Biological effectiveness of *Pseudomonas fluorescens* strains against bacterial and fungal diseases of spring rape in field conditions

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Abstract. The influence of four phytopathogens *Fusarium oxysporum*, *Botrytis cinerea, Alternaria brassicicola*, and *Xanthomonas campestris* on the biometric parameters of spring rape plants in field conditions was evaluated against the background of the use of the biological preparation Binoram, including live antagonistic bacteria *Pseudomonas fluorescens.* A significant decrease in economically important parameters of plants as a result of infection with tested phytopathogens and a positive effect on plant indicators of treatment with the biological preparation Binoram in comparison with the infected control is shown.

1 Introduction

The biopreparation Binoram, consisting of a composition of three strains of *Pseudomonas fluorescens* bacteria (7G, 7G2K and 17-2) was developed at the Institute of Cytology and Genetics SB RAS, and registered by ALSIKO-AGROPROM LLC. According to the manufacturer's registration data, the preparation increases the yield of various crops and protects plants from a wide range of diseases [1]. It was registered "as a fungicide on spring wheat, spring barley, potato, white cabbage; and as a plant growth regulator – on spring and winter wheat, spring barley, sugar beet, table and fodder one" [2].

In the course of studying the characteristics of the preparation's effect on the yield of protected crops and the development of plant diseases, a study was conducted on preparation effectiveness on spring rape under controlled conditions against the background of infection with the causative agent of bacteriosis of cabbage crops *Xanthomonas campestris* [3]. The purpose of this work was to assess Binoram effectiveness in the field. In the experiment, plants of two rapeseed varieties (Forum and Griffin) were used, contrasting in resistance to the causative agent of vascular bacteriosis of cabbage crops [4], and susceptible to the causative agents of fusarium *Fusarium oxysporum* [5], Alternaria blight *Alternaria brassicicola* [6], and gray mold *Botritis cinerea* [7].

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2 Materials and Methods

Seeds of rapeseed varieties Griffin (Syngenta Russia LLC, resistance to race 4 *Xanthomonas campestris*) and Forum (Lipetsk Rapeseed Research Institute, medium-susceptible to *Xanthomonas campestris*) were provided by the originators. The plants were grown at the experimental field of VNIISSOK, Moscow in 2017. The soil in the field is light loamy, with a humus content of 1.5%, pH = 6.0. The recommended rates of application of mineral fertilizers for spring rape were used N:P:K 1:0.45:0.85 and regular insecticide treatments were used to control pests. Starting from the 4-5 phase of real leaves, plants were inoculated with pure cultures of phytopathogenic bacteria *Xanthomonas campestris* (strain 1362, isolated from rapeseed) and fungi *Fusarium oxysporum* (strain Fo123), *Alternaria brassicicola* (strain Ab234T), and *Botrytis cinerea* (strain Bc14) at intervals of 3-5 days. To obtain inoculum, mushroom cultures were grown on Petri dishes with agarized Czapek-Dox nutrient medium [8] for 14 days at a temperature of 24°C. Fungal spores were washed off with sterile water with the addition of 0.01% TWEEN 20 as a wetting agent, and brought up by breeding 10^5 colony-forming units (CFU) per ml. The concentration of spores was determined by microscopy in the Goryaev chamber. Bacteria for infection were grown for 48 hours on dextrose-yeast agar medium with calcium carbonate (YDC) [9]. The bacterial mass was removed with a spatula, suspended in sterile water and diluted to a concentration of 10^6 CFU/ml. The concentration was determined by the optical density of suspension using a BioSan DEN-1 densitometer. (Latvia). Inoculation of plants was carried out by spraying (the consumption rate is 15 ml of suspension per plant) with preliminary injury of leaves with scissors before sunset to ensure the maximum length of the leaf humifying period. All treatment options had a threefold, unprotected control had a sixfold repetition. The placement of accounting plots is randomized. Treatment of plants with Binoram was carried out at the recommended dose (4 l/ha) as a protection on options with artificial infection. Treatment with the preparation was carried out when the first symptoms of the disease appeared. A mixture of tebuconazole and triadimephone (150+100 g/l, respectively) was used as a standard. The biometric indicators of plants (height, stem, weight of the aboveground and underground parts, number of leaves, leaf length and width, weight of seeds from one plant, weight of 1000 seeds) and assessment of the development of disease symptoms were carried out in the phases of milk and technical ripeness of seeds. The development of diseases was considered by the percentage of the affected leaf area, estimated using the LeafDoctor program [10]. The analysis of the obtained data was carried out by the method of multivariate variance and correlation analysis using the Statistica software package (ver. 12.5, StatSoft) [11].

3 Results and Discussion

The development of diseases in the options with inoculation and without protection was high due to favorable weather conditions for infection (the average daytime air temperature for the entire period of the experiment (40 days) was 22.3°C, night - 16.9 °C, average humidity – 78.9%, precipitation - 128 mm.

The variance analysis showed a significant decrease in the basic biometric indicators of plants when infected with phytopathogens without the use of protection. (Table 1). For pairs of indicators, average height - number of leaves; average height - leaf length, average height - weight of the aboveground part of the plant, average height - weight of the underground part, a high correlation is shown. Also, a statistically significant correlation was found for the following pairs of treats: number of leaves - weight of the aboveground and underground parts; leaf length – leaf width; weight of the aboveground part – weight of the underground part. At the same time, the reaction of plants to infection based on the weight of the underground part and the aboveground part of the plant and the width of the leaf was variety-

specific. The development of rapeseed diseases caused the most significant decrease in the biometric indicators of plants when infected with phytopathogens *X. campestris* and *B. cinerea*. In these options, a statistically significant decrease in plant height, number of leaves, leaf length, leaf width, and weight of the underground part of the plant was shown. The development of Alternaria blight (caus. *A. brassicicola*) significantly reduced only the leaf length index, and the infection of plants with fusarium (caus. *F. oxysporum*) unexpectedly led to a significant increase in the weight of the underground part of plants, leaf length, and plant height. This result can be explained by the predominant development of fusarium at elevated temperatures [12]. Perhaps this is due to low night temperatures and the release of *F. oxysporum* substances similar to plant hormones. The most noticeable was the stimulating effect on the weight of the underground part on plants of the Griffin variety.

The use of Binoram against the background of the development of rapeseed diseases significantly increased weight of the aboveground and underground parts of plants, plant height, and leaf width. In the Binoram option, the average plant height was 10% higher, the number of leaves increased by 14%, leaf length – by 7%, leaf width – by 14%, and the weight of the aboveground and underground parts of plants increased by 37% and 43% compared to the unprotected variant (Table 2). The use of Binoram led to a significant reduction in leaf damage. On the Griffin variety, this indicator was 55.8% versus 90.0% without protection (biological efficiency of 48%), for the Forum variety – 30.8% versus 82%, respectively (biological efficiency of 62.4%). A significant increase in the weight of seeds from one plant and the weight of 1000 seeds for the Griffin variety was also shown (Table 2).

Table 1. The effect of infection with phytopathogens on the biometric indicators of rapeseed plants. All indicators are averaged for 2 varieties, the assessment was carried out 40 days after infection.

Biometric indicator	**Infection option***	**% to control (Folinor) and the group of significant differences according to the Duncan criterion at 95% significance level**
Average plant height, cm	*X. campestris*	86.3 A
	B. cinerea	89.6 AB
	A. brassicicola	93.1 ABC
	Control**	98.6 BC
	F. oxysporum	103.1 C
Average number of leaves, pcs.	*B. cinerea*	82.0 A
	A. brassicicola	85.0 AB
	X. campestris	95.5 B
	Control	95.5 B
	F. oxysporum	96.9 B
Average leaf length, cm	*A. brassicicola*	80.7 A
	X. campestris	82.1 A
	B. cinerea	84.5 AB
	F. oxysporum	92.4 BC
	Control	95.3 C
Average leaf width, cm	*X. campestris*	70.7 A
	B. cinerea	74.2 AB
	A. brassicicola	75.8 AB
	F. oxysporum	81.3 AB
	Control	84.0 B
Average weight of the aboveground part of the plant, g	*A. brassicicola*	72.0 A
	X. campestris	77.9 A
	B. cinerea	79.1 A
	F. oxysporum	92.7 A
	Control	94.7 A

Biometric indicator	**Infection option***	**% to control (Folinor) and the group of significant differences according to the Duncan criterion at 95% significance level**
Average root weight, g	*B. cinerea*	65.8 A
	X. campestris	67.2 A
	A. brassicicola	91.1 AB
	Control	102.6 AB
	F. oxysporum	106.3 B

**Control without infection and protection

Table 2. Biometric data of plants by varieties and options (as a percentage of the control with Folinor protection).

Option	Average height, %	Average stem weight, %	Average root weight %	Weight of seeds from the plant %	Weight of 1000 seeds, %	Average score of leaf damage %
Forum variety: Without protection	86.90 A	47.63 A	53.27 A	44.29 A	92.50 A	82.00 B
Binoram	97.06 B	87.76 B	84.50 B	77.59 B	98.00 A	30.8 A
Griffin variety: Without protection	88.05 A	47.93 A	57.54 A	47.54 A	87.21 A	90.0 B
Binoram	97.81 B	88.16 B	87.16 B	80.82 B	93.29 B	55.8 A

Groups that are reliably distinguishable by the Duncan criterion are shown in letters to the right of the average values

4 Conclusions

The results obtained showed a significant reduction in the damage of spring rape plants by phytopathogens when using the Binoram preparation. The use of Binoram against the background of artificial infection with pathogens led to a significant increase in the weight of seeds from one plant and the weight of 1000 seeds for the Griffin variety. Rapeseed plants in the variants treated with Binoram developed better against the background of infection with pathogens. Further application of biofungicide Binoram on spring rape and other cabbage crops within the framework of comprehensive plant protection programs is promising.

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