ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Use of Bacillus Spp. Strains for Biocontrol of Ramularia Leaf Spot on Strawberry and Improving Plant Health in Western Siberia.

A.A. Belyaev, N.P.Pospelova, A.A. Lelyak, M.V. Shternshis, and T.V. Shpatova*

Department of Plant Protection, Novosibirsk State Agrarian University, 630039, Russia.

ABSTRACT

This article shows for the first time the antifungal effect of three strains of the *Bacillus* genus (*B. subtilis*, *B. amyloliquefaciens*and *B. licheniformis*) bacteria isolated in local conditions, against the strawberry disease caused by*Ramularia tulasnei*, in Western Siberia in the period of two consecutive years of testing. Creating stress conditions for the plants during cultivation in pots, as compared with the field experiment, revealed the influence of abiotic factors on the activity of the *Bacillus* genus bacterial strains on strawberry plants affected by the disease. Comparative testing of humic formulations and bacterial strains showed that pre-treatment of strawberry root system with the *B. subtilis* and *B. amyloliquefaciens*bacterial strains more effectively inhibited infection of young leaves with ramularia, as compared with the treatment with humic preparation. Strains of *Bacillus spp.* contribute to improving plant health due to both the growth-promoting effect and inducing resistance to ramularia. The results obtained show the prospect of using bacteria as the basis for biological products for strawberry health management in Western Siberia.

Keywords: *Bacillus spp.*, *Ramularia tulasnei*, strawberry, biocontrol, plant health.

***Corresponding author**

ISSN: 0975-8585

INTRODUCTION

Strawberry is the most common soft-fruit crop in the world, which accounts for more than 70% of global production of berries. In cultivation, the problem of biological control of diseases as an alternative to chemical methods of protection becomes very urgent, since the product should not contain residues of chemical pesticides. Bacterial biocontrol agents, i.e., strains of bacteria of the genus *Bacillus*showed sufficiently high effect of suppressing the most common disease of strawberry, the gray moldcaused by*Botrytis cinerea Pers ex Fr*. [1,2] in various geographical areas of crop cultivation [3-6]. In the conditions of Western Siberia, apart from strawberry protection against the gray mold, biocontrol of ramularia caused by*Ramularia tulasnei Sacc.* (*Hyphomycetales*, *Deuteromycota*)) with strains of the *Bacillus* genus is urgent. It isalso important to take into account the influence of these strains on strawberry plants resistance to abiotic stress factors. It is known that acute or chronic stresses lead to physiological weakening of cultivated plants, which leads to increasing their susceptibility to infectious diseases [7,8]. Both organic fertilizers containing humates [9-11], and microbial agents for plant disease biocontrol may reducethe stress, in particular, the soil-borne bacteria that stimulate growth of plants [12,13]. In a whole, improving plant health includes suppression of plant diseases, stimulation of plants nutrition and growth [14,15], and inducing plants resistance to diseases [16,17]. The aim of the study was to evaluate the action of bacterial strains of the *Bacillus* genus*,*on strawberry disease caused by *Ramularia tulasnei*and their contribution to improving plant health with regard to the influence of stress factors.

MATERIALS AND METHODS

Bacterial strains and humic formulation

*Bacillus subtilis*RCAMB-10641, *Bacillus amyloliquefaciens*RCAMB-10642, *Bacilluslicheniformis* RCAMB-10562 bacterial strains isolated from soils of Western Siberia were supplied from culture collection of the Research Center (Koltsovo, Russia). The Phoenix humic preparation was produced byThe TELLURA-BIS Scientific-Production Company (Biysk, Russia).

Plant,disease, pot and field experiments

Strawberry cultivar Junia Smydes was used in all experiments.The strawberry disease caused by *Ramularia tulasnei Sacc.*was observed.

Seedlings of strawberries were grown in 2013 and 2014 in 500 ml plastic pots filled with soil from the experimental plot. Simultaneously, seedlings were grown in the soil from strawberry mother plantation. This experiment included 10 options, 10 plants in each, located on the experimental plot of the production mother plantation situated in the farm "Gardens of Siberia" (55º1' N, 82º55' E) with gray forest soil.

Treatment and estimated parameters

In pot and field experiments, plants were treated with three strains of biological agents and the Phoenix humic preparation, and the control variant was left without treatment. The root system of strawberry seedlings was preliminarily soaked in a suspension containing bacteria at the concentration of 1×10^5 CFU/ml, or Phoenix at the concentration of 0.05% for 2 hours.

The degree of strawberry plants disease severity was evaluated in percent (%) of leaves surface area occupied by necrotic spots.

The growth-stimulating effect of three bacterial strains, compared to the control and Phoenix, was assessed by the parameters of plant height, weight and length of the root system, and the number of strawberry runners (stolons).

Data analysis

Data from the laboratory and field experiments were statistically processed by standard methods using MS Excel and the SNEDECOR for Windows program. The data was compared by calculating LSD_{05}.

RESULTS AND DISCUSSION

The influence of bacterial strains on strawberry damageby ramularia, growth and development of plants in 2013.

In 2013, the plants were planted simultaneously in the pot and field experiments – on June 11, 2013. At the moment of planting, the seedlings had 2 to 3 leaves. These leaves (old) continued to serve for future adaptation and growth, and fell off by the autumn. In addition, on the plants planted in the experiment, new (young) leaves formed, with which the plants passed into the winter. The leaves of the planted seedlings were initially slightly affected by ramularia in the form of single spots with the average prevalence background of 5-10% of the plants, and were the main source of the infection for subsequent pathogen propagation.

The plants planted into pots, experienced more significant changes in humidity and temperature of soil during the growing season, as compared to the plants with the root system in soil. Thus, stress for plants was created in pots. We assumed that the background stress may increase the susceptibility of strawberry to ramularia that would make it possible to model the stress conditions for assessing the effect of bacterial strains on plant growth and their affection with the disease. The influence of bacterial strains was compared to the influence of the preparation based on humic substances (Phoenix). It is known that humic fertilizers increase absorption of nutrients, having a positive effect on the composition of soil microflora [13], plant growth and increasing the biomass of the product, including strawberries [9,18]. Nutrients may reduce development of plant diseases [6, 14]. In turn, the genus *Bacillus* bacteria are capable of enhancing absorption of nutrients from the soil by plants [19, 20].

In both experiments, no significant decrease in the degree of old leaves damage under the influence of bacterial strains at the first estimation on June 22 (12 days after planting) was observed (Fig. 1-2).

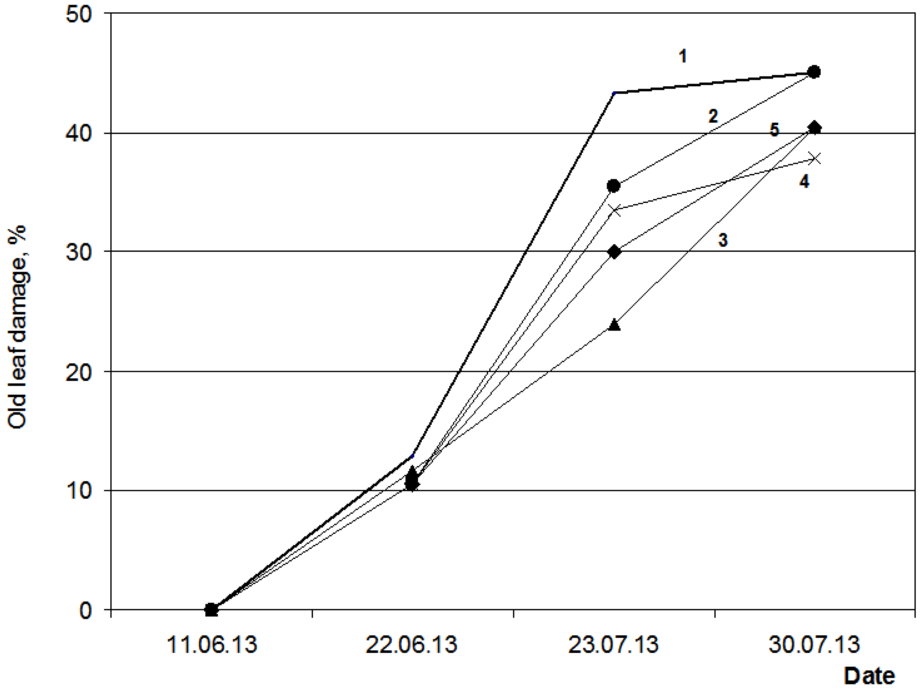


Figure 1: Influence of bacterial strains on strawberry plants' old leaves damage during thepotexperiment in 2013:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis*;

LSD_{05} by variants and dates = 1.9%

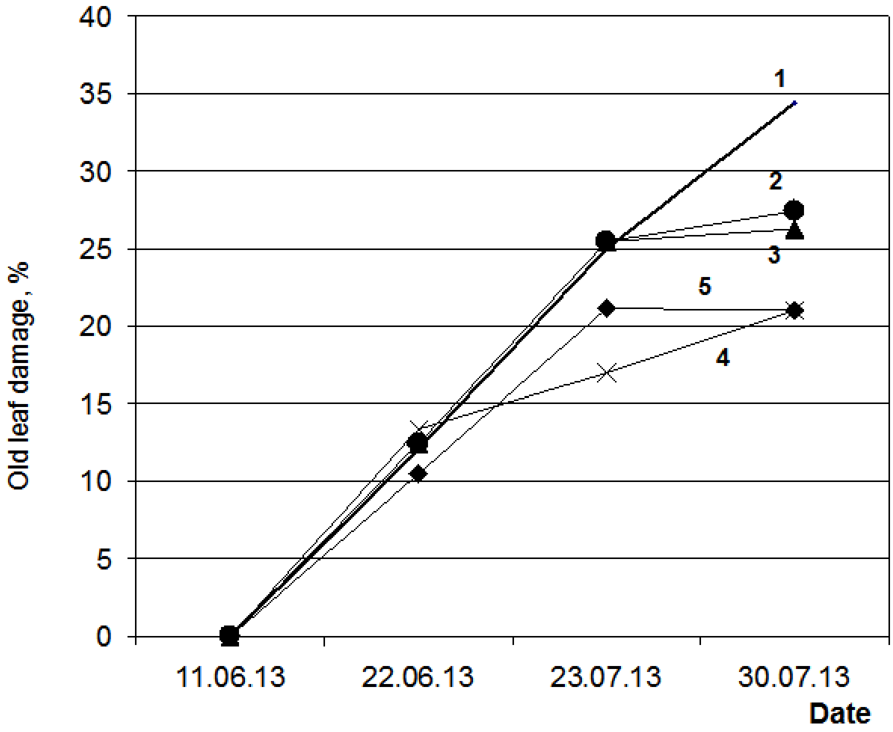


Figure 2: Influence of bacterial strains on strawberry plants' old leaves damage with ramularia during the field experiment in 2013:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens;*
4 – *B. subtilis*;
5 – *B. licheniformis;*
LSD_{05} by variants and dates = 1.9%

By July 23, in the plants in the field experiment, the degree of leaf damage with white spots reliably decreased 1.2-1.5 times when strains of *B. subtilis* and *B. licheniformis* were used, while in the control group, the degree of leaf damage reached 25.0%.

Plants in the pot experiment, in all variants with the use of *B. amyloliquefaciens, B.licheniformis*, *B. subtilis* and Phoenix were 1.2-1.8 times less affected, and in the control group, the degree of damage reached 43.3% (1.7 times higher than in the plants not subjected to stress).

By the end of July the old leaves infectedwith ramularia started to fall off, especially in the stressed plants. In this regard, by July 30, in the potexperiment, the effect of reducing the damage under the influence of bacterial strains considerably weakened 1.1 to 1.2 times. In the field experiment, the effect of reducing the extent of damage remained in the plants treated with *B. subtilis*, *B.licheniformis*, while in the plants treated with the Phoenix and *B. amyloliquefaciens*it was manifested at low level (decreased 1.3 times). Thus, development of ramularia in old leaves was stronger in stress conditions. In case of a high level of disease under stress, all bacterial strains and humic formulation were effective in suppressing its development, with the maximum effect manifested with the use of*B. amyloliquefaciens*and the minimum effect - with the use of Phoenix. In the field experiment, the plants treated with the *B. subtilis* and *B.licheniformis* strains showed a greater disease mitigation.

All experimental plants, as they adapted themselves, started growing young leaves in June, they were mainly damaged by ramularia in July. By July 23, the severity of damage of the young leaves of plants in the potexperiment decreased considerablyunder the influence of *B. subtilis*strainand Phoenix formulation, by 7.8 and 3.2%, respectively. In the plants in the field experiment, significant effects were observed (Fig. 3-4).

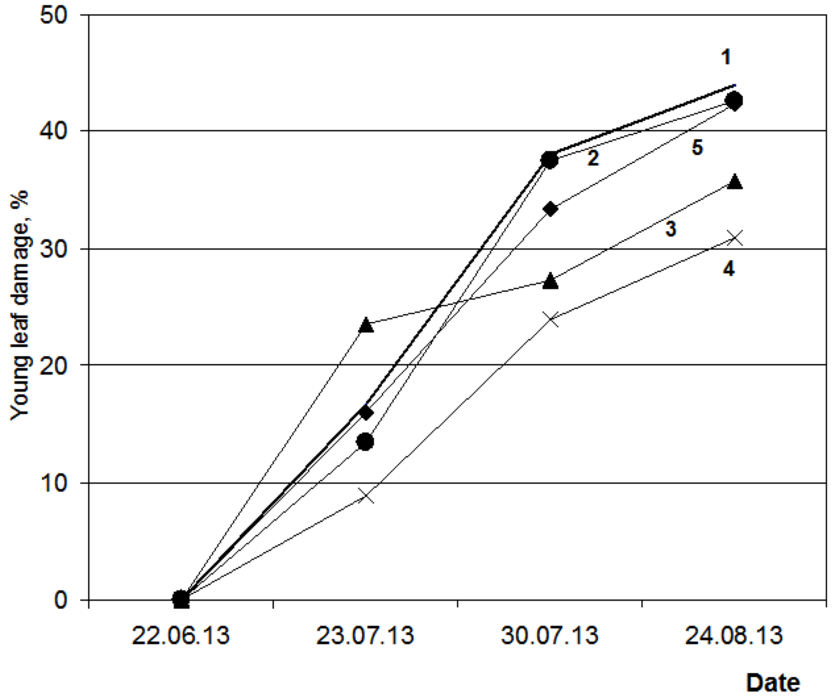


Figure 3: Influence of bacterial strains on damaging young leaves of strawberry plants during the pot experiment in 2013:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis*;

LSD_{05} by variants and dates = 1.9%

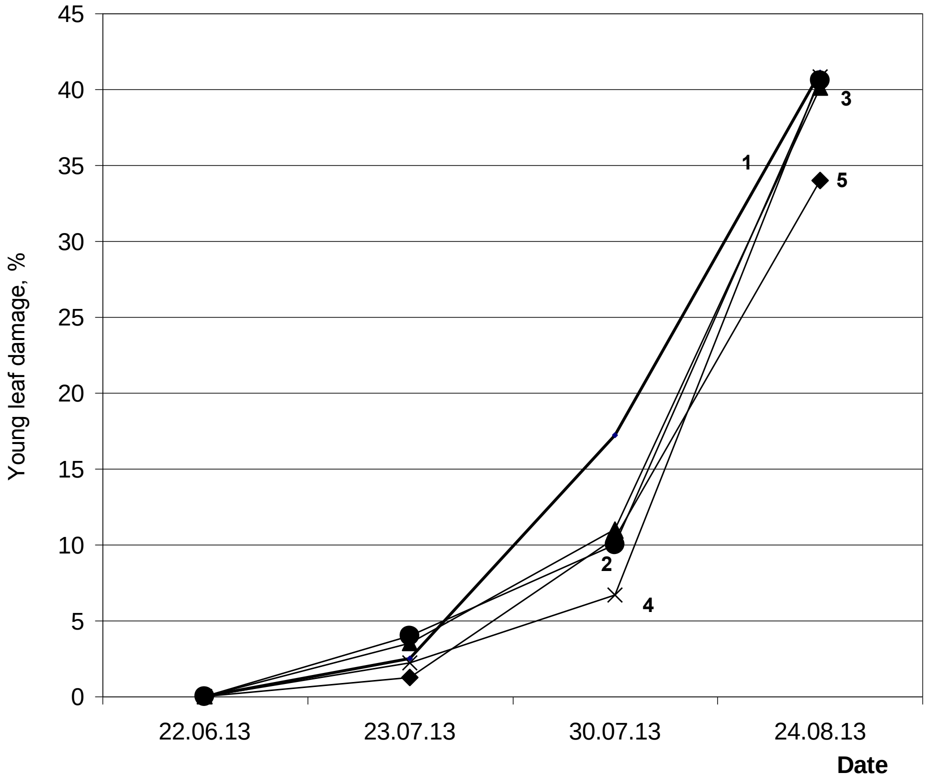


Figure 4: Influence of bacterial strains on damaging young leaves of strawberry plants with ramularia during the field experiment in 2013:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis*;
LSD_{05} by variants and dates = 1.9%

By 30 July, in the field experiment, the degree of young leaves damage decreased considerably - 1.6 to 2.6 times- under the influence of all bacterial strains and Phoenix formulation. In the plants of the pot series, the degree of damage reliably reduced 1.1 to 1.6 times only after treatment with bacterial strains.

By the end of vegetation, the damage of young leaves (due to their physiological aging) increased reliablyin all variants. Against this background, by 21 August in the plants in the field experiment, a reliableeffect of reducing the extent of damage by the disease was preserved only in the case of*B.licheniformis* strain – 7.1%. In the plants in pot experiment, the damage reduced in variants with *B. subtilis* and *B. amyloliquefaciens*, by 13.1 and 8.2%, respectively.

The results show that damage of young leaves (as well as old leaves before falling off) statistically decreased to a greater extent (in absolute values) under the influence of bacterial strains on the background of stress. With that, the maximum effect of reducing leaves damage in various ages was observed in case of preliminary treatment of seedlings roots with strains of *B. subtilis* and *B. amyloliquefaciens*. The parameters of plants growth and reproduction also depended on the abiotic conditions and the biocontrol agents used (Table.1).

Table 1: The influence of bacterial strains on growth and vegetative reproduction of strawberry (final determination - on 05.09.2013)

Type of experiment	The variant of pre-planting treatment	Length of the above-ground part, cm	Root length, cm	Number of runners, pcs.	Number of rosettes, pcs.
Field experiment	Control	27.1	22.0	2.5	0.9
	Phoenix	25.9	21.1	2.9*	1.4
	B. amyloliquefaciens	26.3	21.3	3.2*	1.6*
	B. subtilis	29.4*	20.9	3.3*	2.4*
	B. licheniformis	23.0	27.1*	2.4	2.1*
Pot experiment	Control	18.1	15.4	0.0	0.0
	Phoenix	18.9	17.8*	0.1	0.2
	B. amyloliquefaciens	18.6	15.0	0.1	0.0
	B. subtilis	18.2	16.2	0.1	0.1
	B. licheniformis	21.0*	17.3	0.0	0.0
LSD_{05} by variants		1.7	2.3	0.4	0.7
LSD_{05} by types of experiment		1.0	1.3	0.3	0.4

* - difference from the control by preparations are statistically reliable ($P<0.05$)

The length of the aboveground part of plants in the field experiments increased reliably when the root system was pre-treated with the *B. subtilis* strain - by 2.3 cm, in the plants in the pot experiment after treatment with *B. licheniformis* – by 2.9 cm.

The length of the root system of plants in the field experiment was reliably greater after the treatment with the *B. licheniformis* strain – by 5.1 cm, that of the plants in the pot experiment – after treatment with Phoenix – by 2.4 cm; and under the influence of other strains (except *B. amyloliquefaciens),* a weak trend towards roots elongation by 0.8 to 1.9 cm was observed.

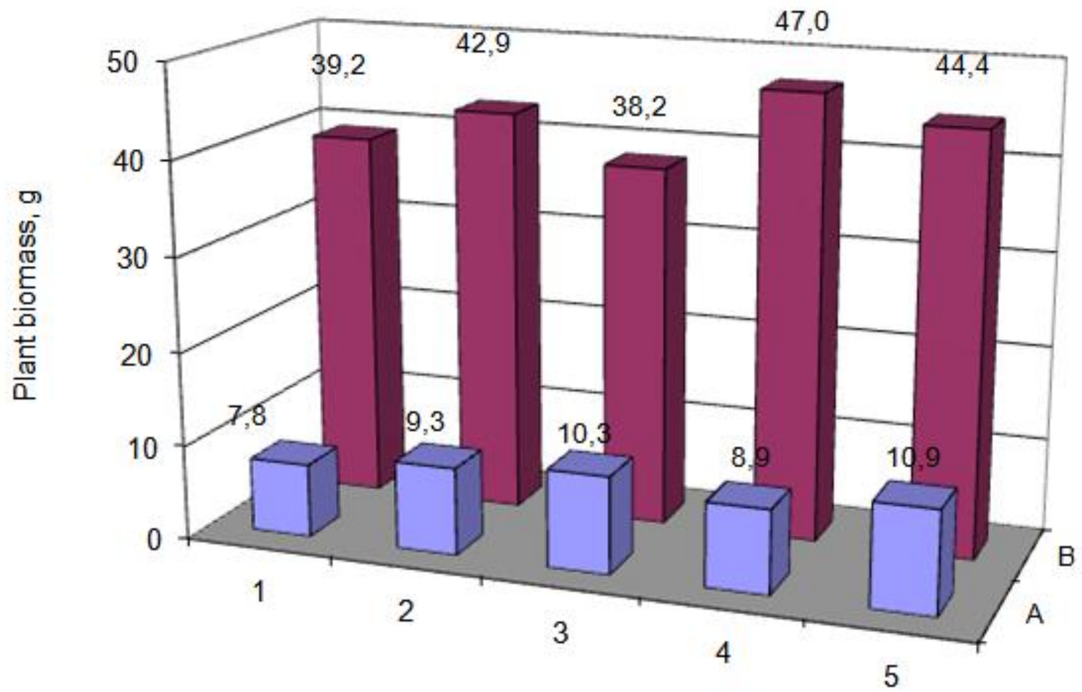
The influence of bacterial strains on the number of runners in plants from the field experiment was reliable when strains of *B. subtilis* and *B. amyloliquefaciens*were used – the number increased by 0.7 to 0.8 runners/plant, the number ofrosettes increased after the treatment with strains *B. subtilis* and *B.licheniformis*

– by 1.5 to 1.2 rosettes/bush. The plants in the pot experiment grew virtually no runners, and rosettes were virtually formed due to the stress conditions.

The value of strawberry plants biomass clearly indicates a significant negative impact of stressful growing conditions on plant health (a sharp decrease in the biomass, 5 times on the average, due to the stress). However, pre-treatment with bacterial strains increased the biomass in both experiments. Biomass of a single plant in the field experiment increased under the influence of the B. *subtilis* strain by 7.8 g/plant, under the influence of *B.licheniformis* - by 5.2 g/plant, and the effect of using these strains was most significant.

Biomass of a single plant in the pot experiment also increased after treatment with *B. amyloliquefaciens*and *B.licheniformis* by 2.5 to 3.1 g/plant (Fig. 5).

Figure 5



1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis*;
A – thepotexperiment;
B – the field experiment;
LSD_{05} by variants = 3.4 g/plant;
LSD_{05} by types of experiment = 2.0

The increase in total plant biomass, relative to the control with pre-treatment with bacterial strains in the field experiment amounted to 9.6% (3.8 g/plant) on average. In the plants in the pot experiment, biomass growth averaged to 28.9 % (2.3 g/plant). This fact also confirms the presence of a pronounced anti-stress action of the bacterial strains tested.

The influence of bacterial strains on strawberry damageby ramularia,growth and development of plants in 2014

The methods of establishing experiments in 2013 and 2014 were identical. No plants in the pot and the field experiments in 2014 and in 2013 were died.

ISSN: 0975-8585

In the plants in the pot experiment, a considerable decrease in the extent of the disease, as compared to the control, was detected in all variants by 26.07.14 (1.5 months after planting). The degree of damage in the control group was 49.5 %, i.e. 2.8 times higher than in the control plants in the field experiment that were not exposed to stress (Fig. 6). Under the influence of *B. amyloliquefaciens*and *B. subtilis* strains, severity of the disease decreased to 25.0% (1.5 times, P<0.05). These strains showed the best effect, which also considerably exceeded the effect of the standard, i.e., Phoenix humic formulation. The *B. licheniformis* strain acted weaker, reduced the damage approximately 1.3 times. By the end of monitoring old leaves (09.08.14, due to their progressing falling off) the degree of damage in the control group reached 63.0%, and the reliable effect of reducing the damage in the best variants with the use of *B. amyloliquefaciens*and *B. subtilis*strains decreased slightly (Fig. 7).

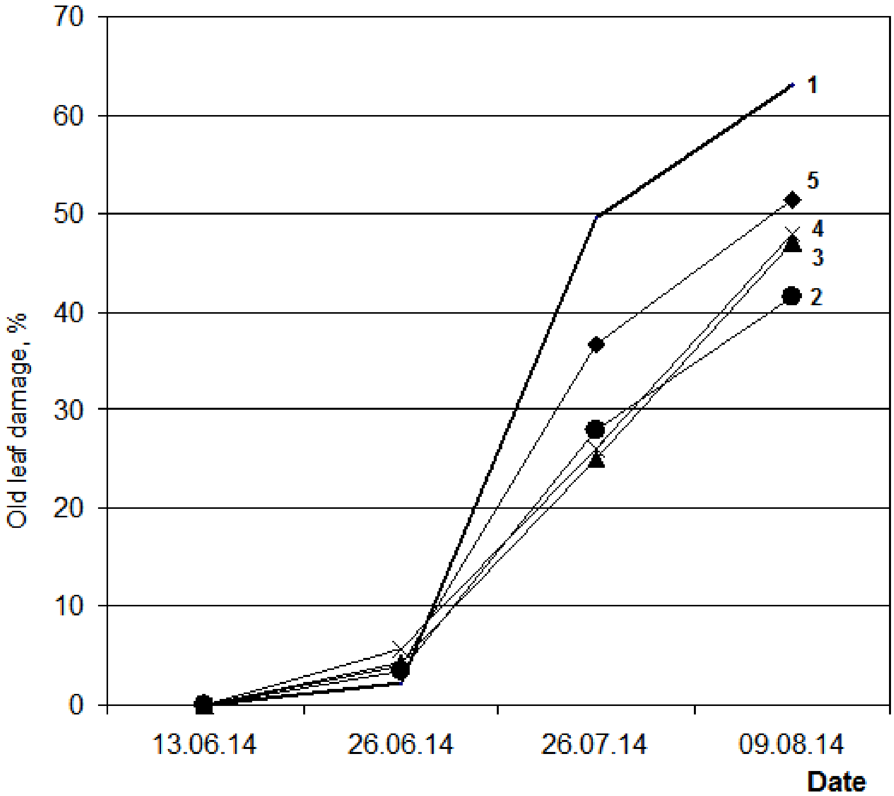


Figure 6: Influence of bacterial strains on damaging old leaves of strawberry plantsduring the pot experiment in 2014:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis*;
LSD_{05} by variants and dates = 2.0%

The plants in the field experiment showed reduction in the young leaves damage, as compared to the control 2 weeks after planting (on 26.06.14). 1.5 months after planting (on 26.07.14), the severity of the disease in the control group amounted to 18.0%, the maximum effect was observed after treatment withthe *B. amyloliquefaciens*, *B.licheniformis* strains– the damage decreased 1.3-1.4 times. By the end of monitoring old leaves, the degree of damage in the control group reached 28.5%, and the highest effect of reducing the damage - 1.4 times - was reached after treatment with *B. amyloliquefaciens*.

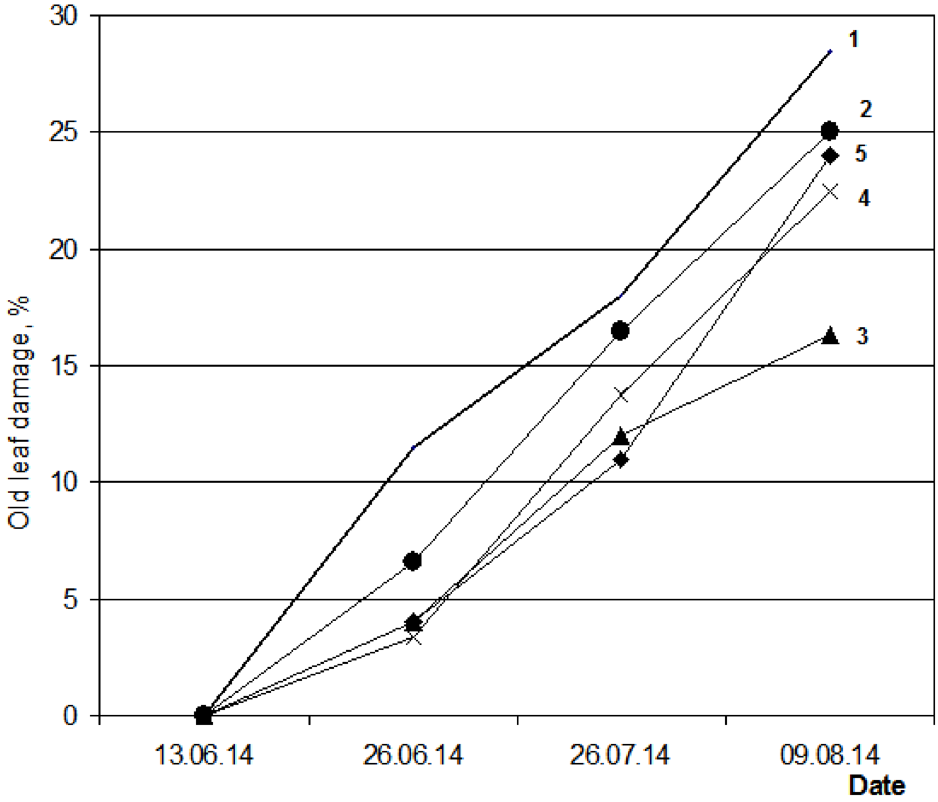


Figure 7 – Influence of bacterial strains ondamaging old leaves of strawberry plants with ramularia during the field experiment in 2014:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis;*
LSD_{05} by variants and dates = 2.0%

After comparing the data obtained in 2014 and in 2013, it can be stated that within two years, a reliable effects of reducing damaging old leaves with ramularia leaf spot was proven when strawberry plants were grown in the pot experiment under the influence of the *B. amyloliquefaciens*and *B. subtilis* strains. In the field experiment, the most considerable decrease of the damage was noted after treatment with the *B. amyloliquefaciens*and *B.licheniformis* strains.

After planting experimental plants in the field experiment (13.06.2014), new leaves remained completely undamaged by ramularia leaf spots for approximately 1 month.

In the plants in the pot experiment, the first symptoms ramularia on young leaves were found after 21 July. By 26.07.14, the severity of the damage in the control group reached 15.6 %.The damage developed in all experimental variants with the use of the *B. amyloliquefaciens, B. subtilis, B. licheniformis*strains and the Phoenix humic formulation (Fig. 8) was lower, 1.3-1.5 times. However, 8 weeks after planting, the level of stressed plants damage increased dramatically to 42.0% in the control group. After preliminary treatment with the *B. amyloliquefaciens*and *B. subtilis* strains, the severity of the disease at this point was 1.6-1.7 times lower. Until the end of the experiment (22.08.14), the situation remained almost unchanged. It should be noted that efficiency of *B. amyloliquefaciens*and *B. subtilis* in reducing ramularia leaf spots was approximately 2 times higher than the corresponding value after treatment with the Phoenix formulation.

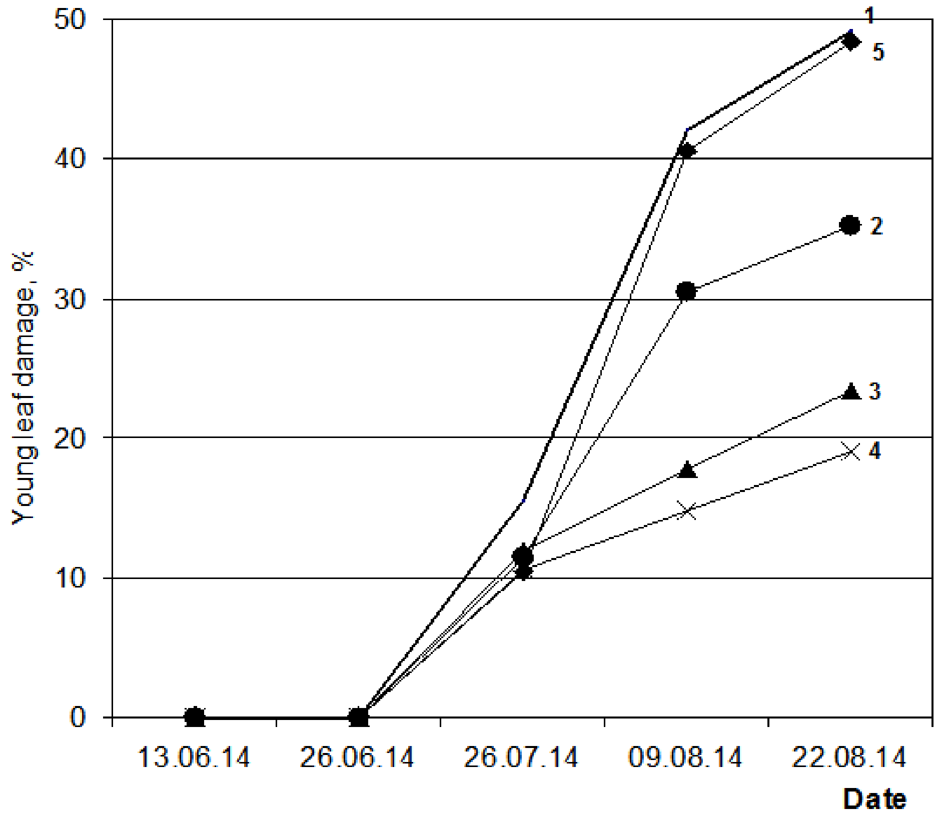


Figure 8: Influence of bacterial strains on damaging young leaves of strawberry plants during the pot experiment in 2014:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens*;
4 – *B. subtilis*;
5 – *B. licheniformis*;
LSD_{05} by variants and dates = 2.0%

In the field experiment, the first symptoms of ramularia on young leaves of the control plants were noted on 26.07.14. In August (09.08.14), the degree of young leaves damage in the control group was 6.6%. In the plants treated with the *B. amyloliquefaciens*and *B. subtilis* strains, the damage in this period was 1.6-1.7 times weaker,as compared to the control group. The effects of these strains mainly remained in place until the end of the experiment (22.08.14) (Fig. 9).

Summarization of the data obtained in 2014 and in 2013 shows that within two years, a reliable effects of reducing damaging new leaves with ramularia leaf spot was proven, when strawberry plants were grown in the pot experiment under the influence of the *B. amyloliquefaciens*and *B. subtilis* strains. With that, the effect of reducing the damage remained stable during the growing season.

The results show that pre-treatment with bacterial strains enhances strawberry plants resistance to ramularia leaf spot, which appears to be the case on the basis of easing the stress states that hinder premature tissues aging. The most pronounced immunizing action on strawberry plants grown in stressful conditionswas shown by the *B. amyloliquefaciens*and *B. subtilis* strains, the effect of which was considerably higher than the effect of the humic Phoenix formulation.

The estimations performed at the end of the 2014growing season showed that the length of the above-ground part of the plants in the field experiment had considerably increased by 3.4 cm under the influence of preliminary treatment with *B. amyloliquefaciens*. The length of the aboveground part of plants in the pot experiment reliably increased in the variants with the use of *B. amyloliquefaciens*and *B. subtilis* by 2.6 to 3.5 cm (Table 2).

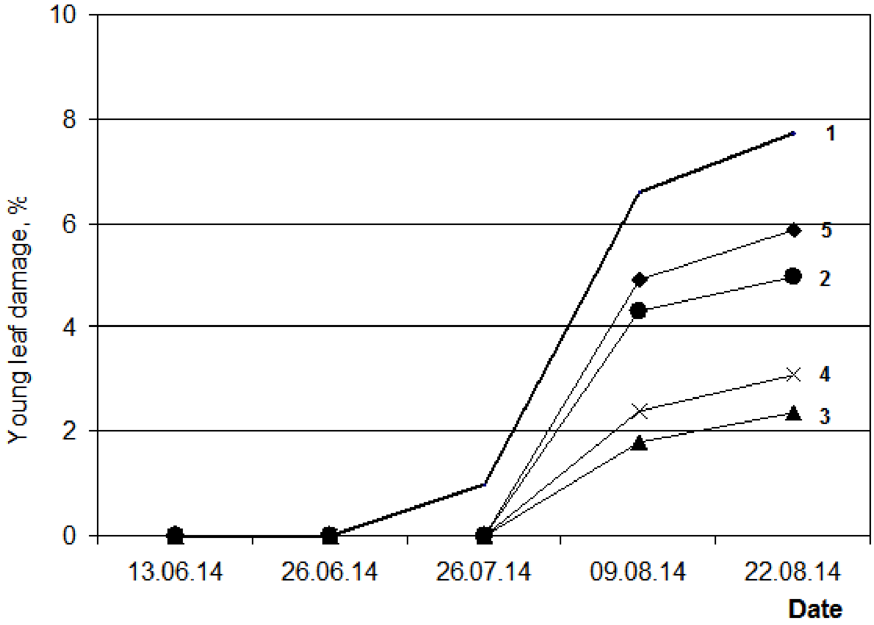


Figure 9: Influence of bacterial strains on damaging young leaves of strawberry plants with ramularia during the field experiment in 2014:

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens;*
4 – *B. subtilis*;
5 – *B. licheniformis;*

LSD_{05} by variants and dates = 2.0%

Table 2: The influence of bacterial strains on growth and vegetative reproduction of strawberry (final determinations - on 22.08.2014)

Type of experiment	The variant of pre-planting treatment	Length of the above-ground part, cm	Number of young leaves, leaves/ plant	Root length, cm	Number of runners, runners/ plant	Length of runners, cm	Number of rosettes, rosettes/ plant
Field experiment	Control	22.3	3.4	14.3	0.8	10.2	0.5
	Phoenix, 0.05%	26.7*	3.5	18.8*	1.2*	16.8*	0.8
	B. amyloliquefaciens	25.7*	3.0	16.9*	1.5*	21.9*	0.5
	B. subtilis	23.6	3.3	19.3*	1.2*	17.4*	0.5
	B. licheniformis	22.1	3.5	20.8*	1.3*	14.5	0.8
Pot experiment	Control	15.6	2.6	11.1	0.0	0.0	0.0
	Phoenix, 0.05%	16.6	2.9*	14.4*	0.0	0.0	0.0
	B. amyloliquefaciens	18.2*	3.2*	14.9*	0.0	0.0	0.0
	B. subtilis	19.1*	2.9*	13.8*	0.0	0.0	0.0
	B. licheniformis	15.7	2.8	11.0	0.0	0.0	0.0
LSD_{05} by variants		1.5	0.3	1.5	0.4	5.9	0.4
LSD_{05} by the type of experiment		0.8	0.2	0.9	0.2	3.4	0.2

The number of young leaves in plants in the field experiment did not reliably change. However, in plants in the pot experiment, the number of young leaves increased after treatment with the *B. amyloliquefaciens*and *B. subtilis* strains – by 11.5 to 23.1%, with 2.6 leaves/plant in the control group.

Length of the roots of plants in the field experiment considerably increased in all experimental variants by 2.6-6.5 cm (1.2-1.5 times). In the stressed plants in the pot experiment, the length of the root system increased after pre-treatment with the *B. amyloliquefaciens*and *B. subtilis* strains by 2.7 to 3.8 cm (1.2 to 1.3 times), and was roughly equivalent to the effect of treatment with the Phoenix humic formulation.

Under the influence of treatment with strains, the biomass of a single plant in the field experiment increased in all variants by 7.5 to 9.5 g/plant (1.4-1.6 times, as compared to the control group)($P<0.05$). In plants in the pot experiment, biomass also increased by 2.6-3.7 g/plant, however, the effect was proven statistically only after treatment with the *B. subtilis* strain where the increase, as compared to the control group, was 1.6 times. The observed effects were at the same level or higher than the stimulating effect of the Phoenix humic formulation (Fig. 10).

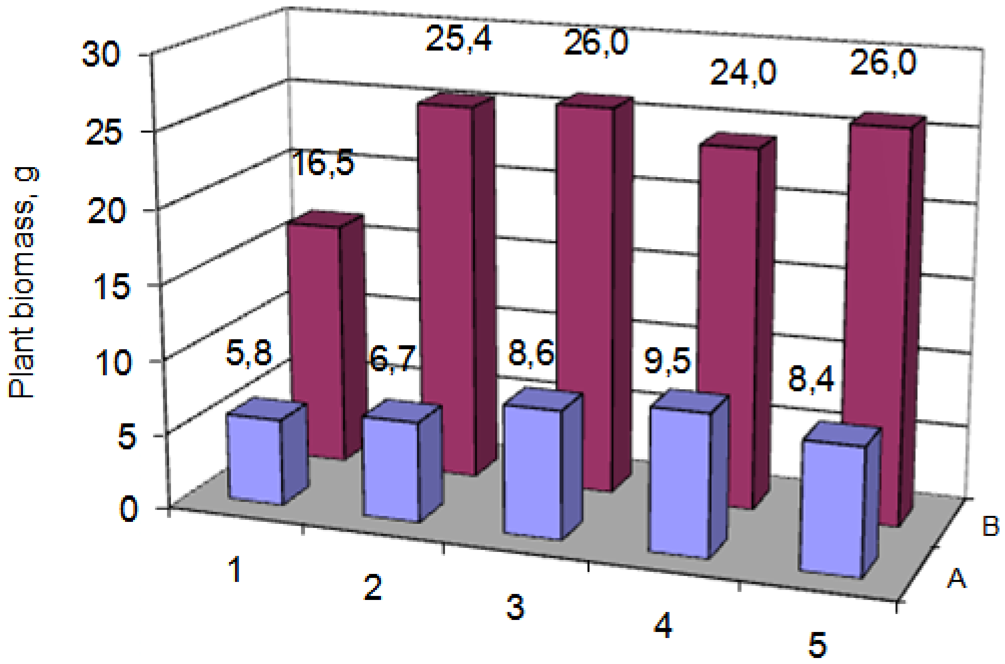


Figure 10: Influence of bacterial strains on the total biomass of strawberry plants (g/plant, final check on 22.08.14)

1 – Control;
2 – Phoenix;
3 – *B. amyloliquefaciens;*
4 – *B. subtilis*;
5 –*B. licheniformis;*

A – the pot experiment;
B – the field experiment;
LSD_{01} by variants = 3.4 g/plant;
LSD_{01} by the type of experiment = 2.0

The influence of strains on the number of runners formed by plants during the field experiment was reliable in all variants - increasing the number of runners by 0.4-0.7 runners/plant (1.5to 1.9 times), as compared to the control group. The maximum effect was observed under the influence of the *B. amyloliquefaciens*strain. The length of the runners increased after the treatment with Phoenix and the *B. amyloliquefaciens*and *B. subtilis* strains by 6.6 to 11.7 cm.

The plants in the pot experiment virtually grew no runners, and rosettes were virtually not formed due to the stress conditions.

Summarization of the data obtained in two types of model experiment performed in 2013 and 2014 shows that under the influence of preliminary treatment, the length of the aboveground part of plants in the field experiment increased after the treatment with the *B. subtilis* strain and with the Phoenix humic formulation by 1.6 to1.8 cm. The length of the aboveground part of plants in the vegetative experiment increased in all variants with the use of bacterial strains by 1.5 to 1.8 cm (Table 3).

Table 3: The influence of pre-treatment of the root system with bacterial strains on strawberry growth parameters (average for 2013-2014)

Type of experiment	The variant of pre-planting treatment	Length of the above-ground part, cm	Number of young leaves, leaves/ plant	Root length, cm	Total biomass, g/ plant	Number of runners, runners/ plant	Length of runners, cm	Number of rosettes, rosettes/ plant
Field experiment	Control	24.7	3.8	18.2	27.8	1.6	26.6	0.7
	Phoenix, 0.05%	26.3*	3.6	20.0*	34.1*	2.1*	31.4	1.1*
	B. amyloliquefaciens	26.0	3.4	19.1	32.1*	2.4*	34.3*	1.1*
	B. subtilis	26.5*	3.7	20.1*	35.5*	2.3*	36.6*	1.5*
	B. licheniformis	22.6	3.5	24.0*	35.2*	1.9*	29.8	1.4*
Pot experiment	Control	16.9	2.9	13.3	6.8	0.0	0.0	0.0
	Phoenix, 0.05%	17.8	3.1	16.1*	8.0	0.05	0.7	0.1
	B. amyloliquefaciens	18.4*	3.2*	15.0*	9.4	0.05	0.5	0.0
	B. subtilis	18.7*	3.0	15.0*	9.2	0.05	1.4	0.05
	B. licheniformis	18.4*	3.3*	14.2	9.7	0.0	0.0	0.0
LSD_{05} by variants		1.5	0.3	1.5	4.0	0.4	5.9	0.4
LSD_{05} by types of experiment		0.8	0.2	0.9	2.3	0.2	3.4	0.2

The number of young leaves in plants in the field experiment did not reliably change. In plants in the pot experiments an increase in the number of young leaves was observed by 0.3-0.4 leaves /plant, with 2.9 leaves/plant in the control group.

The length of roots of plants in the field experiment increased in all experimental variants by 1.9 to 5.8 cm (1.1 to1.3 times), except for thetreatmentwith *B. amyloliquefaciens*. The length of the root system of plants in the pot experiment after treatment with bacterial strains also reliably increased by 1.7 cm, except for variant with the *B.licheniformis* strain.

Under the influence of treatment with strains, the biomass of a plant in the field experiment increased in all experimental variants by 4.3 to 7.7 g/plant (1.2-1.3 times)($P<0.05$). Biomass of plants in the pot experiment increased by 2.6-2.9 g/plant in the form of a statistically unproven trend. The influence of formulations on the number of runners formed by plants during the field experiment was reliable in all variants - increasing the number of runners by 0.3-0.8 runners/plant (1.2-to 1.5 times), as compared to the control group. The maximum effect was observed after the treatment with the *B. amyloliquefaciens*strain. The length of the runners increased after the treatment with the *B. amyloliquefaciens*and *B. subtilis* strains by 7.7 to 10.0 cm. The number of rosettesreliably increased in all experimental variants by 0.4 to 0.8 rosettes/plant.

In plants in the pot experiment, runners and rosettes almost didnot form during both years of the study, which can be explained by the stress conditions of growing.

CONCLUSION

In this study, we have first shown the antifungal effect of three strains of bacteria of the *Bacillus* genustowards the causing agent of strawberry ramularia leaf spot under the conditions of Western Siberia. Creating stress conditions for the plants during growingin pots, as compared with the field experiment, revealed the influence of abiotic factors on the activity of bacterial strains of the genus *Bacillus* on strawberry plants damaged by the disease. Their contribution into improving the plant health by growth-promoting effect and induction of resistance to ramularia has been shown. The bacterial strains tested in this study are the promising biocontrol agentsfor strawberry health management in Western Siberia.

ACKNOWLEDGMENTS

This work was supported by a grant from the Russian Scientific Foundation (project No. 14-16-00101).

REFERENCES

[1] Williamson, B., Tudzinski, B., Tudzynski, P., van Kan, J.A.L. *Botrytis cinerea:* the cause of grey mold disease. *Mol. Plant Pathol.*,2007;8(8), 561-580.

[2] Donmez, M.F., Esitken, A., Yildiz, H., Ercisli, S. Biocontrol of *Botrytis cinerea* on strawberry fruit by plant growth promoting bacteria. *The J. Animal Plant Sci.*,2011; 21(4), 758-763.

[3] Essghaier, B., Fardeau, M.L., Cayol, J.L., Hajlaoui, M.R., Boudabous, A., Jijakli, H. Biological control of grey mould in strawberry fruits by halophilic bacteria. *J. Appl. Microbiol.,* 2009;106(3), 833-846.

[4] Haggag, W.M. Isolation of bioactive antibiotic peptides from *Bacillus brevis* and *Bacillus polymyxa* against *Botrytis* grey mould in strawberry. *Arch. Phytopathol. Plant Protec.*, 2008;41(7), 477-491.

[5] Haggag, W.M., Abd-El-Kariem, F., Abou-Hussein, S.D. Bioprocessing of *Brevibacillus brevis* and *Bacillus polymixa*, a potential biocontrol agent of gray mold disease of strawberry fruits. *Int. J. Eng. Innovat. Technol.,*2013;3(2), 509-518

[6] Nicot, P.C., Bardin, M., Debruyne, F., Duffand, M., Lecompte, F., Neu, L., Pascal, M. Effect of nitrogen fertilization of strawberry plants on the efficacy of defense-stimulating biocontrol products against *Botrytis cinerea.IOBC-WPRS Bull*.,2013;88, 39-42.

[7] Shkalikov, V.A., Dyakov, Y.T., Smirnov, A.N. et al. Plant immunity. Moscow: Kolos, 2005; 190pp.

[8] Ramegowda, V., Senthil-Kumar, M. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. *J Plant Physiol.,*2015;176*,* 47-54.

[9] Arancon, N.Q., Edwards, C.A., Bierman, P., Welch, C., Metzger, J.D. The influence of vermicompost application to strawberry: Part 1. Effect on growth and yield. *Biores. Technol.,*2004;93(2), 145-153.

[10] Senesi, N., Plaza, C., Brunetti, G., Polo, A. A comparative survey of recent results on humic-like fractions in organic amendments and effects on native soil humic substances. *Soil Biol. Biochem.*, 2007;39(6), 1244-1262.

[11] Singh, R., Sarma, R., Satyendra, K., Gupta, R., Patil, R. Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria x ananassa*). *Biores. Technol.*, 2008;99(17), 8507-8511.

[12] Young, J., Kloepper, J.W, Ryu, C.M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci*., 2009;14, 1-4.

[13] Calvo, P., Nelson, L., Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant& Soil*, 2014;383(1), 3-41.

[14] Dordas, C. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agron. Sustain. Development*, 2008;28(10), 33-46.

[15] Cawoy, H., Bettiol W., Fickers, P., Ongena, M. Bacillus-based biological control of plant disease. In: *Pesticides in the modern world- Pesticides use and Management* (Dr. Stoycheva M., ed.),InTech., 2011;pp. 211-246.

[16] Kloepper, J.W., Ryu, CM., Znan, S. Induced systemic resistance and promotion of plant growth by *Bacillus spp.Phytopathology,*2004;94(11), 1259-1266.

[17] Choudhary, D.K, Johri, B.N. Interaction of *Bacillus spp*. and plants – with special reference to induced systemic resistance (ISR). *Microbial Res*., 2009;164, 493-513.

[18] Arancon, N.Q., Edwards, C.A., Lee, S., Byrne, R. Effect of humic acids from vermicompost on plant growth.*Eur. J. Soil Biol.*2006; 42(1), 565-569.

[19] Van Loon, L.C. Plant responses to plant growth promoting rhizobacteria.*Eur. J. Plant Pathol.,* 2007;119(3), 243-254.

[20] Lugtenberg, B., Kamilova, F. Plant-growth-promoting rhizobacteria. *Ann. Rev. Microbiol.,* 2009;63, 541-556.