

# Evaluation of the biological effectiveness of the biofungicide Serenade ASO, SC in the fight against bacterial black spot of tomatoes (*Xanthomonas campestris* pv. *vesicatoria* (Doidge) on indoor tomatoes

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**Abstract.** The article presents the results of two-year studies on the biological effectiveness of biofungicide Serenade, ASO, SC (*Bacillus amyloliquefaciens*, strain QST-713, titer not less than  $1 \times 10^9$  CFU/ml) in the fight against bacterial black spot on the leaves and fruits of indoor tomatoes in the conditions of the II soil-climatic zone of the Crimea. It was found that a five-time treatment with biofungicide Serenade, ASO, SC, the rate of application of 5.0, 6.5 and 8.0 l/ha, contributed to a decrease in the intensity of development of *Xanthomonas campestris* pv. *vesicatoria* on leaves by 2.3-2.9 times, on fruits by 2.2-2.7 times. On the 10th day after the fifth spraying, the biological effectiveness of biofungicide Serenade, ASO, SC in the application rates of 5.0, 6.5 and 8.0 l/ha, with an average development of bacterial black spot on the control variant (3.3-5.2% on leaves, 2.9-3.3% on fruits), on leaves was 76.5-85.1%, on fruits – 71.6-75.0%. On the day of the last harvest, on the 29th day after the last treatment, the biological efficiency on leaves was 57.3-65.4%, on fruits – 54.5-62.6%. The difference of five-time treatment with biofungicide Serenade, ASO, SC in the norm of application of 8.0 l/ha, with the standard is insignificant, within the limits of experimental error. The positive effect of biofungicide Serenade, ASO, SC on yield was noted. The difference with the control (77.9 c/ha) is 34.9, 36.9 and 41.0 c/ha.

## 1 Introduction

Tomato is one of the most common vegetable crops for indoor planting. Currently, various methods are used for the cultivation of tomato plants, which create optimal conditions for intensive growth and development of plants.

One of the main factors limiting the production of products are diseases and pests. The most common and harmful bacterial diseases of tomatoes is bacterial black spot (*Xanthomonas campestris* pv. *vesicatoria*). The aboveground parts of plants are affected at various stages of development, especially young organs and tissues are susceptible. The

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affected fruits may shed, and the quality of the harvested crop is low. In the absence of a competent system of protective measures, crop losses can reach 100%. Up to 50% of seedlings can be affected in the nursery-garden. A favorable condition for the development of bacterial black spot is high temperature and humidity [1, 2, 3].

The tomato plant protection system is based on the use of a significant (up to 25 per growing season) amount of pesticide treatments. The disadvantages of such systems include: high pesticide load, the appearance of strains of phytopathogens resistant to the pesticides used, the accumulation of residual amounts of pesticides in fruits, phytotoxicity, etc. [4, 5].

Organic products are one of the most popular trends all over the world, including in the Crimea. In this regard, recently there has been a reduction in the use of chemicals, a transition to the use of biological plant protection products [6].

To date, the volume of biological plant protection products used is lower than the volume of chemical pesticides used. To develop an effective system of protective measures based on the use of biological pesticides, to expand their list, the biological effectiveness of biofungicides based on various strains of bacteria of the genus *Pseudomonas* and *Bacillus*, as well as their complex combination, strains of the fungus of the genus *Trichoderma*, against mycoses and bacterioses affecting the aboveground, as well as the vascular system of plants, in the fight against root rot [7, 8, 9].

The research results show that the use of biological products has a positive effect on plant growth, as well as on the quality of the resulting crop, without reducing the effectiveness of protective measures

The actual purpose of our research is to study the biological effectiveness of biofungicide Serenade, ASO, SC (*Bacillus amyloliquefaciens*, strain QST-713, titer not less than  $1 \times 10^9$  CFU/ml) in the fight against bacterial black spot of indoor tomatoes.

## 2 Materials and methods

The studies were carried out in 2018-2019 in the II soil and climatic zone of the Republic of Crimea (Saki district, Yevpatoria) in accordance with the methodology of accounting for the development of harmful objects [10]. The biological effectiveness of biofungicide Serenade, ASO, SC (*Bacillus amyloliquefaciens*, strain QST-713, titer not less than  $1 \times 10^9$  CFU/ml) in the fight against bacterial black spot of tomatoes on leaves and fruits was studied.

Variety (F1 hybrid) – Pink Ball F1. Soil: chemoponics (highbog peat with a decomposition degree of 30%, sphagnum moss, tree bark, sawdust, rice husk, coconut substrate). Preceding crop – a cucumber.

Experiment variants:

- I. Serenade ASO, SC, 5.0 l/ha;
- II. Serenade ASO, SC, 6.5 l/ha;
- III. Serenade ASO, SC, 8.0 l/ha;
- IV. Gamair, SC (titer  $10^{10}$  CFU/ml), *Bacillus subtilis*, strain M-22 VISR (standard);
- V. Control – without the use of protection products.

The flow rate of the working fluid is 1000 l/ha. Treatments were carried out every 7 days according to the following phases of vegetation: the first - the first visible inflorescence (the first bud is straight), BBCH, 51; the second – 8 or more visible flower buds, BBCH, 58; the third – the 3rd inflorescence: the first flower is open, BBCH, 62; the fourth - the first fruit cluster: the first fruit has reached a typical size, BBCH, 71; the fifth – 10% of fruits show a typical fully mature color, BBCH, 81.

The account of the bacteriosis lesion was carried out by examining every second leaf and all fruits on each registered plant, without tearing them off. The lesion was assessed by assessing the presence or absence of visual signs of disease development according to the following scales [10]:

Degree of leaf and fruit lesion:

0 points – there is no lesion;

1 point – a very weak lesion, small spots are found on individual leaves and fruits;

2 points – a weak lesion, individual spots on no more than 5% of the examined organs;

3 points – a weak lesion, up to 10% of the examined organs are affected;

4 points – average lesion, up to 15% of the examined organs are affected;

5 points – severe lesion, almost every organ is affected, up to 25% of the leaves are withered;

6 points – a very severe lesion, up to 50% of leaves and fruits die off, the beginning of the stem lesion;

7 points – up to 75% of leaves and fruits die off, the damage to the stem progresses;

8 points – on the plot all the plants completely died.

The determination of the biological effectiveness (B.E.) of the protective measures taken against bacterial diseases was carried out according to the formula:

$$B.E. = \frac{Rk - Ro}{Rk} \times 100\%$$

where B.E. is technical efficiency, %;

R k – the development of the disease under control on the leaves (affected fruits in the sample), %;

R o – the development of the disease on the studied variant on the leaves (affected fruits in the sample), %.

Identification of the bacterium was carried out using molecular genetic methods. DNA extraction was performed using a modified CTAB method from plants with visual symptoms of bacteriosis [11]

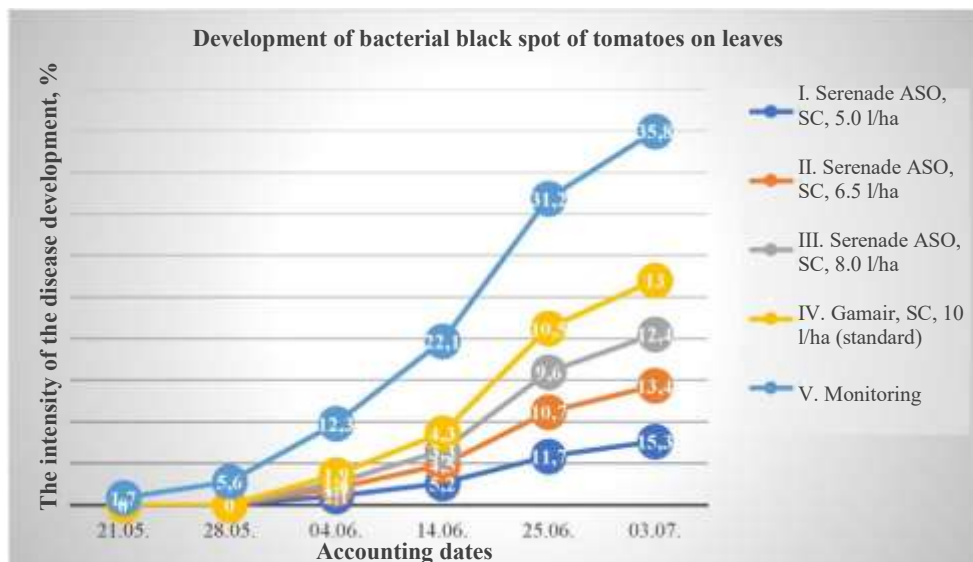
Amplification of gene loci was carried out in "2.5x Reaction mixture for PCR-RV" (Syntol, Moscow). Amplification of fragments of the 16S rRNA gene of bacteria was carried out under the following conditions: initial denaturation – 95 °C - 5 min, then 35 cycles: 95 °C - 30 sec, 55 °C – 60 sec, 72 °C – 60 sec; final polymerization – 72 °C - 7 min.

The analysis of PCR products was carried out using electrophoresis in 1% agarose gel, at a field strength of 6 V/cm by the glow of ethidium bromide in a UV lamp. PCR purification of agarose gel products was carried out using the "ColGen Reagent Kit for DNA isolation from agarose gel and reaction mixtures". DNA sequencing was performed using the Sanger method on an ABI 3130 genetic analyzer [12].

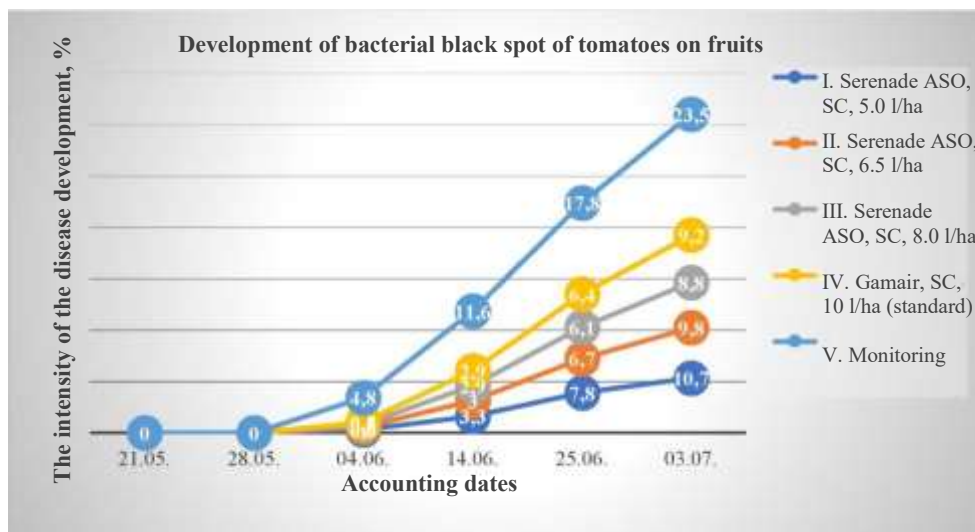
### 3 Results

Before starting the experiment, testing was carried out for the presence of *Xanthomonas campestris* pv. *vesicatoria* in tomato plants by molecular genetic methods. As a result of testing, it was found that about 10% of the plants in the experimental area were affected by bacterial black spot.

Analysis of the data obtained showed that, on average, over two years of research, five-fold treatment of indoor tomato plants with biofungicide Serenade ASO, SC, application rates 5.0, 6.5 and 8.0 l/ha, contributed to a decrease in the development of bacterial black spot on leaves and fruits by 2.3-2.9 and 2.2-2.7 times, respectively (Figure 1, 2). The first visual signs of the development of bacterial black spot of tomatoes on the control variant were noted at the beginning of the third decade of May, 18 days after the seedlings were planted. The first visual signs of *Xanthomonas campestris* pv. *vesicatoria* were noted in the first decade of June, 10 days later than in the control variant, on the variants of the experiment using Serenade ASO, SC and standard (Gamair, SC). Intensive development of the disease was noted in the second decade of June.



**Fig. 1.** Dynamics of development of bacterial black spot on the leaves of indoor tomato, 2018-2019.

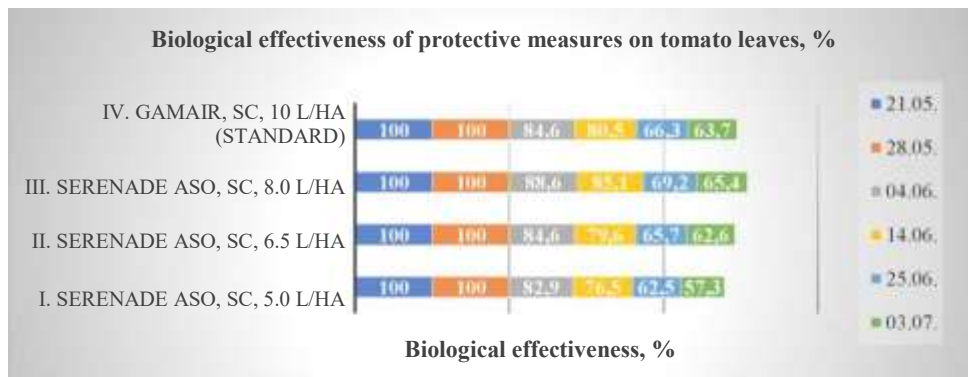


**Fig. 2.** Dynamics of development of bacterial black spot on indoor tomato fruits, 2018-2019.

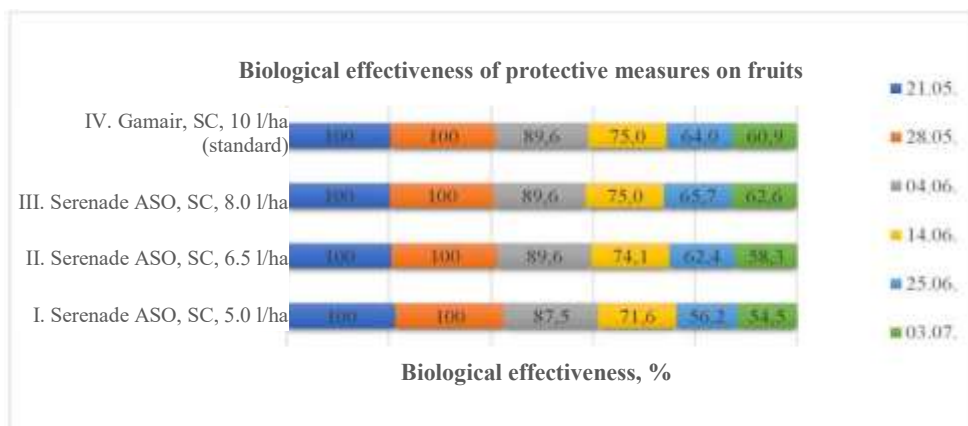
Carrying out protective measures with the biofungicide Serenade ASO, SC, application rates of 5.0, 6.5 and 8.0 l/ha, allowed maintaining high biological efficiency before harvesting on all variants of the experiment.

10 days after the fourth spraying, the effectiveness of the drug Serenade ASO, SC, in the normal use of 5.0-8.0 l/ha was 82.9-88.6% on leaves, 87.5–89.6% on fruits. The difference between variants I-III and the standard (84.6% on leaves and 89.6% on fruits) is within the limits of experimental error (Figure 3, 4).

10 days after the fifth spraying, with the development of bacterial black spot on the control up to 22.1% on leaves and 11.6% on fruits, the effectiveness of the drug Serenade ASO, SC, in the norm of 5.0 l/ha (76.5% on leaves and 71.6% on fruits) was at the level of the standard – Gamair, SC (80.5% on leaves and 75.0% on fruits) and was inferior to the variant with an application rate of 8.0 l/ha (85.1% on leaves and 75.0% on fruits).



**Fig. 3.** Biological effectiveness of biofungicide Serenade, ASO, SC, in the fight against bacterial black spot on the leaves of indoor tomato, Pink Ball hybrid, 2018-2019.



**Fig. 4.** Biological effectiveness of the biological fungicide Serenade, ASO, SC, in the fight against bacterial black spot on indoor tomato fruits, 2018-2019.

Analysis of the data obtained shows that the five-fold treatment with biofungicide Serenade, ASO, SC, application rates 5.0, 6.5 and 8.0 l/ha, has a positive effect on the yield of tomato plants. This is especially evident in the extreme conditions of protected ground in the Crimea – high relative humidity and air temperature (Table 1).

**Table 1.** Tomato yield by experiment variants variety (F1 hybrid) – Pink Ball, 2018-2019

Experiment variant	Average fruit weight, g	Yield		
		kg/bush	t/ha (estimated)	% to control
I. Serenade ASO, SC, 5.0 l/ha	168.5	5.5	112.8	144.8
II. Serenade ASO, SC, 6.5 l/ha	173.3	5.6	114.8	147.4
III. Serenade ASO, SC, 8.0 l/ha	177.8	5.8	118.9	152.5
IV. Gamair, SC, 10 l/ha (standard)	170.5	5.7	116.9	150.0
V. Monitoring	154.3	3.8	77.9	-

Carrying out five-fold treatments with the biofungicide Serenade ASO, SC allowed to significantly increase the yield on the experimental variants, compared with the control variant, where no treatments were carried out. The yield increase on variants with the tested drug Serenade ASO, SC in 3 application rates was close to the standard: 47.4% (Serenade

ASO, SC (5.0 l/ha)); 44.8% (Serenade ASO, SC (6.5 l/ha)); 52.5% (Serenade ASO, SC (8.0 l/ha)); 50.0% (Gamair, SC – standard).

## 4 Conclusion

The results of our research show that the five-fold treatment with biofungicide Serenade ASO, SC, application rates 5.0, 6.5, 8.0 l/ha, allows us to obtain high biological efficiency in protecting leaves and fruits of indoor tomato, at the level of 76.5-85.1% and 71.6-75.0%. % (on the 10th day after the fifth spraying), respectively. On the day of the last harvest, on the 29th day after the fifth spraying, the biological effectiveness of the five-time use of the biofungicide Serenade ASO, SC, application rates 5.0, 6.5, 8.0 l/ha, was 57.3-65.4% on leaves and 54.5-62.6% on fruits. The increase in the yield harvested at the control was 44.8, 47.4 and 52.5%.

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