The effect of bacterial products and mineral fertilizers on growth and productivity of *Brassica juncea Czern*

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Abstract. The paper reports the results of field experiments that analyzed changes in growth indicators and productivity of Sarepta mustard (*Brassica juncea* Czern.) of the Start variety (k-4259) in response to inoculation and increasing doses of mineral nitrogen. Morphometric parameters and productivity of dry mass were measured during the stage of harvesting maturity, i.e., the full flowering stage. The seeds were inoculated with the following bacterial products: Agrofil (*Agrobacterium radiobacter*, strain 10), Mizorin (*Arthrobacter mysorens*, strain 7), Mobilin (*Pseudomonas fluorescens*, strain PG-5), and Flavobakterin (*Flavobacterium sp*., strain 30). The amount of nitrogen ranged from N_{30} to N_{120}. It was enhanced with the application of PK at the rate of 60 kg/ha. The experiments showed that the application of bacterial products and increasing doses of mineral nitrogen has a stimulating effect on plants. The fertilizer formulation $N_{120}P_{60}K_{60}$ and rhizobacteria strains *Flavobacterium sp*., strain 30, and *Pseudomonas fluorescens*, strain PG-5, proved to be the most effective. The obtained data were roughly consistent with the application of N_{90} and N_{120}. The use of mineral fertilizers resulted in the highest productivity of dry shoot mass.

1 Introduction

Growing environmental awareness and efforts to increase sustainability of agriculture worldwide have encouraged the introduction of new biotechnologies that are gaining momentum [1]. Among them a special focus is given to soil microflora, in particular, associative nitrogen-fixing bacteria [2-3]. It is known [4-5], that apart from improving mineral nutrition, nitrogen-fixing bacteria have a positive impact on root health. Therefore, the application of growth-promoting bacteria to the plant root zone increases plant productivity and resistance to such adverse external factors as soil drought even during the

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critical period of plant development [6]. This is especially relevant for the productivity of non-legumes, whose needs in nitrogen under favorable conditions have been shown to be provided for by almost 45% [7].

Hence, such eco-friendly bacterial tools are increasingly seen as an alternative to the traditional use of chemical fertilizers. According to some reports, modern biotechnologies can partially [8, 9] or even completely [10] replace chemical fertilizers. On the other hand, the application of a small amount of nitrogen enhances biological nitrogen fixation of root-associated bacteria (2 to 4 times), and inhibits nitrogen starvation [11].

2 Materials and Methods

The study was conducted in 2018-2019 and 2021 at the agrobiological research station of Herzen University using a common methodology of experimental research [12]. Field experiments were conducted on soddy podzolic sandy loams with an average quality of cultivation. The soil reaction with pH_{KCl} - 5.7 was close to neutral. Average humus content amounted to 1.5%. The content of mobile phosphorus (147 mg/kg) and potassium (120 mg/kg) on the research site was determined using A.T. Kirsanov's methodology [12]. The sampling area of each plot was 1 m^2. Prior to the experiments, the research site was planted with a mix of crops (barley, oats, and wheat) for three years. During the years of study, weather conditions were similar—average monthly temperatures and precipitation did not show much difference (Table 1).

Table 1. Meteorological conditions of Sarepta mustard vegetation periods (2018-2019, 2021).

Month	Ten-day period	Air temperature, °C				Precipitation, mm			
		per year			Average for 3 years	per year			Average for 3 years
		2018	2019	2021		2018	2019	2018	
5	2	7.3	10.9	7.9	8.7	7.9	7.2	7.0	8.6
	3	13.2	16.2	11.9	13.8	12.1	13.7	10.2	12.0
ten-day period 1 and 2		10.3	13.6	10.3	11.3	20.0	20.9	17.2	20.6
6	1	13.1	13.9	13.5	13.5	10.7	11.0	12.7	11.5
	2	14	16.6	14.7	15.1	11.3	11.1	4.5	9.0
	3	17.3	16.7	20.7	16.9	9.3	9.8	1.5	6.9
one month		14.8	15.7	16.3	15.2	31.3	31.9	18.7	27.4
7	1	16.7	18.9	18.1	17.9	5.5	0	0	5.5
ten-day period 1		16.7	18.9	18.1	17.9	5.5	0	0	5.5

Note: * – average monthly temperature; ** – monthly precipitation. Source: Compiled by the authors.

The study focused on Sarepta mustard (*Brassica juncea* Czern.) of Start variety (k-4259). It is a minor crop which, however, is quite important as a forage and green manure crop in the North-West of Russia [13]. The seeds for the experiment were provided by the N.I. Vavilov All-Russian Institute of Plant Genetic Resources. In one experiment, plant seeds were inoculated with products based on plant-associated rhizobacteria strains: Agrofil (*Agrobacterium radiobacter*, strain 10), Mizorin (*Arthrobacter mysorens*, strain 7), Mobilin (*Pseudomonas fluorescens*, strain PG-5), and Flavobakterin (*Flavobacterium sp*., strain 30). Bacterial products were provided by the Laboratory of Ecology of Symbiotic and Plant-Associated Rhizobacteria of the All-Russian Research Institute for Agricultural Microbiology. The seeds were treated with pre-mixed suspension with a titer of 107 CFU/ml immediately before sowing. The seeds from the control site (uninoculated) were treated with water.

Another field experiment was related to the application of mineral fertilizers. We used the most common fertilizers and did mineral recalculation per active ingredients (a.i.): ammonium nitrate (34.4% N as a.i.), simple granular superphosphate (26% P_2O_5 as a.i.) and potassium sulfate (50% of K_2O as a.i.). We also introduced a single dose of $N_{60}P_{60}K_{60,}$ i.e., 60 kg of active ingredient per a hectare of soil. This amount is used for most crops grown in climatic and soil conditions of the North-West of Russia. Fertilizers were scattered before sowing. The dose was applied according to the experimental design: 1) control site untreated with fertilizers ($N_0P_0K_0$); 2) $N_{30}P_{60}K_{60}$; 3) $N_{60}P_{60}K_{60}$; 4) $N_{90}P_{60}K_{60}$; 5) $N_{120}P_{60}K_{60}$.

Fertilizers were applied four times, each time following the indicated experimental design. Morphometric parameters and plant productivity were measured during the full flowering stage, i.e., the stage of harvesting maturity. The experiment lasted from the sowing stage (second ten days of May) to the flowering stage (first ten days of July). This made 54 days in total. Statistical processing of data was carried out using the dispersion method [14].

As was shown earlier, white mustard inoculated with bacterial products had a positive impact on the revenue of a farming enterprise relative to the control group, i.e., it showed a better economic efficiency [15].

3 Results

Field experiments have shown that inoculation of Sarepta mustard with bacterial products and the application of high doses of nitrogen ($N_{90}P_{90}K_{60}$) has a positive impact on the plant height (Table 2). During the flowering stage, plants inoculated with Flavobakterin and Mobilin were found to be taller by 13.4 cm and 13.2 cm, respectively, than the control plants (87.3 cm). When applying a double dose of nitrogen (120 kg/ha), plants showed an increase in linear growth to almost the same values—104.0 cm, however, this increase was much more significant against the control plants (by 36%). The increase in height (80.1 cm) when applying a dose of N_{30} is reliably comparable to N_{60} (81.5 cm), while the use of N_{90} resulted in a more pronounced increase (88.9 cm).

Table 2. Changes in growth indicators of Sarepta mustard in response to the inoculation of seeds with bacterial products and increasing amounts of mineral nitrogen (a three-year average).

Type of impact	Plant height		Number of leaves	
	cm	%	pcs/plant	%
Application of bacterial products				
Control plants	87.3	100	4.6	100
Agrofil	97.4	112	6.7	146
Mizorin	100.1	115	7.1	155
Mobilin	100.5	116	8.6	187
Flavobakterin	100.7	119	7.9	173
LSD_{05}	6.8	-	2.1	-
Application of mineral fertilizers				
Control plants	76.5	100	5.0	100
$N_{30}P_{60}K_{60}$	80.1	105	5.9	119
$N_{60}P_{60}K_{60}$	81.5	106	7.5	151
$N_{90}P_{60}K_{60}$	88.9	116	8.7	175
$N_{120}P_{60}K_{60}$	104.0	136	9.3	185
LSD_{05}	3.6	-	1.8	-

Source: Compiled by the authors.

A plant leaf is instrumental in assimilation; it is also a key structural element of green mass productivity.

The experiment with rhizobacteria showed that the application of Mizorin, Flavobakterin and Mobilin significantly increased the number of leaves per plant. The highest values were observed after the pre-sowing treatment of seeds with plant-associated strains of pseudomonads. The application of Mobilin increased the number of leaves to 8.6 pcs/plant which is 87% higher than 4.6 pcs/plant in the control group. However, these values are not significantly different from those obtained after the application of Flavobakterin.

The obtained data corresponds to the application of 90 kg/ha of mineral nitrogen—8.7 leaves per plant, which is 75% more than in the control plants. At the same time, reliable increases were noted already after the application of $N_{60}P_{60}K_{60}$ (7.5 pcs/plant). They were comparable to the use of 90 kg/ha nitrogen (8.7 pcs/ plant). The biggest increase in the number of leaves (9.3 pcs/plant) was observed after the application of the highest doze of nitrogen—N_{120}.

An increase in the height of plants and the number of leaves is associated with an increase in the number and length of internodes. Therefore, plants with pronounced metamerism (an increase in the number and length of internodes) tend to develop shoot mass more intensely, which increases plant productivity. An increase in the number of internodes is associated with an increase in the number of leaves, hence, changes in the number of internodes result in qualitative changes in shoot mass.

Our experiments (Table 3) showed that the application of rhizobacteria resulted in the reliable increase of internodes against the control plants (5.4 pcs/plant). This effect was especially pronounced with a 11-14% increase after the application of Flavobakterin (5.9 pcs/plant) and Mobilin (6.0 pcs/plant). However, we did not observe any reliable differences between the two bacterial products.

The number of internodes reliably increases at 60 kg/ha and 90 kg/ha (5.8 pcs/plant) of mineral nitrogen, exceeding the control indicator by 5% (5.5 pcs/ plant). The application of 120 kg/ha of nitrogen increased the number of internodes to 6.1 pcs/plant (13% against the control plants). This result was significantly higher than the data obtained at lower nitrogen doses.

Table 3. Number and length of Sarepta mustard internodes depending on the bacterial product and the dose of nitrogen fertilizer (a three-year average).

Type of impact	Number of internodes		Length of internodes	
	pcs/plant	%	cm	%
Application of bacterial products				
Control plants	5.4	100	7.5	100
Agrofil	5.7	106	7.7	103
Mizorin	5.7	106	7.7	103
Mobilin	6.0	114	7.8	104
Flavobakterin	5.9	111	8.1	107
LSD_{05}	0.2	-	0.3	-
Application of mineral fertilizers				
Control plants	5.5	100	5.5	100
$N_{30}P_{60}K_{60}$	5.6	102	6.8	121
$N_{60}P_{60}K_{60}$	5.8	105	7.3	129
$N_{90}P_{60}K_{60}$	5.8	105	7.2	126
$N_{120}P_{60}K_{60}$	6.1	113	7.7	134
LSD_{05}	0.3	-	1.2	-

Source: Compiled by the authors.

The experiment with pre-sowing seed inoculation was reliably effective with the use of Flavobakterin (8.1 cm) and Mobilin (7.8 cm) which amounts to 107% and 104% relative to the control plants (7.5 cm). Inoculation with Agrofil and Mizorin also contributed to an increase in internode length almost to the same extent (7.7 cm), however, this increase is insignificant.

The experiments with mineral fertilizers showed an increase in the length of mustard internodes. The length increased by 21-34% relative to the control group (5.5 cm) in all experimental plants. A significant increase was noted already at 30 kg/ha nitrogen application—6.8 cm (21% higher than the control plants). The application of $N_{60}P_{60}K_{60}$ and $N_{90}P_{60}K_{60}$ correlated with the respective internode length of 7.3 cm and 7.2 cm, (29% and 26% longer than the untreated plants). The biggest increase in the internode length of 7.7 cm (34% longer than the control plants) was due to the high nitrogen dose of 120 kg/ha.

The abundance and retention of buds and flowers in crops is an important forward-looking indicator of future seed yields. Our experiments assumed that Sarepta mustard is a green manure crop. Hence, it was reasonable to estimate the number of buds during the peak of flower-bud formation and the number of flowers during the peak of flowering.

Budding makes the reproductive organs highly vulnerable. As compared to flowers, buds are more sensitive to external factors which cause them to drop: changes in night temperatures, lacks of phosphorus, etc. Therefore, the number of retained buds that later develop into flowers is an important indicator.

The reported study found that bacterial products contributed to an increase in the number of formed buds, with the highest efficiency shown by Mizorin, Flavobakterin, and Mobilin (Table 4). They facilitated a 20-26% increase in the number of buds compared to the control plants.

Table 4. Number of Sarepta mustard flowers and buds depending on the bacterial product and the dose of nitrogen fertilizer (a three-year average).

Type of impact	Number of buds		Number of flowers	
	pcs/plant	%	pcs/plant	%
Application of bacterial products				
Control plants	10.0	100	7.2	100
Agrofil	11.0	110	8.4	117
Mizorin	12.6	126	11.2	156
Mobilin	12.0	120	9.4	131
Flavobakterin	12.4	124	8.6	119
LSD_{05}	0.6	-	0.8	-
Application of mineral fertilizers				
Control plants	8.4	100	7.4	100
$N_{30}P_{60}K_{60}$	9.2	110	7.8	105
$N_{60}P_{60}K_{60}$	9.4	112	8.8	119
$N_{90}P_{60}K_{60}$	11.0	131	8.8	119
$N_{120}P_{60}K_{60}$	14.6	174	11.6	157
LSD_{05}	0.4	-	0.5	-

Source: Compiled by the authors.

Increasing doses of mineral nutrition also stimulated the formation of buds during plant development. Interestingly, N_{60} and N_{90} returned almost the same results: 9.2 pcs/plant and 9.4 pcs/plant, respectively. The highest increase was observed at N_{120}, a double dose of nitrogen with 14.6 pcs/plant, which is 74%, higher than the control data with 8.4 pcs/plant.

Our data also showed a high bud retention rate, i.e., an increase in the number of buds that formed flowers. This number was significantly higher than that for the uninoculated seeds. Mizorin was found to be a top performing bacterial product with a 56% increase relative to the control plants (11.2 pcs/plant and 7.2 pcs/plant, respectively).

The experiments with different doses of mineral nitrogen showed a significant increase in the number of flowers at a dose of N_{60} and N_{90} with 8.8 pcs/plant. This figure exceeds the control data of 7.4 pcs/plant by 19%. The most significant increase in the number of flowers in Sarepta mustard was observed after the application of $N_{120}P_{60}K_{60}$ with 11.6 pcs/plant. This result is 57% higher relative to the control plants. It is also significantly higher than the effect produced by lower doses of nitrogen.

Apart from the plant height and the number of leaves, plant density is yet another important element of shoot mass productivity. It may be defined as a plant's ability to form a certain number of side shoots. According to [16], plant-associated rhizobacteria are capable of producing phytohormones that actively stimulate the development of a plant's vegetative organs. As is shown in [17], mineral nitrogen also contributes to the intensive development of shoot mass and an increase in plant yield.

The reported study found that, against the control plants, all the inoculated experimental plants formed an equally large number of side shoots (Table 5). On average, all the bacterial products used in the experiment produced an increase of 3.3-3.6 pcs/plant relative to the uninoculated plants (2.1 pcs/plant).

Table 5. Number of Sarepta mustard side shoots and dry shoot mass depending on the bacterial product and the dose of nitrogen fertilizer (a three-year average).

Type of impact	Number of side shoots		Dry shoot mass	
	pcs/plant	%	c/ha	%
Application of bacterial products				
Control plants	2.1	100	28.1	100
Agrofil	3.3	158	28.9	110
Mizorin	3.3	158	29.7	120
Mobilin	3.3	158	30.6	130
Flavobakterin	3.6	174	31.8	135
LSD_{05}	0.9	-	1.1	-
Application of mineral fertilizers				
Control plants	1.5	100	29.8	100
$N_{30}P_{60}K_{60}$	2.1	139	34.4	115
$N_{60}P_{60}K_{60}$	2.2	144	41.4	139
$N_{90}P_{60}K_{60}$	2.2	144	43.0	144
$N_{120}P_{60}K_{60}$	2.3	150	46.0	154
LSD_{05}	0.6	-	4.3	-

Source: Compiled by the authors

The application of nitrogen significantly increased the numbers of side shoots event at the dose of 30 kg/ha: 2.1 pcs/plant relative to the control plants with 1.5 pcs/plant. No significant increase in the number of shoots was observed at higher doses, however, the dose of 120 kg/ha returned the maximum number of side shoots with 2.3 pcs/plant.

Pre-sowing inoculation of Sarepta mustard resulted in higher productivity of shoot mass in all the experimental plants. Top-performing bacterial products as regards productivity were Flavobakterin, Mobilin, and Mizorin. As compared to the control plants (28.1 c/ha), Flavobakterin (31.8 c/ha) and Mobilin (30.6 c/ha) were found to have the highest efficiency in enhancing plant productivity: 35% and 30%, respectively.

Increasing doses of nitrogen (60 kg/ha) improved the productivity of dry matter by

39%: up to 41.4 c/ha relative to the control plants with 29.8 c/ha. The dose of 90 kg/ha nitrogen produced a similar effect of 43.0 c/ha. A further increase in dry mass to 46 c/ha after the application of a double dose of nitrogen ($N_{120}P_{60}K_{60}$) was insignificant. According to [18], such values of shoot mass are associated with the accumulation of impermissible levels of nitrate nitrogen. However, this effect is not observed after the application of bacterial products [19].

The above factors have an impact on productivity (Table 5) and the economic efficiency of Sarepta mustard (Table 6).

Table 6. Economic efficiency of bacterial products and mineral fertilizers applied to Sarepta mustard: Data based on dry shoot mass.

Bacterial products	Effect, in % relative to the control plants	Mineral fertilizers	Effect, in % relative to the control plants
Agrofil	102.85	$N_{30}P_{60}K_{60}$	115.44
Mizorin	105.69	$N_{60}P_{60}K_{60}$	138.93
Mobilin	108.90	$N_{90}P_{60}K_{60}$	144.30
Flavobakterin	113.17	$N_{120}P_{60}K_{60}$	154.36

The analysis of data on economic efficiency showed that Flavobakterin was the most effective product. Its application resulted in a 13.17% increase against the control plants. The experiment showed that the application of increasing doses of nitrogen (N_{30}-N_{120}) improved economic efficiency by 39-54%.

4 Discussion

The study showed that bacterial products based on associative nitrogen-fixing strains of rhizobacteria are effective stimulators of growth and shoot mass productivity of Sarepta mustard. Their efficiency is very similar to that of mineral fertilizers at a dose of $N_{60}P_{60}K_{60.}$ These findings are a point of departure for future research.

It remains unclear if bacterial products are equally effective at low doses of nitrogen fertilizers. It is also not clear if the experimental results are valid for other annual Brassicaceae crops, e.g., oilseed radish, Abyssinian mustard, black mustard, etc. Despite their low popularity in the North-West of Russia, these crops definitely hold promise as they boast high productivity of shoot mass.

At the same time, not only different species of plants within the same family, but even different plant varieties may have a different response to plant-associated rhizobacteria. Therefore, it remains unclear whether the obtained experimental data are indicative of varietal or species-related responsiveness of Sarepta mustard. This means further experiments with other varieties of *Brassica juncea* Czern are needed.

5 Conclusion

The experimental study concluded that inoculation of Brassica juncea Czern plants of Start variety with bacterial products based on plant-associated rhizobacteria strains and the application of increasing doses of mineral fertilizers stimulates plant growth and dry shoot mass productivity. Thus, the application of rhizobacteria resulted in the following: plant height increased by 7-5%, the number of leaves by 8-73%, the number of side shoots by 74%, dry weight by 30-35%.

In this regard, Flavobakterin (Flavobacterium sp., strain 30) and Mobilin (Pseudomonas sp., strain PG-5) showed top performance. The effect of their application was similar to that of nitrogen at the dose of N60 and N90. The highest increase in dry mass productivity of mustard (39-44%) was noted after the application of 60-90 kg/ha of mineral nitrogen.

6 References

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