A STUDY OF THE COMPLEX EFFECTIVENESS OF BIOLOGICAL AGENTS IN APPLE ORCHARDS IN TERMS OF CLIMATE CHANGE IN THE REPUBLIC OF MOLDOVA

STÎNGACI Aurelia, SCERBACOVA Tatiana, SAMOILOVA Anna

Abstracts. Discussion on the impact of climate change on various aspects of life is of increasing concern to the scientific community. Providing food for the growing population in adverse climatic conditions is a major challenge. Applying environmental approaches in this direction is key to restoring ecological balance. The paper presents how the foliar fertilization with biological products in different doses and combined with two types of foliar fertilizers, applied to an apple tree orchard. The research was carried out within the Institute of Genetics, Physiology and Plant Protection of the MSU of the Republic of Moldova. The article presents the results of the study of efficiency of fruit-cultures treatment with aqueous suspensions of entomopathogenic bacteria *Bacillus thuringiensis* subsp. *kurstaki* and *Bacillus thuringiensis* var. *thuringiensis* with addition of para-aminobenzoic acid derivatives solutions in concentrations 10^{-2}, 10^{-4}, 10^{-6} Mm. An increase in the average weight of fruits and a reduction in disease and insect damage were observed in all variants of the experiment compared to the control. The studied derivatives exhibited higher biological activity at the lower concentration than *para*-aminobenzoic acid (pABA). The best trial was treatment with a mixture of *Bacillus thuringiensis* subsp. *kurstaki* with the addition of an aqueous solution of pABA at concentrations of 10^{-4} and 10^{-6} Mm. It was shown that the use of *Bacillus* spp. with the addition of the para-aminobenzoic acid derivatives solutions leads to an improvement in the functional state of plants, contributing to the activation of the active resistance mechanisms to the action of the stress factors. The obtained results demonstrate the possibility of using entomopathogenic bacteria in a tank mixture with pABA for the creation of biological preparations as alternatives to pesticides and the development of biological methods of plant protection.

Keywords: *Cydia pomonella, Bacillus thuringiensis* subsp. *kurstaki*, para-aminobenzoic acid (pABA), apple orchard, entomopathogenic bacteria.

Rezumat. Studiul eficacității complexe a agenților biologici din livada de meri la schimbările climatice în Republica Moldova. Discuțiile cu privire la impactul schimbărilor climatice asupra diferitelor aspecte ale vieții reprezintă o preocupare tot mai mare pentru comunitatea științifică. Furnizarea de hrană pentru populația în creștere în condiții climatice nefavorabile este o provocare majoră. Aplicarea abordărilor de mediu în această direcție este cheia restabilirii echilibrului ecologic. Lucrarea prezintă modul în care fertilizarea foliară cu produse biologice în doze diferite și combinată cu două tipuri de îngrășăminte foliare este aplicată pe o livadă de meri. Cercetarea s-a desfășurat în cadrul Institutului de Genetică, Fiziologie și Protecția Plantelor al USM al Republicii Moldova. Articolul prezintă rezultatele studiului eficienței tratării culturii mărului cu suspensii de bacterii entomopatogene *Bacillus thuringiensis* subsp. *kurstaki* cu adaos de soluții de derivați ai acidului para-aminobenzoic în concentrații 10^{-2}, 10^{-4}, 10^{-6} Mm. O creștere a greutății medii a fructelor și o reducere a bolilor și daunelor insectelor au fost observate în toate variantele experimentului comparativ cu martor. Derivații studiați au prezentat o activitate biologică mai mare la o concentrație mai mică decât acidul para-aminobenzoic (pABA). Cel mai bun studiu a fost tratamentul cu un amestec de *Bacillus thuringiensis* subsp. *kurstaki* cu adăugarea unei soluții de pABA la concentrații de 10^{-4} și 10^{-6} Mm. S-a demonstrat că utilizarea *Bacillus* spp. cu adaosul de solutii de derivați ai acidului para-aminobenzoic, conduce la o îmbunătățire a stării funcționale a plantelor, contribuind la activarea mecanismelor active de rezistență la acțiunea factorilor de stres. Rezultatele obținute demonstrează posibilitatea utilizării bacteriilor entomopatogene într-un amestec de rezervor cu pABA pentru crearea de preparate biologice ca alternative la pesticide și dezvoltarea metodelor biologice de protecție a plantelor.

Cuvinte cheie: *Cydia pomonella, Bacillus thuringiensis* subsp. *kurstaki*, para-aminobenzoic acid (pABA) livadă de meri, bacterii entomopatogene.

INTRODUCTION

The global climate changes observed in the recent decades affect the phonological cycle and crop productivity. At the same time, the current state of the art in plant protection does not resolve all crop production problems, as pesticides cannot protect plants from abiotic stresses. Therefore, agronomic practices must be developed to attenuate the negative impact of external conditions and enable consistently high yields.

Organic agriculture farming has traditionally existed, with modern practices beginning in the 1920s as a result of pesticide use that was popularized in 1960s. In the 1970s, this conventional farming has been increasing with greater knowledge and developments of technology. In addition to the production of healthy food, organic farming also contributes to the protection of the natural environment At a high level of ecological effectiveness and harmlessness of biological preparations, their use does not always have a stable effect in relation to the negative influence of climatic factors on the host plant (PAINI & SHEPPARD, 2016; SKENDŽIĆ & ZOVKO, 2021).

The current level of plant protection does not solve all the problems of crop production, since pesticides are not able to protect plants from abiotic stress. Global climate changes, which have been observed in recent decades, have an impact on the phenological cycle and productivity of agricultural crops, so agricultural techniques must be developed that weaken the negative impact of external conditions and allow obtaining consistently high yields. Such methods include the treatment of plants with biological preparations, a biological method of plant protection (PETROVSKII & CARACTOV, 2017).

The application of pesticides that have increased toxicity on a large scale produces a harmful effect on the environment, which causes pollution of ecosystems. A serious consequence of the use of these classical methods of control is the loss of the biosphere's ability to self-regulate. As a result, regarding the disadvantages of using classical control methods, the use of modern control methods should not be ignored, as they are based on both natural control methods and ecological methods. All control actions should be applied in accordance with the new ecological concept, of integrated control, which consists of a system of regulation of pest populations, taking into account the specific flight and dynamics of pest and zoophagous species, with a harmonious use of all control methods (agrophytotechnical, physical-mechanical, biological and chemical), in order to maintain the density of pests or their attack, at a level that does not cause crop losses. The aim of our research is to identify and centralize the arthropod fauna existing in walnut plantations in order to establish the useful and harmful fauna as accurately as possible; following these determinations, we will be able to establish the control method that can be used efficiently in these experimental lots.

The famous Report I.A established that the positive effect of pABA on living systems is based on the previously unknown phenomenon of its interaction with enzymes. The positive effect of para-aminobenzoic acid on plants, animals and humans has been argued (COJEVNICOVA, 1983). The excessive use of chemicals has had the effect of reducing friendly predators and increasing residual hazards to both human health and the environment. The priority direction of biological protection is the creation and use of complex mixtures of biological means with synergistic action amplifying the effectiveness. One of the promising directions for increasing both resistance against diseases and pests and crop productivity is the application of the complex of entomopathogenic bacteria and plant growth activators, such as para-aminobenzoic acid (pABA), which is involved in the synthesis of RNA and DNA and is a "growth factor" for many bacteria. It is known that pABA is able to absorb UV rays that negatively affect the bacterial reproduction mechanism, so the use of pABA in bacterial preparations for the treatment of aerial parts of plants can be particularly effective (EIGEIS, 2015).

The aim of the research was to establish the possibility of using a tank mixture of entomopathogenic strains of *Bacillus thuringiensis* subsp. k*urstaki* with para-aminobenzoic acid (pABA) derivatives to determine the synergistic effect in increasing biological efficacy in the *Cydia pomonella* control. In this review of *Bacillus thuringiensis* subsp. *kurstaki* and para-aminobenzoic acid (pABA) we discuss how the evolution of biological preparations, host range determination and pathogenesis have contributed to their inherent safety for non-target organisms including humans. The article represents a generalization aimed at obtaining viable preparations and improving active strains.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of IGFPP of the MSU. Suspensions of entomopathogenic bacteria *Bacillus thuringiensis* subsp. *kurstaki* and para-aminobenzoic acid derivatives in concentrations of 10^{-2}, 10^{-4}, 10^{-6} mM were used for the protection of apple varieties “Renet Simerenko” and “Gala” against *Cydia pomonella* L., in the arthropods from apple orchards where a conventional cultivation technology was applied, as well as in orchards where ecological methods of protection against diseases and pests were applied. At the first stage of the research, we determined the compatibility of bacterial suspensions and PABA derivatives for use in tank mixtures. The experiment was conducted on potato-glucose agar using the filter disc method [4]. Agar plates were inoculated with bacterial suspensions, after which filter discs saturated with pABA derivatives at concentrations of 10^{-2}, 10^{-4}, and 10^{-6} mM were placed in the center of Petri dishes. As a result of the experiment on determining the compatibility of *B. thuringiensis* var. *thuringiensis* and derivatives of para-aminobenzoic acid in concentrations of 10^{-2}, 10^{-4}, 10^{-6} mM on potato-glucose solid medium, it was found that the formation of sterile zones of the growth absence did not occur between bacterial lawns and discs saturated with PABC derivatives, no biological agents growth suppression was observed, hence, they can be used in tank mixtures.

RESULTS AND DISCUSSIONS

The effectiveness of bacterial suspensions depends on the air temperature +24+28ºC. When choosing terms for spraying, their characteristics must be taken into account. The rapid death of the larvae is indispensably related to the concentration of bacterial bodies in the body of the larva. At low temperatures, the larvae move and feed little or not at all within a few days. As a result, they receive small amounts of bacterial bodies. The mortality of the larvae in this case increases to several days and the effectiveness of the suspension is low. The optimum air temperature for *Bacillus thuringiensis* var. *kurstaki*+Microcom for fruit plants+chitinase was +18+30ºC. The treatment was carried out at an average air temperature of +25.5ºC. The constant increase in global temperatures will change the way we manage the growth of fruit trees (Figs. 1; 2).

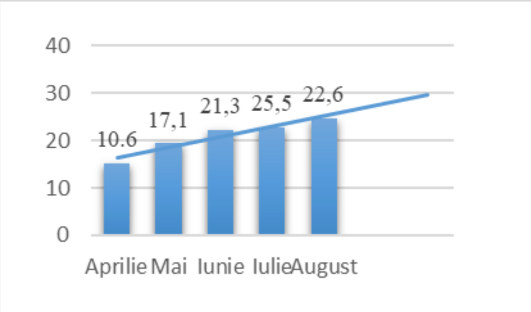


Figure 1. Medium temperature.

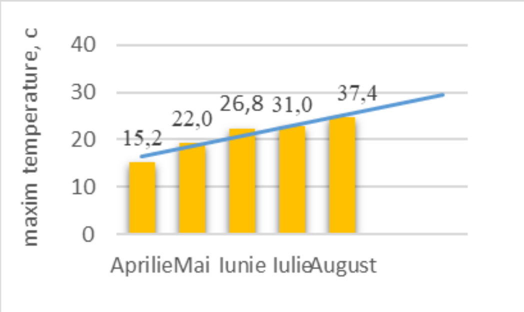


Figure 2. Maximum temperature.

In the first stage of the research, the compatibility of bacterial suspensions and pABA derivatives for use in tank mixes was determined. Experiments were performed on potato glucose agar using the filter disc method of Egorov N. S., 2004. Agar plates were inoculated with bacterial suspensions, after which filter discs impregnated with pABA derivatives at concentrations of 10^{-2}, 10^{-4}, 10^{-6} mM were placed in the centre of the Petri dish, separately for each bacterium.

In apple orchards, treatments were carried out with the bacterial suspension Bacillus thuringiensis subsp. kurstaki and pABA derivatives in the control of apple bollworm (*C. pomonella* L.) larvae by hand spraying. The experiment was carried out in 8 variants with 3 repetitions, in each repetition 3 trees. Experience options: 1). Control 2). Chemical standard Avaunt-15 EC, 0.4 l/ha, 3). Biological standard "Actoverm Formula", 4). *B. thuringiensis* subsp. kurstaki 1.5×10^9 + pABA 10^{-2} mM, 5). *B. thuringiensis* subsp. kurstaki 1.5×10^9 + pABA 10^{-4} mM, 6) *B. thuringiensis* subsp. *kurstaki* 1.5×10^9 + pABA 10^{-6} mM. 7) B. thuringiensis subsp. kurstaki 1.5×10^9. 8) pABA 10^{-6} mM (Table 1).

Table 1. The biological effectiveness of the bacterial suspension *Bacillus thuringiensis* var. *kurstaki* against the apple worm (*Cydia pomonella* L), II generation.

No.	**The options**	**Consomption rate, l/ha/CFU titer/ml**	**Number of fruits in evidence**			**Biological efficacy, %**
			Total fruits	**Totally attacked of fruits**	**attack %**	
1.	Control	-	300	213	71,0	-
2.	Chemical standard Avaunt-15EC 0,4 l/ha	0,4	300	21	7,0	95,3
3.	Actoverm Formula, Biological standard	1×10^9	300	34	11,3	87,3
4.	*B. thuringiensis kurstaki* + PABA 10^{-2}	$1,5\times10^9$	300	48	16,0	81,3
5.	*B. thuringiensis kurstaki* + PABA 10^{-4}	$1,5\times10^9$	300	41	13,7	83,0
6.	*B. thuringiensis kurstaki* + PABA 10^{-6}	$1,5\times10^9$	300	31	10,3	84,6
7.	PABA 10^{-6}	10^{-6}	300	186	62,0	20,6
8.	*B. thuringiensis kurstaki*	$1,5\times10^9$	300	54	18,0	73,6
	$DEM_{0,05}$					8,4

Therefore, our results attest that the effective treatment solution for the control of the number of affected apple fruits was 71%. When treating the trees with a suspension of B. thuringiensis subsp. kurstaki, the number of affected fruits was 18%, and the biological effectiveness was 73.6%. The use of mixtures with pABA derivatives increased their biological efficiency in protecting apple fruits from apple bollworm. When using pABA 10^{-2} derivatives in combination with a bacterial suspension, fruit damage decreased to 16% and the efficiency was 81.3%. Low concentrations of pABA 10^{-4} and pABA 10^{-6} in tank mixtures increased the protective effect, fruit damage decreased to 13.7% and 10.3%, and biological efficiency increased to 83.0%, respectively 84 .6%. The effectiveness of the chemical standard was 95.3%, and the biological one was 87.3% (Fig. 3a, b, c, d).

As a result, it can be observed that the bacterial suspensions in mixtures with pABA derivatives in protecting apple fruits from the apple worm (*Cydia pomonella* L.) showed a higher biological activity at lower concentrations. The best options were treatments with mixtures of B. thuringiensis subsp. kurstaki with the addition of pABA solutions at concentrations of 10^{-4} and 10^{-6} mM. In these variants, a synergistic effect of the suspension of *B. thuringiensis* subsp. *kurstaki* and low concentrations of pABA 10^{-4} and 10^{-6} mM can be seen, as the biological efficiency in reducing apple damage increases from 73.6% when using bacteria without pABA derivatives in a tank mix with 10^{-6} mM pABA - at 84.6% (Fig. 4).

Figure 3a, b, c, d. Treatments with para-aminobenzoic acid derivatives (pABA) mixed with the bacterial suspension with *Bacillus thuringiensis* var. *kurstaki*, in the IGFPP orchard of the MSU (original).

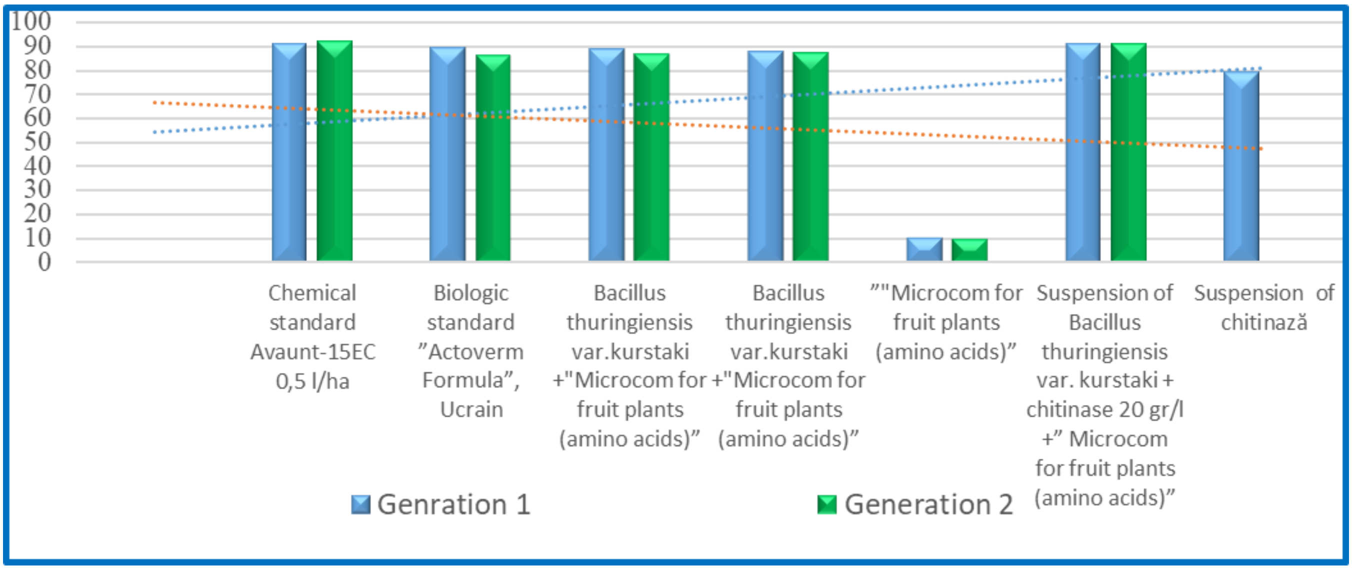
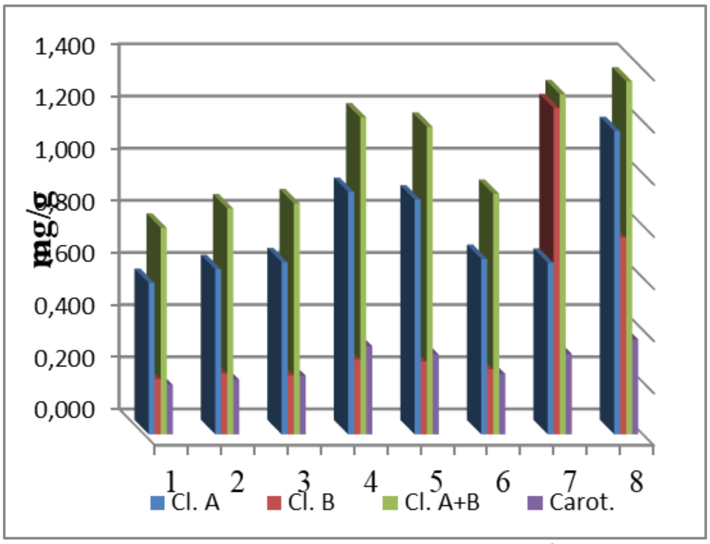


Figure 4. The biological effectiveness of the product *Bacillus thuringiensis* var. *kurstaki* against the apple worm (*Cydia pomonella* L.) generation I, II (Number of fruits taken into account per tree – 300).

Thus, at doses of *Bacillus thuringiensis* var. *kurstaki* +"Microcom for fruit plants" (1.5x10^9UCF/ml), the effectiveness is 15.3%, at the dose of *Bacillus thuringiensis* var. *kurstaki* +"Microcom for fruit plants" (3.7x10^9 UCF/ml)- 91.61%. Compared to the biological standard "Actoverm Formula" with a concentration of 1x10^9, an effectiveness of 86.61% was recorded. The statistical processing of the results obtained with the biological products for the first generation made it possible to reveal an insignificant difference between the variants of the experiment compared to the untreated control variant. Actual >F95% = 3.89< 5.88

Also, the biological effectiveness was argued through the biochemical analysis of the plant mass with the help of the listed biological indicators. This can be explained by the fact that the product inhibits the biosynthesis of chitin in insects during the molting stage, disrupting the formation of a new cuticle. It has a pronounced and moderate contact intestinal action, as well as ovicidal activity. The product is effective against the larvae of lepidoptera and coleoptera that eat leaves, it is an inhibitor of the synthesis of insect chitin, it has an intestinal and contact effect, it reduces the fertility of apple worm females in subsequent generations, it has an ovicidal effect (prevents hatching of larvae from eggs), prevents the passage of larvae to older ages. Also, the product is characterized by a long-lasting protective effect at high temperatures, high resistance to rain and the absence of a negative effect on beneficial arthropods. The statistical processing of the results obtained with the biological products for the first generation made it possible to reveal an insignificant difference between the experiment variants compared to the untreated control variant Freal >F95% = 2.99< 4.87, in the second generation Freal >F95% = 3 .89 < 5.88 (Fig. 5).



Variants: I- Witness; II-Suspension of *Bacillus thuringiensis* var. *kurstaki* (titre 1.5x10^{9}UFC/ml) + "Microcom for fruit plants (amino acids)"; III-Suspension of *Bacillus thuringiensis* var. *kurstaki* (titer 3.7 x10^{9}UFC/ml); IV-Biological standard "Actoverm Formula"; V-Et. chemical "Avaunt" - 15EC; VI-Suspension of "Microcom for fruit plants (amino acids)"; VII-Chitinase solution VIII-Suspension of *Bacillus thuringiensis* var. *kurstaki* (titer 3.7 x10^{9}UFC/ml) + Chitinase suspension + "Microcom for fruit plants (amino acids)".

Figure 5. The content of photosynthetic pigments in dynamics in the leaves of the "Gala" variety apple, after foliar fertilization with microelements and bacterial suspension, mg/g m.p.

When analysing the results of determining the content of photosynthetic pigments in the leaves of apple trees, a more intense accumulation of photosynthetic pigments was noted. Also, during the period of intensive plant development, depending on the fertilization of the plants with these derivatives and bacterial products, the largest amount of photosynthetic pigments (carotenoids) was found in the variant where the bacterial strain *Bacillus thuringiensis* var. *kurstaki* + Microcom for fruit plants" 3.51±0.10. This is very important for apple culture, because the ripening phase takes place during this period, followed by the maturation of the shoots.

The slight differences between the bacterial suspension variant and the tank mixture variants with 10^{-2} and 10^{-4} mM PABA derivatives may have been impacted by the weather conditions of the growing season. The lack of precipitations in May was 21 mm with the required 54 mm (39% compared to the norm), and maximum temperatures in the middle of the month in the range of 25-28^{0}C had a negative impact on the development of plants. In June, 7 mm of precipitation fell, compared to the norm of 65 mm, with a precipitation deficit of 89% and temperature deviations from the norm of +1.2^{0}C (***. Weather and Climate). Plants weakened by the drought developed poorly and were infested by the pest.

CONCLUSIONS

The use of complex mixtures of the entomopathogenic bacterium *B. thuringiensis* subsp. *kurstaki* with derivatives of para-aminobenzoic acid managed to increase the biological effectiveness in the protection of apple fruits of the "Renet Simerenko" variety in combating the apple worm pest (*Cydia pomonella* L.). The use of lower concentrations of pABA 10^{-4} and pABA 10^{-6} mM in the mixture also increased the protective effect: fruit damage was reduced to 13.7% and 10.3%, and the biological efficiency increased to increase to 83.0 %, respectively, 84.6%. The effectiveness of the chemical standard was 95.3%, and the biological one was 87.3%.

The obtained results confirm the possibility of using entomopathogenic bacteria in a mixture with pABA to create biological preparations that can replace pesticides and develop biological methods of protection in potatoes and apples.

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