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MECHANISM AND ACTIVITY SPECTRUM OF MICROBIOLOGICAL PREPARATION BATSIKOL WITH PHYTOPROTECTIVE ACTION

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Abstract

Various groups of agents are involved in biological crop protection to control pests and diseases. Of them, *Bacillus* genus possessing activity against harmful insects and phytopathogens is most promising and widely used. In this, the biologicals based on *Bacillus thuringiensis* (Bt) dominate. More than 70 varieties of Bt have been identified. These bacteria can survive for a long time after treatment. Preparations based on three Bt serovars (A, B, C) are mostly used for insects' biocontrol. Serovar A Bt subspecies can form crystal endotoxins which are active against *Lepidoptera*; serovar B Bt subspecies attack the larvae of mosquitoes and black flies, and phytophagous *Diptera*; and serovar C Bt subspecies are active against *Coleoptera* beetles. A new serovar F (fungi) of this bacillus was identified. Physiological and biochemical properties of *Bacillus thuringiensis* provide the assimilation of nutrient substrates and antibiosis against biocenosis partners. Batsikol, the biological preparation based on *B. thuringiensis* var. *darmstadensis* (H₁₀) with entomopathogenic action, was created at All-Russian Research Institute of Agricultural Microbiology (St. Petersburg). Batsikol contains components of culture liquid, spores, insecticidal and fungicidal exo- and endotoxins, due to which it possesses multifunctional properties. The article presents the mechanisms of entomopathogenic and antifungal action of microbial preparations based on Bt. Results of testing Batsikol effectiveness against various pests and diseases in field trials and vegetation experiments are shown. Liquid form of biological product was used in the study (spore titer of 3.5×10^9 /ml). Field and vegetation tests were carried out in 1994-2013 in different regions of Russia (Leningrad, Novosibirsk, Volgograd region, North Ossetia, Stavropol and Primorsky regions). Batsikol was sprayed against phytophagous pests on vegetating plants. The efficacy against pests varied from 50 to 100 %. Different modes of application against phytopathogen were tested according to the type of parasitism and environmental characteristics of fungi (i.e., spraying, irrigation, seed treatment). In field experiments the efficacy of spraying strawberry plants against gray mold was 60-74 %. Soil watering was used against Fusarium wilt on tomatoes and flax with efficacy of 74-87 % and 34-42 %, respectively. When seeds were treated prior to sowing the efficacy was 66-71 % in case of soaking barley seeds against root rot, and 40-45 % while soaking potato tubers against damping-off. Based on the tests conducted with Batsikol in different regions of Russia, the spectrum of its activities against wide range of phytophagous pests and pathogenic fungi was revealed on different crops. The obtained data expand the understanding of Bt biology and, in particular, the action spectrum against various pests and diseases dangerous for many cultivated plants. Presented materials allow considering *Bacillus thuringiensis* as the basis of microbiological preparations with a multifunctional activity. The obtained data will allow expanding the scope of its application, and it will help to improve ecological situation.

Keywords: *Bacillus thuringiensis*, Batsikol, phytophagous insects, phytopathogenic fungi, biological efficiency.

Various groups of agents are involved in biological crop protection to control pests and diseases. Currently, much attention is paid to the bacteria with phytoprotective function. Of them, the *Bacillus* genus is most promising and widely used for creation of biological preparations. They possess activity against harmful phytophagous insects and phytopathogens [1-14]. The biologicals based on *Bacillus thuringiensis* (Bt) are predominant. In world practice, Bt is used as a

safe insecticidal agent, its share in the market of biopesticides is about 90-95 %.

High adaptive capacity of aerobic spore-forming *thuringiensis* bacteria under various extreme conditions determine their widespread in nature. The greatest number of bacilli is isolated from the soil, they are often found in water and extracted from sick and dead insects. Over 70 Bt varieties greatly effective against *Lepidoptera*, *Coleoptera*, *Diptera* and *Hymenoptera* phytophages have been identified. Bt bacteria can survive for a long time after treatment of plants. They have high selectivity of action, are effective against target objects, safe for human, warm blooded animals and beneficial organisms [15]. Antifeedant, teratogenic and dereproductive features provide their high biological effectiveness. Bt-based products are processible in manufacture and use.

Preparations based on three Bt serovars are mostly used for insect biocontrol in agrocenoses. Bt subspecies of serovar A form endotoxin crystals that are most active against lepidopterous insects (*Lepidoptera*). They are producers of drugs such as Bitoxybacillin (Bt var. *thuringiensis*), Dendrobacillin (Bt var. *dendrolimus*), Entobacterin (Bt var. *galleria*), Lepidocide (Russia), DIPel (USA), Bactospeine (Bt var. *kurstaki*) (France), etc. Over 70 insect species are sensitive to this serovar. Bt subspecies of serovar B (Bt var. *israelensis*) are used as producers of larvicidal biologics, such as Bactoculicide, Bacticide (Russia), Bactimos (France), Teknar (Switzerland), Vectobac (USA), etc., that attack the larvae of mosquitoes and black flies, and phytophagous mosquitoes (rice and champignon mosquitoes) (*Diptera*). Bt subspecies of serovar C (Bt var. *tenebrionis*, Bt var. *darmstadiensis*) are active against beetles (*Coleoptera*), are used as producers of Decimide, Colorado, Batsikol (Russia), Novodor (Denmark), and other biological preparations [2]. A new serovar F (fungi) [1] of this bacillus has been identified.

Batsikol, an entomopathogenic biological preparation based on *B. thuringiensis* var. *darmstadiensis* (H₁₀) containing the components of liquid culture, spores, insecticidal and fungicidal exo- and endotoxins, due to which it possesses multifunctional properties, was created at All-Russian Research Institute of Agricultural Microbiology (ARRIAM). The following Batsikol forms are available: dry powder, paste and liquid form.

Batsikol is similar to Bitoxybacillin (BTB) in its purpose and effectiveness. The latter possesses insecticidal effect and is active against a wide range of pests phytophages. It is recommended against cabbage moth (*Mamestra brassicae* L.), large and small whites (*Pieris brassicae*, *P. rapae* L.) caterpillars in cabbage; meadow moth (*Loxostege sticticalis* L.) caterpillars in beet, alfalfa, sunflower, carrot, and cabbage crops; grape berry moth (*Polychrosis botrana* Schiff.) in grapes; corn earworm (*Heliothis zea* F.), turnip moth (*Scotia segetum* Schiff.), and lappet moth (*Laphigma exigua* Hb.) caterpillars in cotton plants; apple ermine moth and small ermine moth (*Yponomeuta malinellus* Zell., *Y. padellus* L.), brown oak (*Aporia crataegi* L.), fall webworm (*Hyphantria cunea* Drury) caterpillars in fruit trees and berry plants; brown oak tortrix (*Archips crataegana* Hb.), gypsy moth and lackey moth (*Ocneria dispar* L. and *Malacosoma neustria* L.), winter moth (*Operophtera brumata* Cl.), mottled umber (*Erannis defoliaria* L.), brown-tail moths (*Euproctis chrysorrhoea* L., *E. karghalica* M.) in fruit and woody plants, Colorado beetle larvae (*Leptinotarsa decemlineata* Say) in potatoes, tomatoes, aubergine; spider mite (*Tetranychus urticae* Koch) in cucumbers in greenhouses, small gooseberry sawfly (*Pristiphora pallipes* Lep.), common gooseberry sawfly (*Pteronidea ribesii* Scop.) in currants, gooseberries and other pests [2]. In addition, BTB is active against jewel beetle (*Agrilus ribesi* Schaefer) [16]. Extensive BTB testing conducted in various regions of the Russian Federation and the CIS (Krasnodar and Stavropol' Territories, North Ossetia, Lenin-

grad region, Republic of Crimea, Transcarpathia, Belarus, Lithuania) showed its high efficiency (from 80 to 100 %) [2].

Both pathogen and insect characteristics should be taken into account for the successful use of biologics for protection measures. In this regard, it is important to know the mechanism of their interrelations as well as of their relations with the environment. Like other bacilli, *B. thuringiensis* possesses physiological and biochemical features to ensure the absorption of nutrient substrates and antibiosis against biocenosis partners. Bacteria of the genus *Bacillus* are characterized by polyenzymatic features. Various enzymes of the class of hydrolases are found in them which results in simultaneous activities against pest insects and pathogenic fungi [1, 17-19].

Bt effect on pests results from its toxicity to insects, entomopathogenic and metatotoxic effects due to the presence of crystals of endotoxin, exotoxin, phospholipase C and spores. This set of virulence factors is active against different insect species in varying degrees and in varying combinations. Bacteria cause diseases that are accompanied by septicemia, the severe lesions, in which the hemolymph and its phagocytic and non-specific immune mechanisms are no longer able to suppress the proliferation of microorganisms that penetrate it continuously. Cells of infected tissues are broken and the large quantity of parasitic bacteria enter hemolymph thus causing septicemia. Bacteria penetrate the intestinal epithelium where they proliferate rapidly and cause the insect death [2].

The mechanism of Bt antifungal action is linked to a number of factors. Bacteria produce and excrete lytic enzymes into the external medium, particularly protease and chitinase that lyse the cell walls of phytopathogenic fungi [20-24]. With lysis, the content of fungal hyphae becomes the source of power and energy for the bacilli. Moreover, bacilli can produce antibiotics that have a depressing effect against fungi [25].

Recently, much attention is given to the research related to the formation of cyclic lipopeptide antibiotics by the *Bacillus* bacteria; these antibiotics are responsible for the antagonistic effect [26-32].

Effect of *B. subtilis* on *Fusarium oxysporum* is explained by the combined effect of mycolitic enzymes and antibiotic substances [33]. Some authors suggest a link between the antibiotic activity and δ -endotoxin; perhaps, the *B. thuringiensis* var. *thuringiensis* antifungal effect is due to the disconnection of oxidative phosphorylation and respiration processes in the target objects [34].

Significant crop losses due to pests and diseases combined with the need for environmentally friendly products make the use of microbiological preparations extremely important. However, compared to chemical pesticides, their assortment is smaller, therefore it is necessary to create and use the new formulation.

Our research made it possible to estimate the spectrum of action and to identify the effectiveness of the Batsikol biopreparation developed in ARRIAM against mass pests, especially phytophage coleopteran pests (*Coleoptera*) [35], and against the phytopathogens that cause plant diseases [36], which extends the possibilities of its application.

In this paper, we summarized the results of the study of Batsikol efficiency against pests and phytopathogens in a number of crops in various areas and at different cultivation technologies.

Technique. Field and pot tests were performed in 1994-2013 in different regions of Russia. Liquid form of insecticidal biological preparation Batsikol (ARRIAM) was used in the study (spore titer of 3.5×10^9 /ml). Experiments were performed in 3 replicates.

Batsikol activity against Colorado potato beetle (*Leptinotarsa decemlineata* Say) was studied in potatoes (Nevsky, Lugovskoi, Elizaveta varieties) at

the farms of Leningrad and Novosibirsk regions. Potato planting area of 200 m² infected with Colorado potato beetle was sprayed with the preparation at the rate of 12-15 l/ha and working fluid flow of 400 l/ha. Counting was performed with 25 plants selected along the area diagonal prior to treatment and at post-treatment days 5 to 10.

The plants of the strawberry variety Tsarskoselskaya were sprayed during budding in Leningrad Region in the plots of 100 m² area. The rate of the preparation use was 15 l/ha. Buds infected with strawberry blossom weevil (*Anthonomus rubi* Hbst.) were counted prior to the treatment and at post-treatment days 10 and 20 in 25 plants. Berries infected with grey mould were evaluated at the day 20.

Preparation efficiency (12 l/ha) against cruciferous flea beetles (*Phyllotreta*) and cabbage leaf beetle (*Phaedon cochleariae* F.) was studied in rutabaga, rape, mustard, and cabbage in Leningrad, Novosibirsk, and Volgograd regions in the plots of 50 m² area. Counting was performed prior to treatment and at the post-treatment days 5 and 10; 20 plants were examined, and the percentage of occupancy was calculated.

Carrot was treated (15 l/ha) against carrot psyllid (*Trioza apicalis* Frst.) at the plots of 10 m² (counts prior to the treatment and at the post-treatment day 10 in 20 plants).

Raspberry plants were treated with batsikol (15 l/ha) against raspberry mite (*Eriophyes gracillis* Nal.). Mites were counted before and after treatment in the samples of 10-30 leaves.

Flower crops were sprayed (20 l/ha) against thrips (*Thysanoptera*) in greenhouses. Percentage of occupied plants was calculated prior to treatment and at day 10 after treatment.

Batsikol activity (20 l/ha) was tested in buckwheat Emerald variety in Primorsky Territory (Ussuriisk) against buckwheat weevil (*Rhinoncus sibiricus* Faust). Vegetating plants were treated. Leaf infection in the phase of germination and stem infection during flowering and before harvesting was registered.

In estimating Batsikol efficacy against harmful fungi, application techniques appropriate to the parasitism type and environmental pathogen characteristics were used. Vegetating plants of strawberry Tsarskoselskaya variety were treated with Batsikol (15 l/ha) against gray mold (*Botrytis cinerea*), the distribution of which was assessed by the number of infected berries. Soil infected with pathogen (*Fusarium oxysporum*) was watered with the preparation at a rate of 100 ml/kg against fusarium wilt of tomatoes and flax. The efficacy of pre-sowing treatment was studied in flax against fusarium wilt, in barley against Helminthosporium root blight (*Bipolaris sorokiniana*) and in potatoes against rhizoctoniosis (*Rhizoctonia solani*). Seeds were soaked in the preparation for 3 hours. Artificial infections were created in accordance with the guidelines [37, 38].

Proportion (P) and severity (R) of the disease was evaluated according to the formula [39]: $P = A \times 100/N$, where P is a proportion of infected plants, %, A is the number of infected plants, N is the total number of plants in the samples; $R = \Sigma(a \times b)/N \times K$, where R is severity of the disease, %; $\Sigma(a \times b)$ is a sum of diseased plants number (a) multiplied by the corresponding score of disease development (b); N is a total number of recorded plants; K means the highest infection scale score.

Fusarium wilt infection was recorded in flax by N.I. Loshakova et. al scale [40], in tomatoes by S.D. Grishechkina et. al [36]. Prevalence of barley plants with root rot was evaluated on the VIZR scale (All-Russian Institute of Plant Protection, St. Petersburg) [41]. Rhizoctoniosis infection in potato tubers was recorded according to the guidelines [42].

Results. Batsikol effect on Colorado potato beetle has been identified earlier. Treatment of potatoes in Stavropol' and Krasnodar territories, in Volgograd and Leningrad regions and in North Ossetia demonstrated high (to 96-100 %) activity against this pest (2).

Batsikol showed its activity against dangerous mass pests, such as cruciferous fleas of *Phyllotreta* genus [43], oriental mustard leaf beetle *Colaphellus hoefti* Men., flea beetle *Phyllotreta vittula* Redt., pollen beetle *Meligethes aeneus* F., cabbage leaf beetle *Phaedon cochleariae* F., elm leaf beetle *Xanthogaleruca luteola* Müller [44], cereal leaf beetle *Oulema melanopus* L., shield bugs (*Eurydema*), scale insects (*Diaspididae*) [2], strawberry blossom weevil *Anthonomus rubi* Hbst. [45], buckwheat weevil *Rhinoncus sibiricus* Faust [46], carrot psyllid *Trioza apicalis* Frst., raspberry mite *Eriophyes gracilis* Nal. [47], and thrips *Thysanoptera* [48] (Table).

Biological efficacy of the *Bacillus thuringiensis* var. *darmstadiensis*-based liquid Batsikol form (H₁₀) developed by the All-Russian Research Institute of Agricultural Microbiology (St. Petersburg) against pest phytophages in various crops (1994-2013)

Pest	Crop	Test sites	Efficacy, %
Colorado potato beetle <i>Leptinotarsa decemlineata</i> Say	Potato	Novosibirsk and Leningrad Regions	96-100
Cruciferous flea beetles of genus <i>Phyllotreta</i>	Cabbage	Novosibirsk and Leningrad Regions	92-96
Oriental mustard leaf beetle <i>Colaphellus hoefti</i> Men.	Mustard	Volgograd Region	98
Flea beetle <i>Phyllotreta vittula</i> Redt.	Spring cereals	Stavropol Territory	89
Cereal leaf beetle <i>Oulema melanopus</i> L.	Cereals	Stavropol Territory	82-100
Pollen beetle <i>Meligethes aeneus</i> F.	Rape	Volgograd Region	75-80
Cabbage leaf beetle <i>Phaedon cochleariae</i> F.	Cabbage	Volgograd Region	82.5-98.9
Elm leaf beetle <i>Xanthogaleruca luteola</i> Müller	Cabbage	Volgograd Region	86-90
Strawberry blossom weevil <i>Anthonomus rubi</i> Hbst.	Strawberry	Leningrad Region	75-80
Buckwheat weevil <i>Rhinoncus sibiricus</i> Faust	Buckwheat	Primorsky Territory	56
Carrot psyllid <i>Trioza apicalis</i> Frst.	Carrot	Leningrad Region	47.2
Raspberry mite <i>Eriophyes gracilis</i> Nal.	Raspberry	Leningrad Region	96.3
Thrips <i>Thysanoptera</i>	Flower crops	Leningrad Region	80-90

In the study of the product activity against strawberry blossom weevil, its effect on the gray mold pathogen *Botrytis cinerea* Pers. was found in strawberries. Earlier, we observed antifungal Batsikol activity against a number of pathogenic fungi in in vitro experiments. With supplementing medium with the product at the concentration of 10 %, we observed 100 % inhibition of the growth of fungi *Botrytis cinerea* Pers. colonies, 80 % inhibition in *Pythium* sp., 70 % in *Bipolaris sorokiniana* (Sacc.) Shoemaker, 52 % in *Verticillium dahliae* Kleb., 42 % in *Rhizoctonia solani* Kuhn, 51 % in *Fusarium avenaceum* (Fr.) Sacc., 43 % in *F. oxysporum* Schlecht., and 26% in *F. solani* App. et Wr. [49].

The product activity against a number of phytopathogens was confirmed in field and pot experiments. The efficacy of spraying strawberry plants against gray mold was 60-74 % [44]; with soil watering against *Fusarium* wilt it was 74-87 % in tomatoes [50] and 34-42 % in flax [51]; efficacy was 66-71 % in case of soaking barley seeds against root rot [52] and 40-45 % with the treatment of potato tubers against rhizoctoniosis [53].

Thus, our results expand the understanding of *Bacillus thuringiensis* biological features and, in particular, its action spectrum against various pests and dangerous pathogens for many cultivated plants. Along with insecticidal activity, Batsikol possesses antifungal activity as well that has a technological perspective. We found its activity against strawberry blossom and buckwheat weevils, cruciferous flea beetles, carrot psyllid, raspberry mite, thrips, and pathogens *Botrytis cinerea* Pers., *Pythium* sp., *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Verticillium dahliae* Kleb., *Rhizoctonia solani* Kuhn, and *Fusarium avenaceum* (Fr.) Sacc.,

F. oxysporum Schlecht, *F. solani* App. et Wr. Batsikol inclusion in the list of microbiological agents can expand the range of biological plant protection products and their scope, which will contribute to the production of ecologically pure food and to the environment.

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