3000 rpm within 10 min. Zones with concentration of 40–50% were put together and layered in the gradient of 50–60% and after 15 min of centrifugation there was obtained the fraction of SPVC.

For the determination of baculoviral concentration there are used different methods, especially of electronic and optical microscopy (Ciuhrii M 1991.,Volosciuc L. T . 2009). Titration is carried out with the help of Goreaiev chamber or in the fixed and colored preparations. There were elaborated different methods of determination of biological activity of baculoviruses. At the initial phase viral suspension is titrated, determining its concentration. Then there is prepared a series of successive dilutions with the help of which are infected larvae of the second age (it is rational to use 40 larvae of the same physiological state). After the third day there is determined the morality of larvae by options, and is being prepared the diagram of “dose-effect” relation. For that reason there is applied the method of sample analysis. Then are made some additional calculations, which allow transformation of axis for obtaining of the “dose-effect” relation in the form of straight line, and not in the form of asymmetrical curve. Construction of diagram allows us to determine the logarithm of the viral suspension dose, which ensures the death of 50% of the experimental larvae. Knowing the virus concentration and volume of viral suspension it is easy to determine lethal concentration (CL50).The mathematical treatment was registered on the 15th day after contamination; the statistical treatment was made according to (Dospehov., 1985; GAR. 1963).

RESULTS AND DISCUSSIONS

The results obtained within some multiple experiments carried out with larvae of second and third age of H.cunea lead to the conclusion that surviving of insects and baculoviruses has become possible only at obtaining a moderate biological activity. Thus, there were registered substantial results at the examination of biological activity of viral biological mass obtained from larvae which died on different days after infection with viral suspension (Table 1)

Results placed in the above table show the difference between the parameters of biological activity of biological mass obtained on the different years from the infection with baculoviruses. There are not noticed any substantial differences of biological activity in the case of viral suspension with the same concentration (107 pol./ml). Analyzing the biological effectiveness of the baculoviruses we obtained a value of 92%.

In the terms of that aspect, biological mass obtained from dead larvae after these days is characterized by parameters specific to wild strains obtained from natural conditions, that aspect induces the difference of biological activity of biological mass obtained from dead larvae on different years of infection and denotes the possibility of application baculoviral preparation and will be very useful for the baculovirus treatments managment.

Results of experiments are expressed in (Table 2). Production of SVC was not modified in case when larvae are kept at the temperature of 23 °C, 26 °C and 29 °C, between 1 x I09 SVC/larva and 1,04 x I09 SVC/larva.

TL50 decreases together with the temperature increase: at the temperature of 23 °C was 8 days, at 26 °C — 6,5 days,

Fig. 1. (a,b). Damage of lavae – H.cunea

Table 1.

Biological activity Virin-ABB-3 for control of the fall webworm moth (Hyphantria cunea Drury)

Year testing Virin-ABB-3	Virus preparation and biological standard	Treatments, Kg/ha	Biologic efficacy of treatments, %
2013	Virin-ABB-3	0,1	92,56
	Lepedocid	2,0	100,0
2014	Virin-ABB-3	0,1	84,86
	Lepedocid	2,0	100,0
2015	Virin-ABB-3	0,1	97,15
	Lepedocid	2,0	100,0
Media	Virin –ABB-3		91,52

Fig. 2. (a, b). Buterflies of fall webworm H.cunea

Table 2.

Biological activity of the granulosis virus depending on the temperature in the chamber of larvae growth

Temperature, °C	Concentration, Pol./ml	Number of larvae	Biological activity, %	TL30, days	TL50, days
23	107	20	78	7.2	8.2
26	107	20	80	6	6.5
29	107	20	82	4.7	6
32	107	20	62	8.5	12

Table 3.

Storage time of larvae of H. cunea infected depending on the storage method

Option	Storage method	Temperature	Storage time
Option 1	Plastic bags, in fridge	+ 4°C	Maximum 24 hours
	Plastic bags, in freezer	- 10°C	A few days
	Plastic bags, in freezer	-15°C	Two weeks
	Plastic bags, in freezer	- 22°C	One year
Option 2	Vacuum bags, in fridge	+ 4°C	One week
	Vacuum bags, in freezer	- 10°C	One year
	Vacuum bags, in freezer	-15°C	Two years
	Vacuum bags, in freezer	-22°C	3 years >

and at 29 °C — 6 days. In the option with 32 °C in the growth chamber, TL50 was of 12 days, and the percent of obtained infected larvae (62%) and amount of SVC has considerably decreased in comparison with other options. To minimise the loss of larva tissue rupture or of establishment of a superior infection, we have taken into account also TL30, as a transition moment of the infected larvae in plastic bags in order to be stored in freezer till processing. Thanks to the fact that 20–25% of SVC obtained from larva tissue remain in the medium used for filtration, that biological material needs to be recovered by repeated suspension in distilled water, increase and filtration.

Larvae, presenting the symptoms of infection with baculoviruses, could be stored in plastic bags of 0,5 kg, in fridge at temperature of –15–22 °C, before the end of collection process which was carried out in phases. Vacuum compaction in bags was made for longer time storage: from one week in fridge up to at least two years in freezer (Table 3).

In this respect, put into practice of an integrated control system would allow the inclusion of a production process that could be used simultaneously or succeeding, on the background and for the purpose of maximization of the efficiency of restricting action of enthomophagy, pathogens for insurance of big and high quality production, diminishing the registered loss (JOOP VAN LENTEREN, 2011).

Given that all published reviews unequivocally state that baculoviruses are safe and support their use as low-risk biological control agents for the control of insect pests, we propose that human and environmental toxicity tests and studies related to the residual fate of baculoviruses not be required for the registration of baculoviruses.

CONCLUSIONS

The insect virus pesticide is only one method to construct and restore the stable ecosystem, other methods can also be applied as the effective methods as long as they are useful to the control of target pest insects and do not cause destruction of the environment. Treatments should be applied only during the period when larval are the first two age and leafy trees. The method put forward in this paper is the preliminary result of the experiments we have been trying to control the foliage feeding insects for many years. And this method taking the virus as the main measure to restore and construct the stable ecosystem were the pest insects had occurred. Storage of biological activity of baculoviruses and insurance of ecological and economic efficiency of preparations made on their basis, needs application of deep knowledge regarding the creation of optimal technological conditions for their use in control of insect pests, synergic action between the baculoviral preparation and natural virus strains, as well as the application of efficient forms of the elaborated preparations.

Following this article concluded that baculoviruses are safe for animal and human consumption and are, therefore, acceptable for use in the control of insects that cause damage to plants.