

EFFECTS OF BIOLOGICAL TREATMENTS ON PRODUCTION AND QUALITY PARAMETERS OF SOYBEAN (*GLYCINE MAX* L. MERRILL)

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Keywords: Organic farming, Soybean, Biological treatment, Production

Abstract

Innovative approaches such as seed treatment with lactic acid bacteria (LAB), mycoparasitic fungi (MPF), and entomopathogenic fungi (EPF) can improve yield and quality. A plot experiment with the variety Abaca was conducted on organically certified plots at two climatically distinct sites in the Czech Republic. In the early growth stages, MPF and LAB treatments showed slight improvements in root length, weight, and plant dry matter. Other parameters, including plant height before harvest, thousand-grain weight, and yield, showed a positive but statistically inconclusive effect. Regarding quality, a beneficial impact of MPF and LAB on protein content in seeds was recorded. Starch and oil contents were unaffected. The differences were relatively small, suggesting that biological seed treatments support soybean yield and quality, particularly when combined with other agrotechnical measures.

Introduction

In the organic farming system, legumes are one of the key tools that can mitigate ecosystem deficiencies, reduce nutrient imbalances, contribute to ecosystem resilience, and support biodiversity (Didora *et al.* 2022). By enhancing soil organic matter fractions to promote biological nitrogen fixation, legumes are essential components of successful organic agricultural practices. Soybean (*Glycine max* L. Merrill) has assumed a pivotal role as a globally significant crop, supplying 25% of the world's edible oil and approximately two-thirds of livestock protein concentrate and substantially contributing to food security. Organic soybean farming is driven by the growing consumer demand for organic products and the need for sustainable agricultural practices (Mishra *et al.* 2024).

In recent years, research efforts have increased to devise sustainable methods for improving soybean production (Ayilara *et al.* 2023). Several biocontrol agents have been evaluated for their usefulness to control diseases of soybeans, with varying levels of success (Hartman *et al.* 2016). The direct application of biocontrol agents is more likely to control below-ground diseases and pests (Caldwell *et al.* 2005). Mycoparasitism, the direct attack of one fungus on another using it as a nutrient source, plays a significant role in both ecological and agricultural contexts, particularly in the biological control of plant diseases (Steyaert *et al.* 2003). Entomopathogenic fungi play a vital role in insect population dynamics, making them the earliest insect pest control agents (Maina *et al.* 2018). To date, over 750 fungi species are known to infect insects and mites, which represent about all the major classes of fungi (Moorhouse *et al.* 1992, Hajek and St. Leger 1994).

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The use of fungal endophytes to improve yields and protect plants from damage is increasing (Shrivastava *et al.* 2010). The use of lactic acid bacteria (LAB) in soil-plant systems has been the subject of increasing interest in recent years (Murindangabo *et al.* 2023). LAB isolated from have been shown to be effective biocontrol agents against a wide variety of fungal and bacterial phytopathogens. They can also directly promote plant growth or seed germination and alleviate various abiotic stresses (Lamont *et al.* 2017). Historical and current research demonstrates clear potential for LAB as useful, renewable, and safe agricultural inputs for improving plant growth. Despite progress made in the knowledge of the modes of action of these biological control agents, practical application often fails to control disease in the fields (Alabouvette *et al.* 2006). This study aimed to develop and evaluate a biological control strategy that combines lactic acid bacteria, mycoparasitic fungi, and entomopathogenic fungi to enhance disease management in organic soybean production.

Materials and Methods

The experiment was conducted in two locations on a field certified for organic agriculture from 2022 to 2024. Experimental location of the University of South Bohemia in České Budějovice district, Zvíkov municipality (48.9689644N, 14.62318111E): grain production area, altitude 490 m, soil modal cambium - loam. Climatic characteristics: long-term average air temperature 8.7°C, long-term average precipitation 629 mm.

Experimental location of the Czech University of Life Sciences in Prague, Uhřetěves municipality (50.0329700N, 14.6184069E): beet production area, altitude 295 m, modal brown soil - clayey loam. Climatic characteristics: long-term average air temperature 9.8°C, long-term average precipitation 560 mm.

The Abaca soybean variety was selected for the experiment. The experimental plots were 12 m² in three replications and consisted of four combinations: control, mycoparasitic fungi (MPF)-treated seed, lactic acid bacteria (LAB)-treated seed, and entomopathogenic fungi (EPF)-treated seed. EPF treatment was applied for two years, while other treatments were applied for three years. The seeding rate was used at a density of 400 g/plot (33.33 g/seed m²). Standard small-plot mechanization was used to establish, manage, and harvest the experiment.

In the laboratories of the Dairy Research Institute, Ltd. and the Department of Plant Production, Faculty of Agriculture and Technology in České Budějovice, promising strains of mycoparasitic fungi (*Trichoderma virens*), entomopathogenic fungi (*Metarhizium brunneum*), and lactic acid bacteria were selected based on laboratory tests. The seed was treated with an MPF, EPF, and LAB suspension. The suspension was prepared to a standard titer in concentrations: 1×10⁶ spores in 1 ml of suspension (MPF, EPF) and 5×10⁶-10⁷ spores in 1 ml of suspension (LAB). At the beginning of flowering (BBCH 59-61), a foliar application was made using microorganisms produced as a solution (LAB) and applied to a natural substrate (MPF and EPF). A detailed procedure for preparing the suspensions is available in Nguyen *et al.* (2025).

In the initial growth phases, repeated sampling of whole plants was carried out, and the effect of seed treatment on the weight of plants and roots was evaluated. The occurrence of diseases and pests was monitored on a weekly basis during the vegetation period. Before harvesting, the number of plants per square meter and the number of pods on the plant were counted. Subsequently, the crop was harvested using a small plot combine harvester. The yield and weight of a thousand seeds were determined, and the protein, oil, and starch content in the seeds were also measured.

Data were analyzed using the Stat Soft, Inc. STATISTICA, version 12, by one-way ANOVA with a significance level of 5%. The Tukey's HSD test with a significance level of 5% was used to

determine the differences between the individual variants. Box plots showing the effect of different treatments were processed in Excel.

Results and Discussion

The effect of seed treatment with mycoparasitic fungi, entomopathogenic fungi, and lactic acid bacteria was evaluated at the beginning of the vegetation period. Three samples of whole plants were taken from the fourth leaf stage thrice weekly. The values shown in the figures are averages. The results for individual samples in the early growth stages of soybean showed that in the first sample, the seed treatment had practically no statistically significant effect on any of the monitored parameters. At the same time, there were differences in each sample, and treatment by LAB was the most effective. Plant length varied from 9.65 cm (control) to 15.53 cm (LAB), root length varied from 5.27 cm (control) to 13.93 cm (LAB) and weight of plants varied from 1.09 g (control) to 4.19 g (LAB). In the case of the second and third sampling, a specific positive effect of the treatment on the number of tubers was evident, as well as on the length of the plants in the case of MPF application to the seed. These results do not rely with Lamont *et al.* (2017) and Mukherjee *et al.* (2022). However, the differences were relatively erased in the average of the three samplings shown in the graphs.

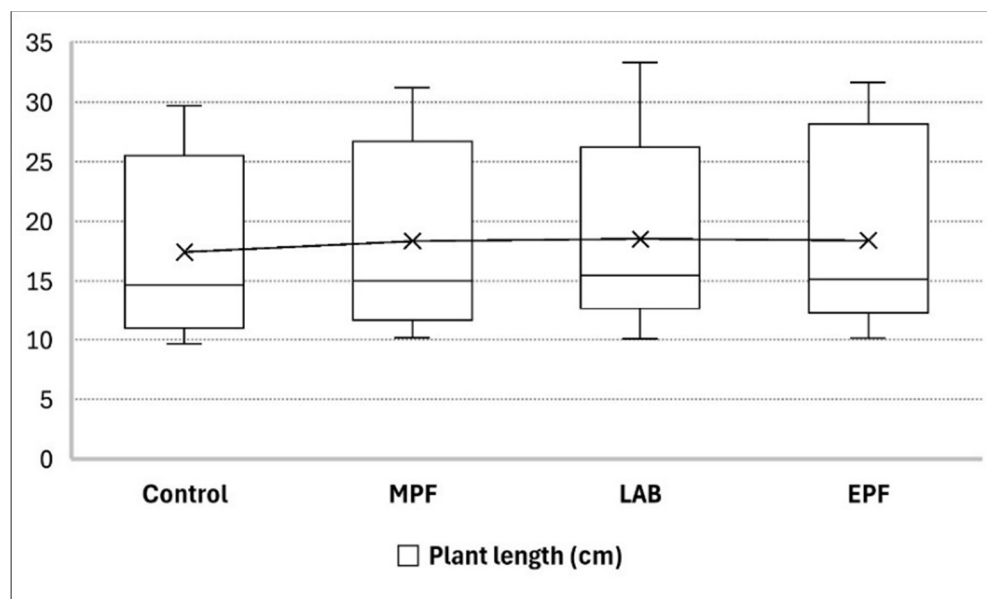


Fig. 1. Effects of seed treatment by mycoparasitic fungi (MPF), lactic acid bacteria (LAB), and entomopathogenic fungi (EPF) on plant length.

Fig. 1 illustrates the effect on plant length, where a relatively positive impact of the LAB application is evident, with more significant variability favoring longer plants. In the case of the length of the roots (Fig. 2), the differences are less pronounced- the most significant variability was evident in the control seed variant. From this, it can be inferred that the treatment with biological preparations had a slight stabilizing effect on the length of the roots, especially in the case of the MPF and EPF seed treatments. Regarding plant weight, the effect was less pronounced (Fig. 3) when the most significant variability and the highest plant weight were recorded during

the EPF seed treatment. The same was true for the impact on the weight of the roots (Fig. 4), where a slight positive effect was evident in the case of the biological treatment. The positive impact of seed treatment on early growth stages of plants and roots was also reported in a study by Nguyen *et al.* (2025) which used a similar biological treatment on peas, resulting in a notable improvement in shoot length and root dry weight.

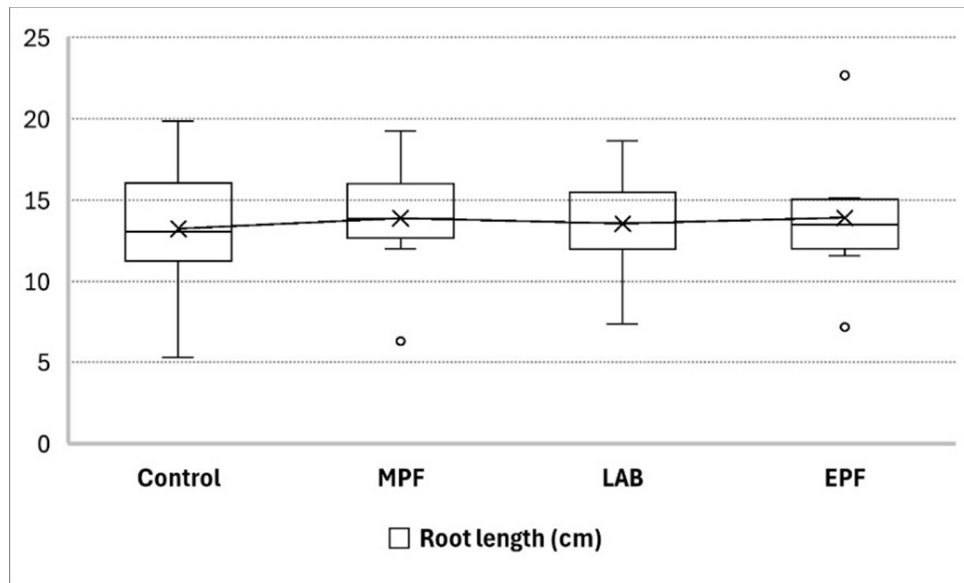


Fig. 2. Effects of seed treatment by mycoparasitic fungi (MPF), lactic acid bacteria (LAB), and entomopathogenic fungi (EPF) on root length.

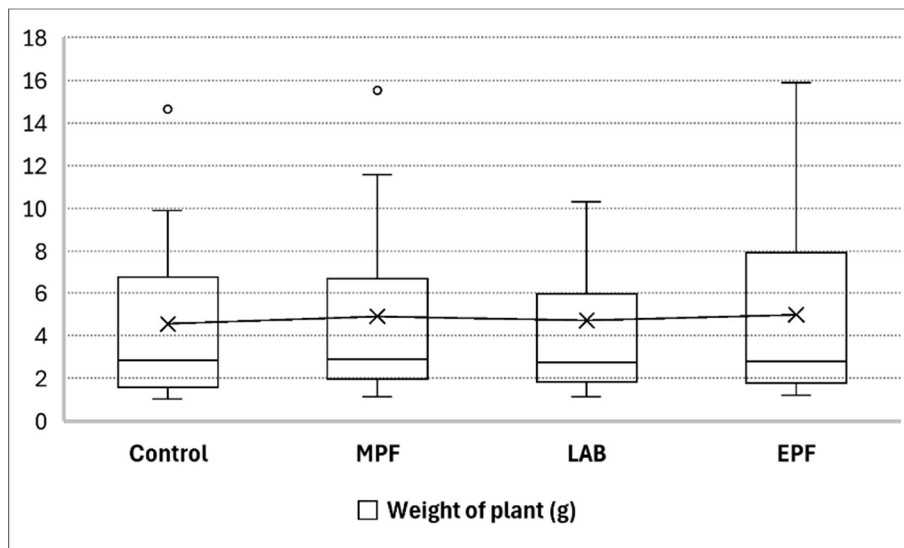


Fig. 3. Effects of seed treatment by mycoparasitic fungi (MPF), lactic acid bacteria (LAB), and entomopathogenic fungi (EPF) on the weight of the plant (dry matter).

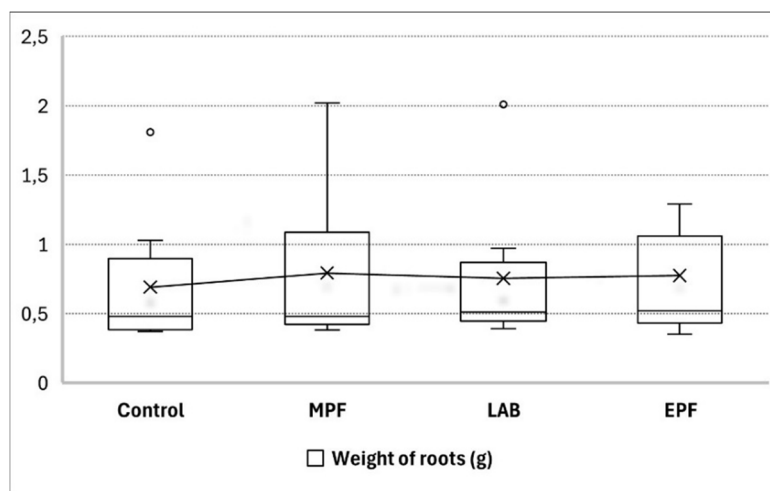


Fig. 4. Effects of seed treatment by mycoparasitic fungi (MPF), lactic acid bacteria (LAB) and entomopathogenic fungi (EPF) on the weight of roots per one plant (dry matter).

A weak infestation by diseases and pests was recorded at both monitored locations. The diseases mainly involved the sporadic occurrence of soybean downy mildew. Field observations showed that treated variants were less affected, but the results were statistically inconclusive.

Just as the occurrence of diseases and pests did not represent the biggest problem, the opposite situation was actual in the case of weed control, especially at the beginning of vegetation, because soybean has a reduced competitive ability against weeds (Andrade *et al.* 2019, Absy and Yacoub 2020, Kumar and Rana 2022). Weed regulation in our experiments was challenging because the critical crop-weed competition period in soybeans varies from 15 to 45 days after sowing, making timely intervention crucial (Kumar and Rana 2022). Based on our experience from experiments, it was appropriate to choose several measures. It is advisable to break up the soil crust before the soil preparation for sowing and support weed germination before pre-sowing preparation, during which young weeds are effectively destroyed. Furthermore, increasing the seeding rate by several percent has proven successful (Baird *et al.* 2009). Lastly, it is necessary to repeatedly harrow the crop and weed regularly during the later growth phases.

Table 1. Production parameters of soybean after different seed treatments.

Treatments	Number of plants after emergence (m ²)	Number of plants before harvest (m ²)	Number of pods per plant	Length of plants (cm)	Yield (t.ha ⁻¹)	Thousand grain weight (g)
Control	65.7 ± 20.3 _b	50.1 ± 28.3 _a	11.4 ± 3.1 _a	49.9 ± 12.3 _a	1.51 ± 0.37 _a	198.9 ± 5.4 _a
MPF	66.8 ± 21.0 _b	56.7 ± 27.8 _b	11.8 ± 3.3 _a	48.9 ± 11.6 _a	1.47 ± 0.17 _a	198.7 ± 6.8 _a
LAB	59.6 ± 16.9 _a	48.4 ± 19.6 _a	12.5 ± 0.8 _b	53.9 ± 14.2 _b	1.51 ± 0.27 _a	235.2 ± 5.5 _b
EPF	61.6 ± 20.4 _{ab}	53.1 ± 26.2 _{ab}	11.9 ± 1.9 _a	50.3 ± 13.7 _a	1.51 ± 0.45 _a	201.1 ± 8.1 _a

MPF: Mycoparasitic fungi, LAB: Lactic acid bacteria, and EPF: Entomopathogenic fungi.

Within the monitored production parameters, a positive effect of MPF application on the seed was evident in the number of plants after emergence and before harvest. Plant length was increased for LAB application. The number of pods per plant was, on average, from 11.4 to 12.5

and was also highest in the case of LAB application (Table 1). The differences between the individual variants' yields were insignificant and statistically inconclusive. The thousand grain weight showed minimal differences but was higher in the case of LAB application.

Qualitative parameters are shown in Fig. 5. The protein content in the seeds was slightly increased following MPF treatment. The starch content was unaffected and remained identical across all treatments. The oil content was similar in all variants, with a slight increase in the cases of EPF and LAB.

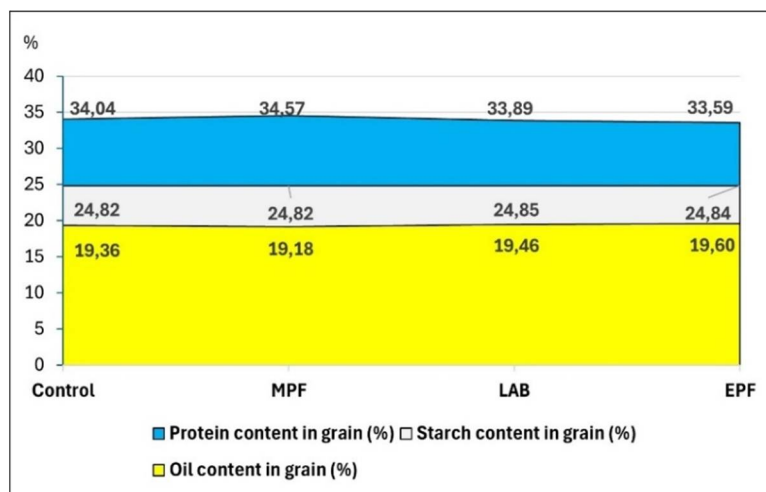


Fig. 5. Effect of treatment on quality parameters of mycoparasitic fungi, lactic acid bacteria and entomopathogenic fungi.

Applying biological preparations to soybean seeds for treatment positively affected early plant growth and several monitored production parameters in organic farming. Although statistically significant differences were recorded only to a limited extent, the application of mycoparasitic fungi and lactic acid bacteria stabilized root length and plant length, and the application of lactic acid bacteria showed a slightly higher number of pods per plant. Therefore, integrating biological seed treatment with agrotechnological measures, particularly optimized weed control through increased seeding density and repeated early-stage harrowing, appears promising for practical application in organic soybean production.

Acknowledgment

This work was funded by the Ministry of Agriculture of the Czech Republic, grant number QK22010255.

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(Manuscript received on 05 May 2025; revised on 11 October 2025)