The efficiency of application of biological preparations with a fungicidal effect against the sunflower diseases in the central zone of the Krasnodar region

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Abstract. Diseases are one of the main factors limiting the reception of high sunflower yields. The use of biological preparations for plant protection will allow to fight diseases successfully and improve the products quality. The article presents the results of research on the efficiency of application of biopreparations under the trademarks of BSka-3 with active ingredient Trichoderma viride, Pseudomonas koreensis, Bacillus subtilis and BFTIM with active ingredient Bacillus amyloliquefaciens against sunflower diseases. We conducted the experiments in 2018-2019 on the fields of the “Berezanskoye” farm in Korenovsky district of the Krasnodar region. We established that biofungicides effectively suppressed seed infection. In 2018-2019, dry rot of heads (Rhizopus spp.), bacterioses (Xanthomonas spp., Pseudomonas spp.) and Verticillium wilt (Verticillium dahliae Kleb.) had the highest prevalence in the sunflower sowing: 49.75 %, 42.0 % and 47.0 %, respectively. The schemes of BSka-3 D + BFTIM I + BFTIM K and BSka-3 A + BFTIM B + BFTIM B showed the best biological efficacy against Rhizopus spp.: 37 and 38 %, respectively. The schemes of BSka-3 D + BFTIM I + BFTIM K and BSka-3 A + BFTIM B + BFTIM B showed the best biological efficacy against the bacterioses: 54 and 51 %, respectively; the standard and variant of BSka-3 B + BFTIM V + BFTIM G showed 32 and 31 % of efficiency, respectively, against Verticillium wilt. The schemes of BSka-3 A + BFTIM B + BFTIM B (2.65 t/ha) and BSka-3 + BFTMI I + BFTMI K (2.61 t/ha) showed the best economic effectiveness.

1. Introduction

A significant increase of the world food production requires further development and expansion of modern agriculture [1]. It is predicted that by 2050 the world’s population will increase by 30 % to 9.2 billion people, and it is estimated that this will inevitably increase demand for food production by 70 % [2].

Sunflower (Helianthus annuus L.) is an important food, fuel and ornamental crop cultivated in many countries [3]. It is mainly used for the production of seed oil but also as a protein crop and fodder. Depending on the purposes of breeding and end use, there are three main types of sunflower: oil, confectionary and decorative [4].

Diseases are one of the main factors limiting the reception of high sunflower yields; they are caused by more than 30 pathogenic microorganisms. Differences in climate, distribution of pathogenic microorganisms, and crop cultivation technology affect the prevalence of individual diseases in each region [5, 6].

Previously, highly effective broad-spectrum chemical pesticides have been widely used to prevent disease outbreaks. Their long-term application caused a number of negative consequences, which required a search for less harsh protection methods in crop cultivation [1, 7].

All over the world, bacterial-based biofungicides draw considerable attention due to their cost-effectiveness, ecological safety, affordability and a wide range of antifungal effect against...
phytopathogens [8]. In addition, antagonistic bacteria are able to secrete enzymes (chitinase and protease) that break cell wall; these enzymes are responsible for the biocontrol potential against many fungal pathogens prior and after harvesting (Alternaria spp., Fusarium spp., Aspergillus spp., Pencillium spp., Rhizoctonia spp., Sclerotinia sclerotium, Botrytis cinerea, Verticillium dahlia) [9].

The biological control of soil pathogens by means of microbial antagonists is gaining more and more popularity in the system of crop protection [10-12]. Powdered and liquid compositions of microorganisms and their metabolites contribute to a plant growth, a significant decrease in disease rate, an increase in seed germination, plant height and productivity indicators, an obtaining of additional productivity and extra revenue [13, 14]. The preparations of multifunctional value with a wide spectrum of antifungal effect are of particular value among the range of biological plant protection products based on phytopathogenic antagonist microorganisms. They include biological products with the active ingredient of micromycetes of the genus Trichoderma spp., which are considered to be potential agents in control of fungal diseases of plants. They are able to interact with roots, increasing plant growth, resistance to diseases and abiotic stress. In addition, Trichoderma spp. can directly destroy fungal pathogens of a plant by means of antibiosis, as well as with the help of mycoparasitism strategies [15].

Chemical products of plant protection are used in cultivation of sunflower in the south of the Russian Federation, in the Krasnodar region. However, there has already been a positive trend towards the use of biological products of plant protection in crop cultivation technology. Therefore, the research aim was to study the effect of fungicides under the trademark of BSka-3 and BFTIM based on microbiological antagonists on the prevalence of diseases (Alternaria blight, Fusarium blight, dry rot, bacterioses, Phoma rot, etc.) in the sowings of confectionery sunflower of SPK variety of VNIIIMK breeding (Krasnodar) and to determine their biological effectiveness.

2. Materials and methods
In 2018-2019, we tested the fungicides based on microbiological antagonists against sunflower diseases (SPK variety) in the central soil-climatic zone of the Krasnodar region on the fields of the “Berezanskoeye” farm of the Korenovsky district.

In total, we tested 10 variants of schemes for protecting sunflower against diseases. Each scheme consisted of: the treatment of seeds with biofungicides of company OOO “Biotechagro” under the trademark of BSka-3 based on Trichoderma viride, Pseudomonas koreensis, Bacillus subtilis – Bska-3 A – Bska-3 K (various antagonist strains), Zn with application rate of 2 l/ha; the treatment of plants in the phase of 2-3 pairs of true leaves with biofungicides under the trademark of BFTIM based on Bacillus amyloliquefaciens (titer 1 × 10^8 CFU/ml) – BFTIM B – BFTIM S (various antagonist strains), Zn – 2 l/ha and in the budding phase with biofungicides under the trademark of BFTIM based on Bacillus amyloliquefaciens (titer 1 × 10^8 CFU/ml) – BFTIM B – BFTIM T (various antagonist strains), Zn – 3 l/ha. We chose the variant with untreated sunflower seeds and plants as a control; as a standard, we chose the variant with seeds and plants of a crop treated with recommended chemical fungicides (for seed treatment: Fludioxonil, SC (25 g/l) + Mefenoxam, WE (350 g/l) – 5.0 + 3.0 l/t; for the first treatment of plants: Azoxystrobine + Cyproconazole, SC (200 g/l + 80 g/l) – 0.8 l/ta; for the second treatment of plants: Dimoxystrobin + Boscalid, SC (200 + 200 g/la) – 0.5 l/ha).

We carried out the sowing with the “GASPARDO-MT 8” seeding machine according to the scheme with 3 replications of the experiment. We determined the biological effectiveness proceeding from the diseases prevalence in the experiment variants [6]. We subjected the obtained data to analysis of variance using SPSS software.

The growth and development of sunflower during the growth season (from April to August) took place against the background of increased daily average air temperatures (exceeding the long-term average data by 2.9-5.2 °C) during both years, critically low precipitation in 2018 – 134.0 mm, or 53.8 % of the long-term norm (249 mm), and increased precipitation in 2019 – 321.5 mm, or 129.0 % of the norm. Consequently, 2018 was characterized as unfavorable, and 2019 – as a moderately favorable year for the crop.
3. Results and discussion
At the first stage of the study of biological fungicides against diseases, we determined their effect on the sowing qualities and on contamination of sunflower seeds. We found that the laboratory seed germination in the control reached 75%, the standard was significantly higher than the control – 95%, as well as the variants with application of BSka-3 K, BSka-3 V, BSka-3 E and BSka-3 Z - 91, 87, 86 and 85 %, respectively. Alternaria spp., Rhizopus spp., Fusarium spp., as well as mold fungi (Penicillium spp., Aspergillus spp., Mucor spp.) were present on seeds and seedlings of sunflower in control; pathogen infection was 25, 12, 15 and 10 %, respectively, and the total infection reached 42 %. The application of biofungicides BSka-3 Z, BSka-3 E and Fludioxonil, SC + Mefenoxam, WE against Alternaria spp. showed high biological effectiveness – 68, 60 and 56 %, respectively. In comparison with the control, the affection of seeds and seedling with Rhizopus spp. decreased by 42-100 % in the variants. The standard, BSka-3 K, BSka-3 V, BSka-3 Z and BSka-3 I showed the maximum efficiency (100 %). The seed treatment with biofungicides BSka-3 V, BSka-3 K and BSka-3 Z contributed to the decrease of the Fusarium spp. infection by 80, 87 and 87 %, respectively, and with BSka-3 B and the standard – by 100 %. We observed the lowest effect (effectiveness did not exceed 50 %) against mold fungi in the preparations of Fludioxonil, SC + Mefenoxam, WE, BSka-3 V, BSka-3 A and BSka-3 G (Table 1).

Table 1. The effect of fungicide treatment of sunflower seeds on the laboratory seed germination and seed infection, V.S. Pustovoit All-Russian Research Institute of Oil Crops (2018-2019).

| Variant                  | Application rate, l/t | Laboratory seed germination, % | Alternaria spp. | Rhizopus spp. | Fusarium spp. | mold fungi |
|--------------------------|-----------------------|--------------------------------|-----------------|--------------|---------------|------------|
| Control (without treatment) | –                     | 75                             | 25 *            | 12 *         | 15 *          | 10 *       |
| Fludioxonil, SC + Mefenoxam, WE standard | 5.0 + 3.0             | 94                             | 68              | 100          | 100           | 50         |
| BSka-3 K, Zh             | 2.0                   | 91                             | 60              | 100          | 87            | 100        |
| BSka-3 V, Zh             | 2.0                   | 87                             | 36              | 100          | 80            | 50         |
| BSka-3 E, Zh             | 2.0                   | 86                             | 56              | 75           | 67            | 80         |
| BSka-3 Z, Zh             | 2.0                   | 85                             | 28              | 100          | 87            | 80         |
| BSka-3 I, Zh             | 2.0                   | 83                             | 48              | 100          | 33            | 100        |
| BSka-3 Z, Zh             | 2.0                   | 82                             | 36              | 91           | 40            | 100        |

* infected seeds in control, %

The total decrease in infection on seeds and seedlings of sunflower according to the experimental variants ranged from 40 to 78 %. The chemical standard showed the best result – 78 %, the biological fungicide BSka-3 K was at the level with it – 74 %.

At the next stage of research, we tested the preparations in the field. We carried out the sowing of sunflower seeds in the last 10 days of April. The full seedlings were obtained at the end of the first 10 days of May. In both years of research, in the variants with application of BSka-3 sunflower seedlings appeared 1-2 days earlier than in the standard and control, which indicates the growth-promoting effect of the preparation of this group. In the phase of sunflower leaf development we did not observe visual signs of disease infection.

The bacterioses (Xanthomonas spp., Pseudomonas spp.), dry rot of heads (Rhizopus spp.), and Verticillium wilt (Verticillium dahliae Kleb.) of sunflower had the highest prevalence in the conditions of 2018-2019. We observed the first signs of bacteriosis infection in the budding phase of sunflower: angular or small spots surrounded by a chlorotic halo and small brown necrosis along the veins appeared on the leaf blades. The prevalence of the disease, depending on the variant of the experiment, ranged from 3.6 to 11 %. It was the lowest in the standard and in schemes of BSka-3 A + BFTIM B + BFTIM B; BSka-3 B + BFTIM B + BFTIM G; BSka-3 G + BFTIM Zh + BFTIM 3 and BSka-3 E +
BFTIM L + BFTIM M – from 3.6 to 4.8 %. By the end of the growth season, we noted the maximum number of sunflower plants affected by bacteriosis in control – 54.1 %, in the variants of application of BSka-3 E + BFTIM L + BFTIM M and BSka-3 + BFTIM P – 47 and 41 %, respectively.

We found dry rot on sunflower at the end of flowering; hot and dry weather contributed to its early manifestation. The disease prevalence, depending on the experiment variant, ranged from 1.6 to 4.2 %, with the control having the highest value. During the seed-filling period, we observed the increase in the prevalence of dry rot of sunflower: it reached 18 % in the untreated variant, and it was the highest in the scheme of BSka-3 K + BFTIM C + BFTIM T – 20 %. At the same time, in the variants of BSka-3 A + BFTIM B + BFTIM B and BSka-3 B + BFTIM G the number of infected heads was minimal – 10/2 and 11.0 %, respectively. By the end of the growth season, the prevalence of dry rot was 30.5-49.5 %. It was the lowest in the schemes of BSka-3 A + BFTIM B + BFTIM V and BSka-3 D + BFTIM I + BFTIM K - 30.5 and 31.0 %, respectively, while in the chemical standard it was 34.5 %.

We observed the infection with Verticillium wilt in the second half of the sunflower vegetation during the seed-filling period. Its prevalence was highest in the control and, on average, reached 10.2 %, in the other variants it ranged from 2.5 to 5.8 %, and by the end of the crop ripening period, its prevalence was highest in the control and, on average, reached 10.2 %, and by the end of the crop ripening period, it reached 32-47 % in the variant BSka-3 B + BFTIM V + BFTIM G with the minimum values and 32 % in the chemical standard.

Based on the results of the diseases prevalence, we calculated the biological effectiveness of fungicides (Table 2).

**Table 2.** The biological and economic effectiveness of the application of fungicide schemes against diseases on sunflower, “Berezanskoye” farm of Korenovsky district of the Krasnodar region (2018–2019).

| Variant | Application rate, l/t, t/ha | Biological effectiveness, % | Yield, t/ha |
|---------|---------------------------|-----------------------------|-------------|
| Control* (without treatment) | - | 49<sup>o</sup> | 54<sup>o</sup> | 47<sup>b</sup> | 2.31<sup>b</sup> |
| Fludioxonil, SC + Mefenoxam, WE; Azoxystrobin + Cyproconazole, SC; Dimoxystrobin + Bosphalid, SC – standard | 5.0 + 3.0 | 30 | 34 | 32 | 2.37 |
| BSka-3 A + BFTIM B + BFTIM B | 2.0; 2.0; 3.0 | 38 | 41 | 24 | 2.65 |
| BSka-3 B + BFTIM V + BFTIM G | 2.0; 2.0; 3.0 | 30 | 46 | 31 | 2.45 |
| BSka-3 G + BFTIM Zh + BFTIM Z | 2.0; 2.0; 3.0 | 11 | 51 | 23 | 2.50 |
| BSka-3 D + BFTIM I + BFTIM K | 2.0; 2.0; 3.0 | 37 | 54 | 6 | 2.61 |
| BSka-3 Z + BFTIM P | 2.0; 3.0 | 30 | 40 | 23 | 2.51 |
| LSD<sub>05</sub> | - | - | - | | 0.15 |

* the disease prevalence in control, %

The variants of protection schemes of BSka-3 A + BFTIM B + BFTIM B (38 %) and BSka-3 D + BFTIM I + BFTIM K (37 %) were the best against dry rot of sunflower heads. The chemical standard decreased the prevalence of disease by 30 %, the schemes BSka-3 B + BFTIM B + BFTIM G and BSka-3 Z + BFTIM P were at the same level with it.

We observed the highest effectiveness against bacteriosis during the application of schemes BSka-3 D + BFTIM I + BFTIM K (54 %) and BSka-3 G + BFTIM Zh + BFTIM Z (51 %), the standard (32 %) and schemes of BSka-3 B + BFTIM V + BFTIM G (31 %) were the most effective against Verticillium wilt. The yield of sunflower seeds was at the level of 2.34-2.65 t/ha, the highest values were reached during applications of schemes of BSka-3 A + BFTIM B + BFTIM B and BSka-3 D + BFTIM I + BFTIM K – 2.65 and 2.61 t/ha, respectively.

**4. Summary**

The conducted research showed that biofungicides based on microbiological antagonists against the sunflower diseases under the trademark of BSka-3 effectively suppressed seed infection. The
fungicides BSka-3 K, BSka-3 Z were the best against *Alternaria* spp., BSka-3 K, BSka-3 V, BSka-3 Z and BSka-3 I were the best against *Rhizopus* spp., BSka-3 V, BSka-3 K, BSka-3 Z and BSka-3 B were the best against *Fusarium* spp. The treatment of sunflower during the growth season with BFTIM B (in the phase of leaf development) + BFTIM B (in the budding phase) decreased the prevalence of dry rot by 38%, while the treatment with fungicides BFTIM I + BFTIM K in these phases decreased the prevalence of bacteriosis by 54%, which is higher than the chemical standard by 8 and 20%, respectively. The schemes of BSka-3 A + BFTIM B + BFTIM B (2.65 t/ha) and BSka-3 D + BFTIM I + BFTIM K (2.61 t/ha) showed the best economic effectiveness.

5. References

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