Journal of Agricultural Technology 2012 Vol. 8(1): 191-203
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Antifungal action of *Bacillus thuringiensis* delta-endotoxin against pathogenic fungi related to *Phytophthora* and *Fusarium*

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Kamenek, L.K., Kamenek, D.V., Terpilowski, M.A. and Gouli, V.V. (2012) Antifungal action of *Bacillus thuringiensis* delta-endotoxin against pathogenic fungi related to *Phytophthora* and *Fusarium*. Journal of Agricultural Technology 8(1): 191-203.

The activity of *Bacillus thuringiensis* subsp. *thuringiensis* delta-endotoxin against pathogenic fungi related to *Phytophthora* and *Fusarium* was investigated. A possible mechanism of the antifungal activity of delta-endotoxin is linked with uncoupling of oxidative phosphorylation and respiration in fungal cells. The toxin caused to increase the respiratory activity of the fungal cultures. This effect was comparable with processes that occurred in insect cells after contact with entomopathogenic bacteria. The antifungal activity of delta-endotoxin against phytopathogenic fungi was dependent on the concentration and time of contact with fungal cells. The protective effects of the endotoxin against *Fusarium oxysporum* f. sp. *lycopersici* as a causative agent of wilt and late blight disease was estimated throughout the storage of tomatoes.

Key words: delta-endotoxins of *Bacillus thuringiensis,* phytopathogenic fungi, antifungal action, crop protection

Introduction

At the present time, microbial insecticidal formulations based on the entomopathogenic bacterium, *Bacillus thuringiensis* (*Bt*) and its metabolites are the most promising and widely used pesticides for the control of arthropod pests. The key of the *Bt* toxic component is the delta-endotoxin, having a protein nature with molecular masses ranging from 60 to 150 kDa. The most usual protein includes polypeptides with molecular masses within the limits of 70-90 kDa which dissociate to the active components in the slightly alkaline surroundings of the intestine of susceptible insects having specific proteolytic enzymes (Schnepf *et al*., 1985; Chestuchina *et al*., 1978). Numerous subspecies

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of *Bt* produce delta-endotoxins that are homologous in composition, but at the same time differ in specificity of action to insects. The synthesis of delta-endotoxins is genetically determined (Debabov *et al*., 1977) and the toxin in *Bt* subsp. *thuringiensis* are the product of genes Cry1Ia10 and Cry1Ba1. The toxin is pathogenic for insects from the order Lepidoptera (Crickmore *et al*., 2007). Morphologically delta-endotoxins are organized as parasporal crystals provoking toxic effects on intestinal epithelial cell membranes of susceptible insects. The contact of epithelial cells with the toxin leads to formation of ion-specific pores in membranes to provide ion transport (Tran *et al*., 2001). As a result, the toxin causes peroxidation of lipids that incorporate into cell membranes, which acts as dissociative factors of oxidative phosphorylation and respiration (Kamenek and Shternshis, 1986). Simultaneously the delta-endotoxins can also activate ATPase, condense of the mitochondrial matrix, cause disruption of the nuclear chromatin, and finally result in cell disintegration. In addition to the insecticidal action of delta-endotoxins, some are active against some bacteria (Yudina and Burtseva, 1997; Kamenek *et al*., 2005). There is also evidence of an antibiotic action of spore and *Bt* complex on phytopathogenic fungi (Grishechkina *et al*., 2002; Kandybin and Smirnov, 1999). There is a supposition that the mechanisms of the antimicrobial action of *Bt* have a likeness with the processes taking place in the case of the insect infections. For example, the stimulatory effect of delta-endotoxin on the respiratory activity of bacteria, *Erwinia* and *Pseudomonas* is apparently a consequence of the uncoupling of oxidative phosphorylation and respiration. The bactericidal action of the toxin was demonstrated in cabbage, cucumber and oats diseases caused by *Erwinia* and *Pseudomonas* (Yudina and Burtseva, 1997). Additional information showed that the delta-endotoxin has a cytostatic effect on several economically important phytopathogenic fungi related to *Fusarium, Bipolaris, Phytophthora, Alternaria,* and *Rhizoctonia* (Tyulpineva, 2003). A single cell structure of eukaryotic organisms demonstrated the universal mechanism of the *Bt* delta-endotoxin action to both insect and fungal cells (Tran *et al*., 2001). However, the mechanism of the antifungal action of delta-endotoxins remains scantly explored. Subsequent study of the relationship of delta-endotoxins with phytopathogenic fungi was of practical importance from the perspective of the development of novel microbial pesticides for control of plant diseases. The aim of this work was to study the influence of the delta-endotoxin *B. thuringiensis* on phytopathogenic fungi, as well as to assess its efficacy as a potential microbial fungicide.

Material and methods

The bacterium *Bt* subsp. *thuringiensis* strain 202 was used as a producer of delta-endotoxins. Bacterial culture for experiments was obtained from the State Research Institute of Genetics and Selection of Industrial Microorganisms (Russia, Moscow). Phytopathogenic fungi, *Fusarium oxysporum* f. sp. *lycopersici* Snyder & Hansen, *Fusarium graminearum* Schwabe and *Phytophthora infestans* (Mont.) de Bary were used as a test subjects. The first fungus, a causative agent of tomato fusariose, was obtained from the Department of Plant Pathology at Moscow Agricultural Academy named after K.A. Timiryazev. Two other fungal cultures, causative agents of cereals rot and late blight of potato accordingly, were received from the Department of Microbiology at Kazan State University (Kazan, Tatar Republic, Russian Federation). *Bt* bacterium was cultivated on fish peptone agar medium (FPA) at 27˚C, and pH 7.2-7.5 over a 4 day period. Biomass of *Bt* containing endotoxin crystals and spores was separated using centrifugation at 3000 RPM for the 20 min and then washed with distilled water to eliminate components from the growth media and water-soluble toxins. The endotoxin crystals were separated from spores through flotation by means of a two-phase medium based on chloroform and an aqueous solution of Na_2 SO_4. As a result, the crystals are concentrated in the aqueous phase, and then the endotoxin is isolated via centrifugation at 3000 RPM for 20 min. Alkaline hydrolysis of the crystals was carried out by the method of Cooksey (Cooksey, 1968; Fadeeva and Kuzmicheva, 1977).

The solution was then dialyzed and diluted with water to the desired concentration. Hydrochloric acid (0.1 N) was used to titrate the pH to 7.8. The solution contained several protein components. Separation of proteins was done using electrophoresis. The dominant faction included proteins of 120-140 kDa (50%), and the other part contained the polypeptide components of 60-90 kDa. The toxic fractions used for the experiments were additionally identified by means of electrophoresis with the following marker proteins: bovine serum immunoglobulin G (Mr 150 kDa), transferrin (Mr 80-90 kDa), albumin (Mr 67 kDa), and cytochrome C (Mr 13, 37 kDa). Finishing purification and sterilization of delta-endotoxins was completed by microfiltration through a bacterial filter (pore diameter 0.4 microns). The protein concentration in the suspension was determined spectrophotometrically by the method of Lowry (1951). The effect of delta-endotoxin on oxygen consumption by fungal cells was assessed the Warburg manometric method using a modified nutrient Starr's medium (Baslavskaya and Trubetskova, 1964; Kamenek *et al.,* 2005). The number of fungal propagules was calculated by direct enumeration techniques and dilution plating (Goettel and Inglis, 1997).

Phytopathogenic fungi were grown on potato dextrose agar (PDA) at 28º C and pH 6.6. For the estimation of the effect of delta-endotoxin on oxygen consumption of fungal cells, the fungal strains were cultivated at 28ºC, and pH 7.0 using a modified Starr medium (Baslavskaya and Trubetskova, 1964). After sterilization the medium was cooled to 40ºC and toxin was added at concentrations of 0.01%, 0.05%, and 0.1% (pH 7.2). Phytopathogenic fungi, after 10 days cultivation were used for the preparation of 10 – 20 mm sized blocks. The blocks were placed on the surface of the medium containing the *Bt* delta-endotoxin. Fungi growing on the same medium without toxin served as a control. The incubation period was 10 days at 28ºC. Observation on the growth of cultures was performed at 2, 4 and 6 days after adding the toxin. The key indicators of the toxin's influence to cultures of fungi were changed in the linear growth of the colonies on the medium surface, morphology and pigmentation of the colonies. The percent of inhibition of the phytopathogenic fungus caused by the delta-endotoxin was determined using the formula:-

$$\frac{D_k - D_o}{D_k} \times 100,$$

Where D_k - The diameter of fungal colonies in the control;
D_o - The diameter of fungal colonies in the experiment.

Tomato fruits, *Lycopersicon esculentum* L. (Madrigal variety) having different degrees of natural damage of wilting disease were selected for estimation of the effect of delta-endotoxin on this disease during storage. Treatment of tomatoes was carried out at toxin concentrations of 0.01% and 0.05% applied at a rate of 10ml/kg. Control groups were treated with sterile distilled water. The effectiveness of the delta-endotoxin was compared with the antifungal antibiotic nystatin at a concentration of 0.01% in distilled water. Each experiment included ten fruits with four replicatiuons. The experiment was repeated three times. Tomato fruits were stored in boxes at 20º±2ºC and a relative humidity of 60% (Fadeeva and Kuzmicheva, 1977). Disease manifestation was determined by visual basis. The final identification of the disease was carried out after isolation of the fungal pathogen in pure culture.

Data analysis

Statistical analyses were computed with the statistical software SAS (SAS Institute, 2002). The evaluation of different delta-endotoxin concentrations on the development of phytopathogenic fungi was realized by means to compare across treatments using one-way analysis of variance followed by the Fisher’s

Least Significance Difference (LSD) test for post-hoc comparison of means (P<0.05%).

Results and discussion

Effect of delta-endotoxin of B. thuringiensis on phytopathogenic fungi in vitro

Electrophoretic separation of protein hydrolizates showed that the polypeptide component fraction with Mr 120-140 kDa was dominant (55%), and the other part includes polypeptide components of 60-90 kDa. All polypeptide complexes were used for experiments linked with the estimation of the cytopathic effect of delta-endotoxin. The assessment of delta-endotoxin activity in two different concentrations using six species of fungi showed that the toxin has a strong antifungal action.

Cultures of phytopathogenic fungi under the influence of delta-endotoxin exhibited significantly reduced growth rates (Table 1), had suppressed sporulation, and changes in the morphology and pigmentation of colonies. The identical situation was noted during cultivation of fungi on the surface of a dense media with chemical components limiting fungal growth (Bilay, 1982). Phytopathogenic fungi showed different levels of sensitivity to delta-endotoxin in the period of the experiments. The magnitude of the effect was dependent on the type of test organism and the concentration of delta-endotoxin. The endotoxin concentration of 0.05% caused a significantly more marked inhibition of growth for all cultures, compared with a low concentration of 0.01%. Nevertheless, the data related to the influence of the low concentration (0.01%) of delta endotoxin to inhibition of fungi was statistically reliable. All cultures of phytopathogenic fungi can be divided into two groups based on their level of sensitivity to the toxin. The first group had the most sensitivity to the toxin and included *F. infestans* (strain 3 and 5)*, B. sorokiniana,* and *R. solani*. Growth inhibition of colonies 2 days after treatment varied from 25.0% to 36.4% at a delta-endotoxin concentration of 0.01% and from 48.1% to 95.8% at a concentration of 0.05% (Table 1). The second group included the fungi *F. oxysporum, F. graminearum* and *A. brassicae* and had relatively low sensitivity to the toxin. The delta-endotoxin inhibited growth of these fungi from 7.4% to 15.6% at a concentration of 0.01% and 23.5% to 48.1% at a concentration of 0.05%. The differences in sensitivity to delta-endotoxin among those 2 groups were statistically significant and highly significant, respectively.

Table 1. The antagonistic effect of delta-endotoxin on phytopathogenic fungi

Concentration of toxin,% Type of fungi	Inhibition of growth of the colonies of the fungus,% per day					
	2		4		6	
	0,01	0,05	0,01	0,05	0,01	0,05
Fusarium oxysporum	9.1 ± 0.8	31.8 ± 0.7**	7.2±0.6	23.8±1.2**	5.9±0.9	17±1.0**
Fusarium graminearum	7.4 ± 0.6	48.1 ± 2.0***	5.0±0.7	61.0±1.8***	4.3±0.6	54.9±1.7***
Fusarium oxysporum f. *lycopersici*	12.0 ± 1.7	40.0 ± 3.1***	8.5±0.6	45.3±1.7***	6.81±1.1	40.5±2.5***
Phytophthora infestans 3	36.4 ± 2.2	75.0 ± 6.2***	30.8±4.2	63.9±2.0***	26.1±2.1	51.1±7.8***
Phytophthora infestans 5	25.0 ± 4.1	95.8 ± 3.6***	8.0±0.9	90.0±4.3***	6.5±0.5	87.0±4.0***
Bipolaris sorokiniana	15.6 ± 2.3	75.0 ± 4.8***	7.6 ± 1.2	68.6 ± 3.8***	6.2 ± 0.7	65.4 ± 4.6***
Rhizoctonia. solani	33.3 ± 3.6	77.0 ± 2.7***	10.0±1.5	70.5±3.7***	0	35.9±2.1***
Alternaria brassicae	11.8 ± 1.8	23.5 ± 2.6***	1.4±0.3	20.8±0.8**	6.9±1.1	6.4±1.0**
LSD_{05}	2.3	3.5	2.1	3.4	1.9	3.5

Note: The significance level for the differences between the concentrations: ** $P < 0.01$; *** $P < 0.001$

Different sensitivities of pathogenic fungi to the delta-endotoxin *Bacillus thuringiensis* subsp. *thuringiensis* may be explained by several reasons. Chemical composition of the cell wall of fungi including chitin or cellulose can play an important role as a natural barrier against of the various external inhibitors. The specificity of the action of antibiotic substances may also be explained by differences in the biological and morphological properties of fungal pathogens such as speed of growth, thermo resistance, pigmentation, size of the propagules etc. There is information about the significantly strong influence of environmental conditions to viability of the fungal mycelium with the light and small spores typical for *Fusarium* fungi (Ivanova and Marfenina, 2001). It is noteworthy that in all cases after reaching a maximum fungistatic effect (2 days) the phytopathogenic fungal cultures started to grow. The effectiveness of growth was inversely proportional to the concentration of the toxin. Toxin concentration of 0.01% for fungal cultures with a lower sensitivity of the inhibitory effect was significantly reduced by day 6. The inhibitory effect caused by the higher concentration of toxin (0.05%) remained throughout the entire experimental period, although after 6 days, cultures of *F. oxysporum, R. solani* and *A. brassicae* had increased growth. The more sensitive cultures had a significant reduction of inhibition, which reached of magnitudes from 6.2% to 26.1% by day 6 at a concentration of delta-endotoxin of 0.01% and from 35.9 to 87% at a concentration of 0.05%.

The obtained data may indicate that the effect of the delta-endotoxin in the sensory cells of the fungus occurs in a short period after the beginning of contact with fungal propagules during which the toxin is embedded in the membrane of sensitive cells. The binding of toxin molecules with cell membranes is irreversible. For this reason, the use of higher concentrations of the toxin leads to an increase in the amount of contact with the cells, each of which has a definite number of toxin-sensitive receptors (Smith and Ellar, 1994). There is also a direct correlation between the rate of growth of mycelium

and the degree of suppression of its linear growth (Tyulpineva, 2003). Micromycetes having a high rate of growth are more sensitive to the toxin, compared with those that are slow-growing. This regularity manifested to a considerable degree at the later stages of cultivation. It should be noted that for all species of fungi there was an increased in the rate of growth of mycelium for the controls over the entire period of observation. Under the action of the toxin, this value was reduced compared with the controls, but mostly the upward trend was preserved, which confirms the nature of the toxin as fungistat.

The stimulating effect of delta-endotoxin on the respiratory activity of the bacterial genera *Erwinia* and *Pseudomonas* has been previously noted (Kamenek *et al*., 2005) as well as in insect intestinal cells (Kamenek, 1998). These are apparently a consequence of the uncoupling of respiration and phosphorylation. A similar effect can be expected in the case of microscopic fungi.

The fungi *P. infestans*, characterized as highly sensitive to the toxin, and *F. graminearum,* with relatively low sensitivity to the toxin, were selected to study the effect of delta-endotoxin on the respiratory activity of the fungal cultures. There is information that the respiratory chains of mitochondria of animals and fungi have both similar and different characteristics. The fungi have cytochromes related to three of the four that are characteristic for eukaryotes; namely cytochrome a_1, a_3, b, b_1, b_2, c and c_1. Also alternative respiratory chains have been identified in fungi. The blocking of the respiratory chains by cyanide, carbon monoxide or azido-cytochrome aa_3 leads to the interruption of the transition from the oxidized to a restored condition and back to the restored components of the chain of cytochromes. In the case of animal cells such blocking leads to complete cessation of breathing. However, in a number of insensitive to cyanide fungi the respiration is not stopped, that is obviously due to the inclusion of an alternative respiratory chain (Bilay, 1977; Akimenko, 1989; Medentsev *et al*., 2001).

Studies of respiratory activity showed that the reseeding of fungi on medium of Starr is accompanied by an increase in respiratory rate associated with the increased content of carbon sources in the environment and the respiratory substrates (Fig. 1). During the first two minutes of observation the rate of respiration *F. graminearum* reduced to a value that is optimal for growth on this medium (0.018 mm / min). Possibly this low rate of respiration is connected with the depletion of substances that stimulate active respiration. The respiratory rate was equal to 0.018 mm / min with no further changes (background rate). Addition of 0.1 ml of the delta-endotoxin at a concentration of 0.05% to the medium at the time of incubation that approached the background rate of respiration of the fungus (2 minutes of incubation) provoked

a dramatic increase in the rate of respiration for the test organism (Fig. 1). Cell respiration caused by the toxin increased more than 40 times that of the background. After 3 minutes into the stimulation of respiration a significant inhibition of oxygen consumption occurred with a subsequent jump in the rate of respiration exceeding that of the background respiratory rate by more than 35 fold. A decreased in the rate of oxygen consumption to zero was observed 30 seconds after reaching the minimum. The initial stimulation of respiration after addition of the toxin is characteristic for uncouples of oxidative phosphorylation and respiration. The emergence of subsequent peaks in the graph is probably due to the inclusion of the alternative respiratory chain of the test organism. A similar effect was observed earlier by the action of delta-endotoxin in bacteria (Kamenek*, et al*., 2005). The alternative oxidase of fungi is a protein with a mass of 36-37 kDa, and is encoded by nuclear genes (Bilay, 1977). The appearance of alternative oxidase in fungi is induced by many factors, including oxidative stress, thermal shock, the presence of respiration inhibitors, depletion of glucose (transition to stationary phase), etc. (Akimenko, 1989). These data suggest that alternative oxidase is a component of a complicated adaptive response of cells to various stress factors. Thus, the second and subsequent maxima were apparently caused by the inclusion of cyanide-resistant electron transport pathways followed by inhibition of oxidative phosphorylation and respiration as a result of the delta-endotoxin action. A similar picture was noted when cell culture *P. infestans* strain 3 was exposed to the toxin on (Fig. 2). Two maximum rates of respiration were observed after adding the toxin, which exceeded the figure for the first stage of the experiment (before the addition of toxin) by 1.5 times. Complete suppression of the oxygen consumption rate occurred after 16 minutes of the experiment (Fig. 2). The sharp decline of the respiratory rate to near zero within 30 seconds was observed for cell culture of the *P. infestans* strain 5. A special feature is that subsequent maxima were not observed for the remaining 9 minutes of the experiment, which was likely due to the complete inhibition of growth of the fungal culture (Table 1). This phenomenon once again emphasizes the specifics of the possible mechanism of action of delta-endotoxin in cell cultures of fungi of different taxonomic groups (Fig. 3). The nature of stimulation of cellular respiration of fungi described earlier corresponds to the epithelial cells of insects and bacteria (Kamenek, 1998; Kamenek *et al*., 2005). Repeated successive peaks of the cellular respiration rate of the test organism under the action of the toxin were apparently related to the inclusion of an alternative respiratory chain of the fungus. This method can be used to quickly establish the presence of sensitivity and assessment of its extent in pathogenic fungi to the delta-endotoxin *B. thuringiensis*.

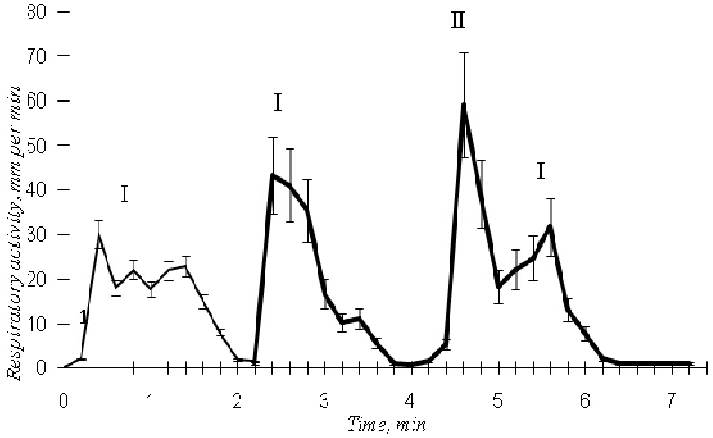

Fig. 1. Stimulatory effect of delta-endotoxin *Bacillus thuringiensis* on cell respiration culture *Fusarium graminearum*: 1 - Background respiration rate, 2 - respiration rate under the action of toxin

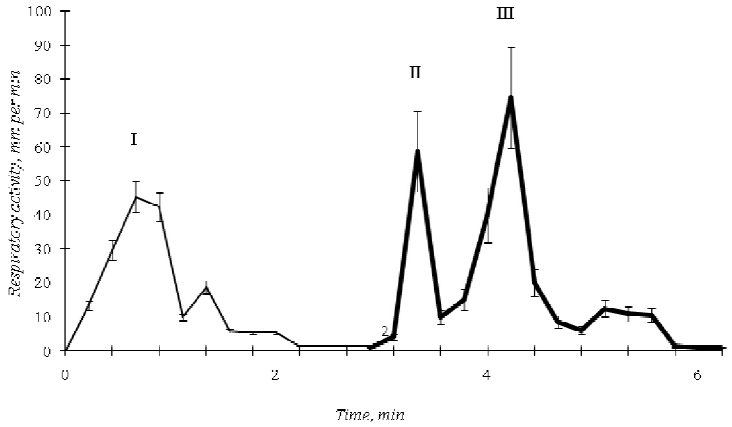

Fig. 2. Stimulatory effect of delta-endotoxin in cell culture respiration of *Phytophtora infestans* strain: 3 1 - Background respiration rate, 2 - respiration rate under the action of toxin

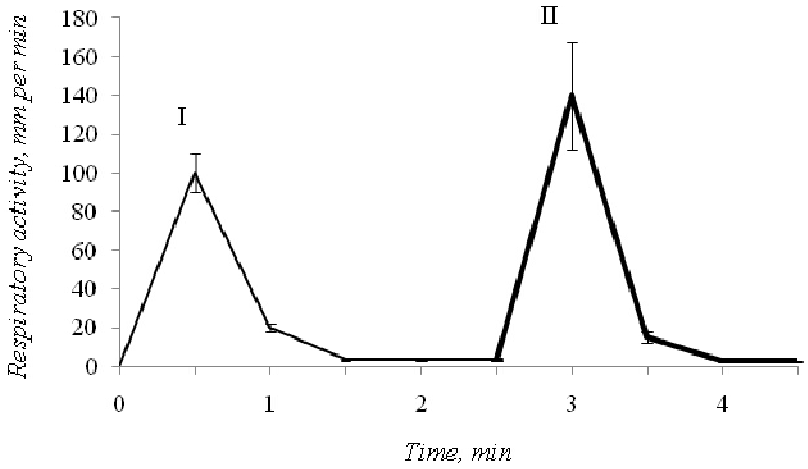

Fig. 3. Stimulatory effect of delta-endotoxin *Bacillus thuringiensis* on cell respiration in culture *Phytophtora infestans* strain 5: 1 - Background respiration rate, 2 - respiration rate under the action of toxin

Effect of delta-endotoxin on the development of late blight of tomato fruits in storage

According to the received information, processed fruit toxin has a pronounced myco-static effect (Table 2). After 18 days of observations, the prevalence of late blight on fruit treated with the toxin did not increase as compared to the first day, but at 25 days was lower than in the controls. Symptoms of late blight were not visually observed for up to 18 days.

According to Table 2, after 18 days the number of fruits in the control group with no signs of damage was 67%, and those with a damage score of 2 was 33%. After 25 days the number of damaged fruits as well as the degree of damage increased: the number of damaged fruits in the 1, 2 and 3 score categories was 65%, 11% and 12%, respectively. Among the delta-endotoxin treated tomato fruits, no damage were observed after 18 days, and insignificant spoilage was detected after 25 days with 42% of fruits having a damage degree score less than 1. Nystatin showed 100% efficacy in the suppression of late blight of tomato throughout 25 days of the study. The effect of nystatin on phytopathogenic fungi have been described previously (Tarr, 1972) but due to the fact that this antibiotic is used for medical purposes, it is unacceptable to apply it in agriculture.

Table 2. Effect of delta-endotoxin *Bacillus thuringiensis* on the development of late blight of tomatoes in storage

№	Variant	18 days						25 days					
		The spread of the disease%	Number of damaged tomatoes, % / point					The spread of the disease%	Number of damaged tomatoes, % / point				
			0	1	2	3	4		0	1	2	3	4
1	Control: * water	33	67±8	0	33±6	0	0	80	12±3	65±2	11±4	12±4	0
2	Delta-endotoxin: 0.1%	0	100	0	0	0	0	52	58±6	42±3	0	0	0
3	Nystatin: 0.1%	0	100	0	0	0	0	0	100	0	0	0	0
4	1 point infestation 0.1% delta-endotoxin	100	0	75±4	0	0	25	100	0	0	0	0	100±0
5	1 point infestation	100	0	0	8±2	16±2	76±7	100	0	0	0	0	100±0
6	LSD_{05}	56.0	58.3	31.7	13.8	6.7	32.5	60.1	45.5	30.8	4.8	5.2	56.6

It should be noted that the effectiveness of delta-endotoxin was inversely proportional to the initial grade of fruit damage. The best results were obtained for relatively healthy fruits and fruits with low damage scores. Tomatoes fruits with an initial grade of fungal disease after treatment of endotoxin only showed 25% damage after 18 days. At the same time in the controls 100% of the fruits were spoiled among the different damage level grades. Thus, the application of delta-endotoxin to tomato fruits with low initial grade lesions (1 point) reduces the degree of development of wilting or late blight compared with the same fruit, not treated with the toxin. Tomatoes with an original 3 grade of fungal damage that were treated with the delta-endotoxin showed only 33% of spots with a 4 grade score, while at the same time the number of fruits in the control with a 4 grade score was 76%.

Endotoxin is able to control the disease within 3 weeks even in the case where the fruits were initially damaged. The endotoxin is considered to be nontoxic for people and warm-blooded animals and this substance can be considered as a very promising product for use on fruits during storage and other perishable agricultural products.

Thus, these data enabled us to establish the sensitivity of some pathogenic fungi in culture to delta-endotoxin *B. thuringiensis* subsp. *thuringiensis* strain 202, as well as state differences in the susceptibility of the studied crops. Delta-endotoxin has been effective in curbing the development of late blight of tomatoes during storage.

Acknowledgment

This research was supported by the Ministry of Education and Science of Russian Federation.

References

Akimenko, V.K. (1989). Alternative oxidases of microorganisms. Publisher: Nauka, Moscow: 1-263 (Russian).

Bilay, V.I. (1977). Fusarias. Publisher: Naukova Dumka, Kiev: 1-442.

Bilay, V.I. (Ed.). (1982). Methods of experimental mycology. Reference book. Publisher: Naukova Dumka, Kiev: 1-550 (Russian).

Baslavskaya, S.S. and Trubezkova, O.M. (1964). Practicum for plant physiology. Publisher: Moscow State University, Moscow: 327 (Russian).

Chestuchina, G.G., Zalunin, I.A. Kostina, L.I., Kotova, T.S. Kastrucha, S.P., Lublinskaya, L.A., and Stepanov, V.M. (1978). Proteolytic enzymes linked with crystals of *Bacillus thuringiensis.* Biochemistry v. 43: 857-864 (Russian, English summary).

Cooksey, K.E. (1968). Purification of a protein from *Bacillus thuringiensis* toxic to a larvae of Lepidoptera. // Biochem. J., v. 106: 445- 454.

Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., van Rie, J., Lereclus, D., Baum, J., and Dean, D.H. (1998). Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiology and Mol.Biol. Rev. vol. 62, №3: 807-813

Debabov, V.G., Azizbekian, R.R., Chlebalin, O.I., Dyachenko, V.V., Galushka, F.P. and Belish, P.A. (1977). Isolation and preliminary characteristics of chromosomal DNA elements of *Bacillus thuringiensis*. Genetics, v.13, № 3: 496-501 (Russian, English summary).

Fadeeva, Y.N. and Kuzmicheva, A.A. (Eds). (1977). Methods of phytopathological and entomological research in plant selection. Moscow, Publisher Kolos: 223 (Russian).

Goettel, M.S. and Inglis, G.D. (1997). Fungi: Hyphomycetes. In Manual of Techniques in Insect Pathology (Ed. L.A. Lacey), Academic Press New York: 213-250.

Grishechkina, S.D., Smirnov, O.B. and Kandybin, N.V. (2002). Fungistatic activity of different subspecies of *Bacillus thuringiensis*. Mycology and Phytopathology, v.36, №1: 58-62 (Russian, English summary).

Ivanova, A.E. and Marfenina, O.E. (2001). Influence of ecological factors to growth possibility of mycelial fragments and spore germination of microscopic fungi. Microbiology, v. 70, n 2: 235–240.

Kamenek, L.K., and Shternshis, M.V. (1986). Disconnecting act of delta-endotoxin of *Bacillus thuringiensis* Integrated plant protection from diseases and pests in Siberia. Siberian Division of USSR Academy of Agriculture, Novosibirsk: 80-85 (Russian, English summary).

Kamenek, L.K. (1998). Delta-endotoxin of *Bacillus thuringiensis:* structure, properties and utilization for plant protection. Doctoral thesis, specialty 06.01.11, Timiryazev Moscow Academy of Agriculture, Moscow: 1-43 (Russian).

Kamenek, L.K., Levina, T.A., Terechin, D.A. and Minacheva, L.D. (2005). Antibacterial activity Of delta-endotoxin of *Bacillus thuringiensis* as a potential mean of plant protection. Biotechnology, №1: 59-67 (Russian, English summary).

Kandybin, N.V. and Smirnov, O.V. (1999). About creation of formulations with poly-functional properties. Agro XXI, №9: .11 (Russian).

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with folin phenol reagent. J. Biol. Chem. v. 193, №1: 265-275.

Medenzev, A.G., Arinbasarova, A.Y. and Akimenko, V.K. (2001). Contain cAMP at condition of induction of alternative oxidase in east cells *Yarrowia lipolytica*. Microbiology, v.70, n1: 29-33.

Schlotzhauer, S.D. and Little, R.C. (1987). SAS System for elementary statistical analysis. SAS Institute Inc., Cary, NC, USA: 1-416.

Schnepf, H.E., Wong, H.C. and Whiteley, H.A. (1985). The amino acid sequence of a crystal Protein from *Bacillus thuringiensis* deduced from the DNA base sequence. J. Biol. Chem., v.260, №10: 6264-6272.

Smith, N.R. and Ellar, D.J. (1994). Mutagenesis of two surface – exposed loops of the *Bacillus thuringiensis* Cry I C delta-endotoxin affects insecticidal specifility. Biochem. J., v 302: 611-616.

Tarr, S.A. (1972). The principles of plant pathology. Winchester Press, New York, 1-632.

Tran, L.B., Vachon, V., Schwartz, J.L. and Laprade, A. (2001). Differential effects of pH on the pore-forming properties of *Bacillus thuringiensis* insecticidal crystal toxins. Appl. and Environment. Microbiol., v.67, №10: 4488-4494.

Tyulpineva, A.A. (2003). Action of delta-endotoxin of *Bacillus thuringiensis* in the direction of several phytopathogenic fungi. Doctoral thesis, specialty: 03.00.16. Ulyanovsk University, Ulyanovsk: 123 (Russian).

Yudina, T.G. and Burtseva, L.I. (1997). Action of delta-endotoxin of four subspecies of *B. thuringiensis* on different prokaryotes. Microbiology, v.66, №1: 25-31 (Russian, English summary).

(Published in January 2012)