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Polyfunctional Properties of the Entomopathogenic Bacterium in Protecting Potato in Western Siberia

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The Colorado potato beetle and the plant pathogen *Rhizoctonia* are the major harmful organisms affecting potatoes in Western Siberia. The present study has shown the possibility of simultaneous biological control of pests and diseases by preventing treatment of tubers with entomopathogenic bacterium *Bacillus thuringiensis* subsp. *darmstadiensis* (BtH_{10}), strain RCAM 01490, followed by spraying with the same suspension during the growing season. The tests of middle-early cultivar "Yuna" were conducted under laboratory and field conditions during the course of two years. Population of the Colorado potato beetle has decreased by 3 times during both years in comparison with the control. The severity of stem canker decreased by 23.8 and 11.4 times, while stolon canker - by 2.6 and 12.4 times in 2014 and 2015, respectively. The growth-promoting action was manifested in increased height of plants, number of stems and stolons, as well as the increase in the proportion of large daughter tubers. As a result, the insecticidal, antifungal and growth-promoting cumulative effect of the entomopathogenic bacterium was revealed, resulting in higher biomass of potato daughter tubers, which exceeded biomass in control test by 1.4-1.6 times.

Key words: Entomopathogenic bacterium, insecticidal and fungicidal activity, plant growth stimulation, potato, polyfunctional properties.

The Colorado potato beetle (*Leptinotarsa decemlineata* Say) and phytopathogenic fungus (*Rhizoctonia solani* Kuhn.) are the main pests that damage potato (*Solanum tuberosum* L.*)* in Western Siberia. The Colorado potato beetle is the main potato phytophage worldwide[1]. In Western Siberia, it appeared much later, from the end of the XX - beginning of the XXI century and quickly became the most dangerous phytophage over the short time period. It is known that the chemical insecticides were the first most effective means to control the Colorado potato beetle[2]. However, studies carried out in various countries revealed gradually acquired resistance of this insect towards chemical insecticides[3,4]. Besides, the adverse effects of chemicals on the environment and human health are well known[5,6]. This has led to the search for alternative environmentally safe methods to control the population of the Colorado potato beetle[7-9]. The use of natural biological agents of bacterial and fungal origin seems to be the most attractive means. Thus, as early as in the middle of the XX century it was proposed to suppress the population of the beetle larvae by spraying potato plants with fungi *Beauveria bassiana* Bals. Vuil[10,11] and later with *Metarhizium spp*.[12,13]. Compared to fungus-based formulations, the Russian Bitoxibacillin preparation, based on exotoxin-

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containing strain *Bacillus thuringiensis* subsp. *thuringiensis*, turned out to be more effective[14]. In addition, in 1983, a new subspecies of *Bacillus thuringiensis* subsp. *tenebrionis*[15] were isolated and identified, which was specific against the Colorado potato beetle and served as the basis for the development of biological preparations.

Along with the Colorado potato beetle, a serious problem in West Siberia is *Rhizoctonia* disease of potato. To control disease caused by phytopathogenic fungus *Rhizoctonia solani* Kuhn., the main method of potato protection in West Siberia, like in other geographical regions of the world, is the application of chemical fungicides. However, a growing body of research is currently focused on the development of microbiological methods for protection against *Rhizoctonia* disease[16-18].

At present time, the biological preparations not always are competitive with chemical pesticides, and one of the reasons is the high selectivity of the biological control agent, forcing the use of two or more biological preparations instead of one chemical pesticide. To improve competitiveness it is desirable to use biological preparations of polyfunctional action, possessing, for example, both insecticidal and fungicidal properties.

The biological agents of such effect include bacteria of the genus *Bacillus*: *B. subtilis*[19, 20] or *B.thuringiensis*[21-24]. Previously, it was shown that strain B. *thuringiensis* ssp. *darmstadiensis* (BtH_{10}), isolated from the Colorado potato beetle, exhibited insecticidal activity against the Colorado potato beetle of northwest and south populations[25]. In addition, this strain has shown antifungal activity against *Rhizoctonia* disease of potato at a single treatment of potato tubers when planting "Lubava" and "Lugovskoy" potato cultivars[26]. However, it is important to reveal the dual effect of this bacterium, supplementing the treatment of tubers before planting by spraying with a bacterial suspension during the growing season. Due to the fact that early potato "Yuna" is a promising cultivar for Western Siberia region, it was challenging to use it for carrying out the study on the polyfunctional properties of the entomopathogenic bacterium.

The objective of the present work consisted in evaluation of the insecticidal, antifungal and growth-promoting action of *B. thuringiensis* subsp. *darmstadiensis* at treatment of the potato tubers before planting followed by subsequent spraying during the growing season under the natural conditions of Western Siberia.

MATERIALS AND METHODS

The research objects were the plants of "Yuna" potato cultivar, the Colorado potato beetle, *Rhizoctonia* disease of potato, *Bacillus thuringiensis ssp. darmstadiensis* (BtH_{10}), the strain RCAM 01490 taken from the collection of the All-Russian Institute for Agricultural Microbiology.

The efficacy of the bacterial strain against the Colorado potato beetle in a laboratory experiment was tested on larvae of all instars, taken from natural populations. Larvae were planted on the leaves of "Yuna" potato cultivar in Petri dishes and sprayed with a bacterial suspension (10^6 CFU/ml). The leaves were substituted while drying. Larval mortality was estimated on the 3rd, 5th, 7th and 10th day. Biological efficacy was calculated by the Abbot formula.

Field experiments were conducted on potato experimental plots, located in the Novosibirsk District of the Novosibirsk Region ($55^0$1´N, $82^0$55´E). The experiments were performed in three replicates; the area of each plot was 30 m^2 and included 360 plants (70õ35 cm planting system). The vegetation period in 2014 was characterized as arid, while in 2015 – as humid.

Field experiments included 2 variants in three replications: 1) control (without treatment); 2) treatment of tubers with BtH_{10}, followed by spraying during the growing season with bacterial suspension (10^6 CFU/ml). The mortality of the Colorado potato beetle larvae was checked 7 and 10 days after spraying the plants.

The potato stem canker and stolon canker were evaluated 6 and 10 weeks after planting. Disease severity was determined on a Frank scale[27], calculating the disease incidence (P) and disease severity (R) by the formulas: $P = \frac{R \times 100}{N}$, where R – is the number of diseased plants, N – is the total number of record plants; $R = \frac{\sum (a \times b) \times 100}{N \times K}$ where Σ(a×b) – is the sum of the products of the number of diseased plants (a) over the score of the lesion (b), where N – is the total number of record plants, K – is the highest score of the accounting scale. Six weeks after potato planting, samples of potato leaves were collected from the control and experimental sites to determine the peroxidase activity [28].

The degree of black scurf of the daughter tubers was estimated by the ratio of the mass fraction of healthy and diseased tubers[29]. Fractional composition of tubers was determined by weighing. Depending on mass, tubers were grouped into 3 fractions: small fraction – up to 35 grams, medium fraction – from 35 to 80 grams, and large fraction – over 80 grams. Growth rate and development of plants were monitored during the growing season. The effect of BtH_{10} on plant height, number of stems and stolons, as well as tubers mass was evaluated during the experiments.

Statistical data processing was performed by standard methods using MS Excel and ANOVA program for Windows. Data were compared by calculating LSD_{05} (Least Significant Difference).

RESULTS AND DISCUSSION

The mortality of 1st and 2nd- instar larvae under the action of BtH_{10} in laboratory occurred on the 1st day, while on the 5th day reached 100%, significantly differing from larval mortality in the control ($p<0.05$). Since older larvae are more resistant to bacteria, their mortality, checked on the 10th day, was by 2.6 and 1.6 times less as compared to younger instar larvae ($p<0.05$) (Table 1). Such dependence is typical for populations of the Colorado potato beetle in different geographic regions under the action of different *B. thuringiensis* subspecies.

Table 1. Biological efficacy of BtH_{10} against the Colorado potato beetle larvae in laboratory experiment

Larval instar	Biological efficacy at daily accounting, %			
	3	5	7	10
L_1	80.0	96.7	100.0	100.0
L_2	66.7	93.3	93.3	100,0
L_3	30.0	35.7	36.9	58.8
L_4	10.0	11.5	16.1	34.5

Table 2. Influence of BtH_{10} on disease severity and incidence of potato stem canker and stolon canker, %

Weeks after planting	Test variants	Average score of the stems lesion		Disease severity on stems, %		Disease incidence on stolons, %	
		2014	2015	2014	2015	2014	2015
6	Control	0.5	0.68	12.6	13.3	18.2	9.5
	BtH_{10}	0.1	0.08	1.9	1.5	9.1	0
10	Control	1.5	0.44	59.6	40.0	36.0	28.6
	BtH_{10}	0.1	0.16	2.5	3.5	13.8	2.3
	HCP_{05}	0.25	0.30	4.0	3.3		

Table 3. Growth-promoting action of strain BtH_{10} at potato protection

Treatment	Year	Plants height, cm			Number of stems Weekly accounting			Number of stolons		
		4	6	10	4	6	10	4	6	10
Control	2014	8.2	20.0	41.2	2.5	3.1	3.1	2	11	25
	2015	11.6	31.0	31.2	3.0	3.4	3.4	3	21	21
BtH_{10}	2014	11.3	22.7	48.3	3.1	3.9	4.2	3	16	29
	2015	13.9	37.1	40.8	4.2	5.0	5.2	4	40	42
	HCP_{05}	through 2014 – 3.4 through 2015 – 4.2			through 2014 – 0.6 through 2015 – 0.9			through 2014 – 3.6 through 2015 – 4.9		

Table 4. Influence of BtH_{10} on fractional composition of daughter tubers

Treatment	Year	Fractional composition of tubers, %		
		Small fraction	Medium fraction	Large fraction
Control	2014	4.8	34.9	60.3
	2015	13.1	63.7	23.2
BtH_{10}	2014	1.2	16.2	82.2
	2015	6.1	45.9	48.0

During two years of research in the field environment, the mortality of the Colorado potato beetle larvae was not less than 60% seven days after spraying bacterial suspension, and at least 70% ten days after spraying (Fig. 1).

At the same time bacterial strain BtH_{10} at the initial treatment of planting material and subsequent spraying of plants during the growing season caused a significant reduction in the infestation of potato stems and stolons by pathogen *Rhizoctonia* (Table 2). Thus, in 2014 the average score of infestation by stem canker statistically significantly ($p<0.05$) decreased by 5-15 times in 6-10 weeks, while in 2015 – by 8.7-2.7 times in comparison to the control. Disease severity on the stems also decreased on average during two years by 9.4-16.6 times over concerned periods. Comparing the data obtained during treatment the tubers of early potato cultivar in 2014 with just the same bacterial suspension without spraying[26], it can be stated that the impact of additional spraying during the growing season on *Rhizoctonia* disease severity is much higher.

Induction of resistance in potato plants under the influence of entomopathogenic bacterium was confirmed by measurement of the

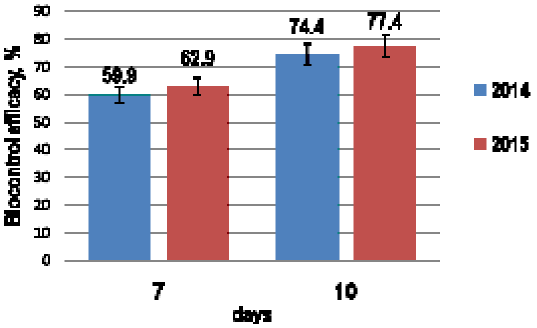

Fig. 1. Biocontrol efficacy (%) of BtH_{10} towards the Colorado beetle larvae in the field

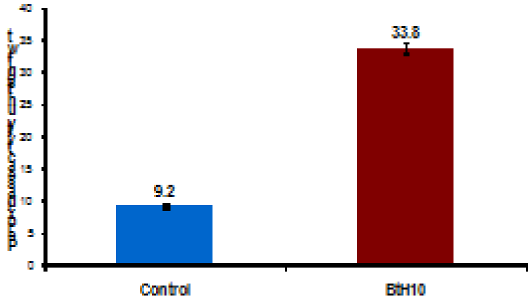

Fig. 2. Peroxidase activity in potato leaves under the influence of BtH_{10} six weeks after planting

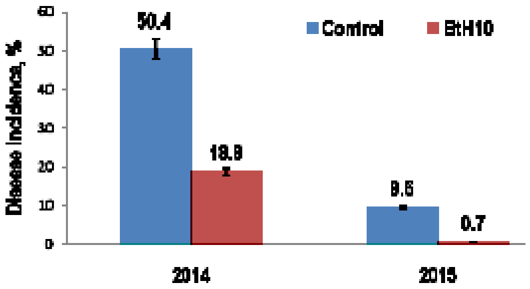

Fig. 3. Disease incidence (%) on the daughter tubers under the influence of BtH_{10} treatment

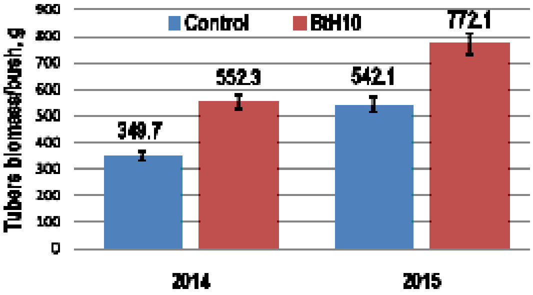

Fig. 4. The effect of BtH_{10} on biomass of the daughter tubers per one bush, g peroxidase activity in potato leaves sampled from control and treated plants (Fig. 2).

Application of BtH_{10} by described two techniques provided also a reduction in the disease incidence on daughter tubers by 2.9 times in 2014 and has led to almost complete release from the disease in 2015 (Fig. 3).

In addition to insecticidal and antifungal activity of bacteria, we revealed also its growth-promoting action on potato. In a field experiment, potato plants have formed bushes faster under the influence of BtH_{10}. In 2015, statistically significant increase ($p<0.05$) of potato plant height in comparison to control was observed 6 and 10 weeks after planting, while in 2014 a significant increase in the height was observed 10 weeks after planting. In both studied years, statistically significant increase ($p<0.05$) in the number of stems in the bush exceeded that of control by 1.2-1.5 times (Table 3). The number of stolons produced was also increased under the influence of BtH_{10}, especially in the year of 2015, which was more favorable in terms of weather conditions (twofold increase).

The proportion of large fraction tubers of the new crop increased under the influence of bacterial strain ($p<0.05$) (Table 4). The increase in tuber mass was due to formation of larger size fractions (exceeding the control by 1.4-2.0 times) as well as reduction in amount of small tubers (by 2-5 times). The mass of tubers per one bush after treating with BtH_{10} was 1.4-1.6 times higher than in the control (Fig. 4).

Our data are consistent with the results on the effect of different *B. thuringiensis* strains on phytopathogenic fungus *R. solani*, damaging other plants[30,31]. Antifungal action of entomopathogenic bacteria the authors explained by the production of different secondary metabolites, including chitinases[32], as well as by inducing systemic resistance of plants[33]. In addition, an antagonistic effect may be due to the production of lipopeptide biosurfactants by the entomopathogenic bacterium[34,35]. The biosurfactants were found also in strains of antagonistic bacteria of the genus *Bacillus*, selected for biological control of plant diseases, including *Rhizoctonia* disease[36-38].

CONCLUSION

The pathogenic effect of *Bacillus thuringiensis* subsp. *darmstadiensis* (BtH_{10}), strain RCAM 01490, on local population of the Colorado potato beetle larvae was shown based on preliminary laboratory experiments. The spraying of planted potato with BtH_{10} suspension significantly reduced the number of beetle larvae. At the same time, the initial treatment of potato tubers with a bacterial suspension, followed by spraying of plants, resulted in reduced stolon canker, stem canker, and black scurf of the daughter tubers. In addition, induction of systemic plant resistance was confirmed by measuring the peroxidase activity in potato leaves. The growth-promoting action of the entomopathogenic bacterium resulted in increased plant height, number of stems and stolons. Thus, in case of damage to potato plants by the Colorado potato beetle and *Rhizoctonia* disease under the natural conditions of Western Siberia, the polyfunctional properties including antifungal, insecticidal and growth-promoting action of *B. thuringiensis* subsp. *darmstadiensis,* strain RCAM 01490, were revealed by treatment of potato tubers before planting followed by spraying with bacterial suspension during the growing season.

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