

Article

Response of Soybean (*Glycine max* (L.) Merr.) to Vermicompost Fertilization and Foliar Application of *Methylobacterium symbioticum*

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Abstract

In the cultivation of leguminous plants, various fertilizers and microbiological preparations are used to increase nutrient availability or stimulate plant development. A pot experiment was conducted to examine the response of soybean to vermicompost fertilization and foliar application of *Methylobacterium symbioticum*. The experiment was conducted in a completely randomized design with four replicates. Vermicompost fertilization was found to increase the SPAD (Soil Plant Analysis Development) value and improve selected physiological parameters of the plants (Fv/Fm, Fv/F₀, PI, RC/ABS) compared to the control. The most optimal results were obtained for vermicompost from sewage sludge, regardless of *Methylobacterium symbioticum* application. Fertilization with this variant significantly increased seed weight per plant and seed protein content compared to the control. Therefore, vermicompost fertilization, particularly with sewage sludge, can be beneficial in soybean cultivation, as it reduces the need for chemical fertilizers. However, foliar application of *Methylobacterium symbioticum* generally did not modify the tested parameters.

Keywords: soybean; vermicompost; nitrogen-fixing bacteria; nodulation; physiological measurements; yield components; protein; fat



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1. Introduction

In sustainable agriculture, it is recommended to reduce the use of chemical fertilizers and increase the application of organic fertilizers [1–3] or to combine organic and mineral fertilization in appropriate proportions [4,5]. The aim is to minimize the negative impact of inorganic fertilizers on the environment while maintaining high and good-quality yields [6–8]. Farmers already use alternative fertilizers, such as composts and vermicomposts, which allow the recycling of various organic wastes [9]. Vermicomposts, processed by earthworms, generally have a higher nutrient content compared to composts. Therefore, vermicomposting is more beneficial than composting alone and can be recommended for the recovery of nutrients from organic waste, including agricultural waste [10,11].

Khare et al. [12] demonstrated that organic fertilizers have a positive effect on crop plants and recommended their use in agroforestry systems. Numerous studies [13–16] have shown that vermicompost application positively affects both yield and seed quality. An additional benefit of this fertilizer is the improvement of soil chemical properties [17,18]. Alkobaisy et al. [19] confirmed that the use of organic fertilizers alters soil chemical composition, including pH and EC, while Debela et al. [20] have concluded that integrated

nutrient management is becoming increasingly important in modern agriculture, closely linked to climate change and the need to adapt to its effects.

Soybean (*Glycine max* (L.) Merr.) is a widely cultivated crop worldwide and an important source of food, feed, and even biofuels. The consistently high demand for plant-based protein and fats results in large-scale soybean seed production in many regions of the world [21]. Additionally, advances in the genetics and agronomy of this species have enabled the production of high-yielding seeds of good quality [22]. In agricultural practice, soybean is highly valued for its symbiosis with *Bradyrhizobium japonicum*, which is of significant economic importance as it eliminates or greatly reduces the need for nitrogen fertilization. Moreover, cultivation of this species positively affects soil chemical composition and subsequent crops [23]. Soybeans and other legumes have moderate requirement for nutrients other than nitrogen and are primarily supplemented with mineral fertilizers [24,25]. However, information regarding soybean fertilization with vermicompost derived from sewage sludge or garden waste is limited or inconclusive.

Vermicompost production is an effective technology for recycling various organic materials, including agricultural waste. However, the chemical composition of vermicompost, and thus its fertilizing value, varies depending on the type of waste and the species of earthworms used [26–28]. Coulibaly et al. [29] demonstrated that vermicompost is a good alternative to inorganic fertilizers, and its agricultural application is both justified and cost-effective. According to Şahin et al. [30], vermicompost fertilization positively affected plant growth and development, while also improving soil structure and chemical composition. Pathma and Sakthivel [31] and Nonthapa et al. [32] confirmed that vermicompost promotes plant growth and development and additionally reduces the incidence of soil-borne diseases, which is particularly important in organic farming. Vermicompost was found to improve the soil microbiological status, including bacteria, and fungi, thereby increasing its fertility [33]. Vermicompost is considered an effective and environmentally friendly organic fertilizer, offering several advantages over commonly used chemical fertilizers [10,13]. For example, it can increase crop yields without causing excessive environmental pollution. Lim et al. [34] demonstrated that the nutrient content of vermicompost is generally higher than that of traditional compost. However, they emphasized that the use of vermicompost in excessively high doses could be detrimental, for example, due to high salt concentrations. In addition, heavy metals contained in sewage sludge pose a potential threat to human health if the sludge is not properly managed and safety rules are not adhered to in waste management [35].

In this context, Khutate et al. [36] and Paliwal et al. [37] recommend the use of vermicompost combined with a reduced dose of mineral fertilizer. This agronomic practice resulted in a high soybean yield and reduced cultivation costs. On the other hand, Azarpour et al. [38] and Rana et al. [39] obtained the highest soybean seed yield after applying vermicompost and inoculating the seeds with symbiotic bacteria. In this variant, they also recorded the highest plant height, number of pods per plant, number of seeds per pod, and 1000-seed weight. Vermicompost was further shown to increase the protein content of soybean seeds, an effect important for agri-food processing [40].

Biological nitrogen fixation (BNF) plays a crucial role in legume cultivation, as it provides a more effective means of supplying nitrogen to the soil than chemical fertilizers. Consequently, a range of biological products is now available to farmers [41–43], although their effectiveness in supplying nitrogen varies [44].

Methylobacterium are methylotrophic microorganisms that are physiologically well characterized, although their evolutionary history and taxonomy remain unresolved [45]. Microbial preparations using *Methylobacterium symbioticum* have the potential to stimulate plant growth, increase seed yield, and improve their quality parameters. Their effective-

ness, however, is not consistent and depends on the crop species, agronomic practices, and growing conditions. Application of these bacteria enabled a 50% reduction in nitrogen fertilization in maize and 25% in strawberry, while increasing photosynthetic capacity under all fertilization regimes. In maize, reduced nitrate reductase activity indicated a marked effect on plant metabolism. In strawberries, plants treated with *Methylobacterium symbioticum* and 25% less nitrogen fertilizer had higher leaf nitrogen concentrations than controls under optimal nutrient supply, suggesting that the bacteria provide an additional nitrogen source [46]. Cardone et al. [47] demonstrated that a commercial preparation containing *Methylobacterium symbioticum* improved both the yield and quality of *Thymus vulgaris* L. On the other hand, Arrobas et al. [48] reported a limited plant response to the application of *Methylobacterium symbioticum*. Similarly, studies by Rodrigues et al. [49,50] showed that *Methylobacterium symbioticum* was not effective in the tested plants. Nysanth et al. [51] and Arrobas et al. [52] stressed the need for broader research on commercial biological products before they can be confidently recommended to farmers.

One such innovative biological product recommended for foliar application is BlueN®. This preparation contains the bacteria *Methylobacterium symbioticum*, which fixes nitrogen from the air in a unique way. Unlike other nitrogen-fixing bacteria, they inhabit the above-ground parts of plants. Nitrogen assimilation by plants occurs through the leaves, using nitrogenase. The bacteria enhance plant growth and development throughout the entire vegetation cycle by supplying nitrogen from the air, regardless of the amount of nitrogen available in the soil [53].

In summary, soybean is one of the main sources of plant protein and oil, and its ability to fix atmospheric nitrogen symbiotically places this species among the most important crops worldwide. The dissemination of new soybean varieties and sustainable agronomic practices, including organic fertilization, can maintain high soybean production without negatively affecting the environment with chemical fertilizers [54].

The aim of the study was to determine whether fertilization with vermicompost, alone or combined with foliar application of *Methylobacterium symbioticum*, is an appropriate practice in soybean cultivation. The research hypothesis assumed that these treatments would result in a higher and better-quality seed yield compared to the control. Furthermore, it was assumed that neither vermicompost nor *Methylobacterium symbioticum* bacteria would interfere with nodulation or plant physiological processes.

2. Materials and Methods

2.1. Assumptions of the Pot Experiment

The pot experiment was conducted under controlled conditions in the Laboratory of the Institute of Agricultural Sciences, Environmental Protection, and Management. The single-factor experiment was established in 2024 using a completely randomized design with four replicates. The treatments were designated as follows:

- I—control,
- II—vermicompost (70% sewage sludge + 30% sawdust),
- III—vermicompost (70% garden and park waste + 30% sawdust),
- IV—vermicompost (35% sewage sludge + 35% garden and park waste + 30% sawdust),
- V—treatment II with foliar application of *Methylobacterium symbioticum*,
- VI—treatment III with foliar application of *Methylobacterium symbioticum*,
- VII—treatment IV with foliar application of *Methylobacterium symbioticum*.

The sewage sludge was obtained from the wastewater treatment plant in Leżajsk. Garden and park waste was collected from the urban green areas of the Leżajsk municipality. Composting followed by vermicomposting was carried out at the waste disposal site in Giedlarowa using *Eisenia fetida* earthworms and 1 m³ containers. Vermicomposting

lasted from September to December 2023. Mechanical mixing and measurements of the vermicompost's temperature and moisture were performed every seven days (Table 1).

Table 1. Measurements of the vermicompost temperature and moisture.

Weekly Measurement	Temperature (°C)			Moisture (%)		
	II	III	IV	II	III	IV
1	16.6	19.3	18.4	62.3	58.3	60.3
2	18.2	19.9	18.8	61.3	56.3	58.3
3	17.6	18.9	18.2	58.3	55.3	56.7
4	17.3	18.5	17.9	66.4	61.1	63.8
5	19.6	20.5	20.1	65.2	60.1	62.7
6	19.2	20.1	19.5	63.4	59.3	61.6
7	18.9	19.6	19.3	60.6	57.3	58.4
8	18.6	19.2	18.9	67.9	62.3	65.9
9	19.4	19.9	19.6	65.2	60.1	62.8
10	19.0	19.5	19.3	65.0	59.4	63.4
11	18.8	19.1	18.9	63.8	56.8	60.1
12	19.2	19.5	19.4	69.2	62.5	65.9
13	17.8	17.9	17.8	62.3	60.8	61.4
14	18.3	18.9	18.4	60.2	58.3	59.8
15	17.6	18.3	17.9	58.3	56.3	57.4
16	16.3	16.6	16.5	57.4	55.9	56.8

II–IV—fertilization variants.

The chemical composition of the vermicomposts was analyzed at PETROGEO (Przedsiębiorstwo Usług Laboratoryjnych i Geologicznych Sp. z o.o., Jasło, Poland) using standard analytical methods and in accordance with applicable Polish standards. The heavy metal content in the vermicomposted substrate was below the permissible limits for sewage sludge (Journal of Laws 2015, item 257; Directive 86/278/EEC on environmental protection, particularly regarding sewage sludge utilization) [55]. According to this document, the permissible content of heavy metals in sludge intended for agricultural purposes should not exceed (mg/kg): Cd—20, Cu—1000, Ni—300, Pb—750, Zn—2500, Hg—16, Cr—500.

2.2. Chemical Composition of Vermicomposts

Vermicompost from sewage sludge (II) had a lower pH and contained less organic matter, potassium, calcium, and magnesium compared with compost from garden and park waste (III). On the other hand, the content of nitrogen, phosphorus, and micronutrients was higher in the vermicompost from sewage sludge. In vermicompost IV, the levels of the analyzed components were generally intermediate between those in variants II and III (Table 2).

Table 2. Chemical composition of vermicomposts.

Parameter	Unit	II	III	IV
pH in 1 mol/L KCl	-	5.7	7.9	6.7
Dry matter	%	27.2	44.8	35.2
N	%	2.5	1.4	1.9
C:N	%	27.8	31.5	29.3

Table 2. Cont.

Parameter	Unit	II	III	IV
P ₂ O ₅	g·kg ^{−1} d.m.	9.3	3.5	6.3
K ₂ O		5.7	7.2	6.4
Ca		2.1	2.3	2.2
Mg		0.3	0.5	0.4
Fe	mg·kg ^{−1} d.m.	4634.1	2481.3	3551.2
Zn		324.4	85.7	203.1
Mn		391.9	85.1	236.7
Cu		72.2	46.7	58.3
Cd		0.48	0.38	0.42

II–IV—fertilization variants.

2.3. Pot Experiment Conditions

The plastic pots had a capacity of 15 L and a diameter of 30 cm. Each variant was replicated four times, and the positions of the pots were randomly rearranged every seven days. The plant developmental stages were recorded according to the BBCH scale (Bundesanstalt, Bundessortenamt und Chemische Industrie) [56]. The doses of vermicomposts were established so that an amount equivalent to 30 kg N ha^{−1} was applied to each pot, due to the low N_{min} content in the soil. In turn, a higher nitrogen dose could interfere with nodulation. The remaining macronutrients were not balanced, assuming they would increase soil fertility for subsequent crops.

Soil for the experiment was collected from an arable field belonging to the Institute of Agricultural Sciences, Environmental Protection, and Management at the University of Rzeszów in Krasne near Rzeszów. The soil was classified as Haplic Cambisol-Cmha [57]. The chemical analysis of the soil before and after the experiment was performed in the laboratory of the Faculty of Technology and Life Sciences at the University of Rzeszów in accordance with Polish standards. Before the start of the experiment, the soil was slightly acidic, with a medium organic matter content and low mineral nitrogen (N_{min}) levels. The content of macronutrients was high, while micronutrient levels were moderate.

The chemical composition of the soil was also analyzed after fertilization. In the control (variant I), certain soil components (N_{min}, P, K, Mg) decreased significantly, whereas micronutrient levels showed only a slight downward trend. Soil pH and organic matter content were higher following the application of vermicompost III compared with variant I. The application of vermicompost II, however, increased the levels of N_{min}, phosphorus, potassium, and magnesium relative to variant I (Table 3).

Table 3. Chemical analysis of soil before and after setting up the experiment.

Parameter	Unit	Before the Experiment	After the Experiment—Variants			
			I	II	III	IV
pH in 1 mol/L KCl	-	5.9 ab	5.7 b	5.8 ab	6.1 a	5.9 ab
Humus	%	1.6 b	1.5 b	1.7 ab	1.9 a	1.8 ab
N _{min}	kg·ha ^{−1}	52.8 a	45.5 b	53.6 a	48.3 ab	50.8 ab

Table 3. Cont.

Parameter	Unit	Before the Experiment	After the Experiment—Variants			
			I	II	III	IV
P ₂ O ₅	mg·100 g ^{−1} soil	18.8 a	15.5 b	19.2 a	18.3 a	18.6 a
K ₂ O		21.6 a	17.3 b	20.3 a	21.8 a	21.1 a
Mg		8.7 a	7.2 b	8.5 a	8.8 a	8.6 a
Fe	mg·kg ^{−1} soil	2439.3 a	2422.9 a	2541.9 a	2431.1 a	2486.5 a
Zn		17.8 a	17.6 a	17.9 a	17.6 a	17.8 a
Mn		376.4 a	375.2 a	377.2 a	375.2 a	376.1 a
Cu		5.4 a	5.3 a	5.5 a	5.3 a	5.4 a
B		1.8 a	1.5 a	1.7 a	1.6 a	1.6 a

I–VII—fertilization variants. Different lowercase letters in the columns indicate significant differences ($p < 0.05$).

Before sowing, soybean seeds were treated with the inoculant HiStick[®] Soy (BASF, Ludwigshafen am Rhein, Germany) according to the manufacturer's instructions. The variety Abelina (Saatbau Polska Sp. z o.o., Środa Śląska, Poland), recommended for cultivation in southeastern Poland, was selected for the experiment. Five seeds were sown in each pot on 7 April 2024. Subsequently, the pots were transferred to growth chambers (Model GC-300/1000; JEIO Tech Co., Ltd., Seoul, Republic of Korea) at 20 °C, 60% relative humidity, 16/8 h (L/D) photoperiod, and a maximum light intensity of approximately 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the day.

Three plants were left in each pot at the stage BBCH 13. The pots were then transferred to a growth chamber with natural lighting and a temperature of 20 °C. Soil moisture was maintained at 60% of field capacity.

Methylobacterium symbioticum (SB0023/3 T, 3×10^7 JTK/g) was applied at the 6-leaf stage (BBCH 16). The preparation was sprayed at the dose recommended by the manufacturer (Corteva Agriscience Poland Sp. z o.o., Warszawa, Poland), i.e., 333 g ha^{−1} in 250 L of water.

SPAD measurements were taken twice on fully developed leaves at the BBCH 21 and BBCH 23 stages for each treatment and each plant. The measurements were performed using a SPAD 502P chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan).

Chlorophyll fluorescence was measured using a Pocket PEA instrument (Hansatech Instruments, King's Lynn, Norfolk, UK). Six chlorophyll fluorescence measurements were taken per pot at the BBCH 23 stage. The following parameters were measured: maximum quantum yield of primary photochemistry (F_v/F_0), maximum quantum yield of photosystem PSII (F_v/F_m), total number of active reaction centers per absorption (RC/ABS) and photosynthetic performance index (PI). Fully developed leaves were dark-adapted for 20 min using leaf clips attached to the axial leaf blades.

The experiment was conducted until the full seed maturity stage. After the experiment, the roots were removed from the pots and rinsed on sieves to count the nodules. Plant measurements included height, number of pods per plant, number of seeds per pod, 1000-seed weight (TSW), and total seed weight per plant (at 14% moisture). Seed moisture was determined using the gravimetric oven-drying method, and total protein content was measured by the Kjeldahl method at the Department of Crop Production, University of Rzeszów.

2.4. Statistical Analyses

A one-way ANOVA was performed, followed by Tukey's post hoc test ($p \leq 0.05$) to assess the significance of differences between the mean values of the measured parameters.

The calculation of Pearson correlation coefficients was done using TIBCO Statistica 13.3.0. (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Physiological Measurements of Plants

All treatments with vermicompost fertilization significantly increased SPAD values compared to the control (Figure 1). It should be noted, however, that fertilization with vermicompost from sewage sludge (II and V) resulted in more favorable effects than fertilization with vermicompost from garden and park waste (III and VI). The application of *Methylobacterium symbioticum* had no significant effect on the SPAD index. Slightly higher SPAD values were recorded in the second measurement (BBCH 23) compared with the first measurement (BBCH 21).

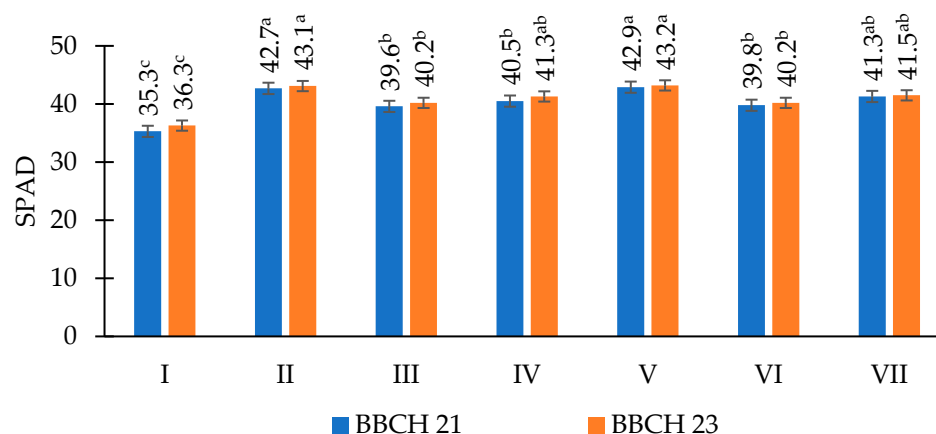


Figure 1. Soil plant analysis development (SPAD). I–VII—fertilization variants. Different lowercase letters in the columns indicate significant differences ($p < 0.05$). The standard error is marked on the columns.

Joshi et al. [13] and Meena et al. [58] demonstrated that vermicompost fertilization increased plant and root biomass, leaf chlorophyll content, and consequently the yield and quality of crop seeds. However, they emphasized that further studies were needed in this area, including the cost-effectiveness of vermicompost fertilization. Similar conclusions were drawn by Mohammadalizade et al. [1], who found that vermicompost increased leaf chlorophyll content and seed yields, while also improving the biological, chemical, and physical properties of the soil. Biswas et al. [59] showed that combined chemical and organic fertilization was an effective practice, as it allowed plants to take up and utilize nutrients more efficiently.

Fertilization with vermicomposts significantly increased the maximal PSII photochemical efficiency, particularly with sewage sludge vermicompost combined with *Methylobacterium symbioticum* application. The maximum quantum yield of primary photochemistry was higher in all organic fertilization treatments compared with the control.

The Performance Index was significantly higher after the application of vermicomposts, particularly in variants V and VII. A comparable trend was observed for the total number of active absorption reaction centers relative to the control. These results indicate that organic fertilizers, not *Methylobacterium symbioticum*, had a beneficial effect on the physiological processes of soybean plants (Table 4).

Table 4. Plant physiological measurements.

Fertilization Variant	Maximal Photochemical Efficiency of PSII (Fv/Fm)	Maximum Quantum Yield of Primary Photochemistry (Fv/F0)	Performance Index (PI)	Total Number of Active Reaction Center for Absorption (RC/ABS)
I	0.77 ± 0.01 c	4.03 ± 0.06 b	8.51 ± 0.08 c	1.98 ± 0.06 b
II	0.85 ± 0.01 ab	4.56 ± 0.12 a	11.3 ± 1.69 ab	2.32 ± 0.06 ab
III	0.81 ± 0.02 b	4.44 ± 0.09 a	10.2 ± 0.83 b	2.15 ± 0.05 ab
IV	0.83 ± 0.02 b	4.51 ± 0.21 a	10.8 ± 1.61 b	2.27 ± 0.07 ab
V	0.88 ± 0.01 a	4.62 ± 0.14 a	12.6 ± 0.83 a	2.38 ± 0.06 a
VI	0.83 ± 0.03 b	4.49 ± 0.17 a	11.8 ± 1.09 ab	2.19 ± 0.11 ab
VII	0.85 ± 0.03 ab	4.53 ± 0.09 a	12.1 ± 0.56 a	2.34 ± 0.07 a

Different lowercase letters indicate significant differences ($p < 0.05$); $\pm$ standard deviation (SD).

Sobiech et al. [60] demonstrated that appropriately selected biostimulants had a positive effect on plant growth, physiological parameters, and maize grain yield. However, the effects obtained varied between the years of the study, depending on the conditions during the growing season. According to Tsoumanis et al. [61], *M. symbioticum* improved nitrogen use efficiency by promoting early photosynthetic activity, increasing biomass accumulation, and enhancing nutrient redistribution during key stages of fruit development. As a result, it was possible to reduce nitrogen fertilizer doses at specific stages of peach cultivation. In another study, the application of *M. symbioticum* delayed leaf senescence, improved photosynthetic activity, stomatal conductance, and PSII efficiency under reduced nitrogen doses [62]. Meanwhile, Moghadam et al. [2] reported that plants at the flowering stage achieved the highest relative water content and leaf chlorophyll levels after the application of 10 tons of vermicompost. The use of vermicompost in arid areas was reported to improve plant physiological parameters and enhance resistance to environmental stresses [63].

3.2. Biometric Measurements of Plants and Yield Components

Vermicomposts increased plant height and the number of pods per plant compared to the control. Plants fertilized with sewage sludge vermicompost were taller than those fertilized with garden and park waste vermicompost. The use of sewage sludge vermicompost combined with *Methylobacterium symbioticum* increased the number of pods per plant compared with fertilization with garden and park waste vermicompost. The number of seeds per pod was higher following the application of sewage sludge vermicompost compared to the control. TSW increased under all tested fertilization conditions compared to the control treatment. It should be noted that the application of *Methylobacterium symbioticum* had a minor effect on the measured parameters (Figure 2).

Research conducted by Aritonang and Sidaurok [64] and Alkobaisy et al. [19] indicates that fertilization with vermicompost significantly increased the number of pods and seed mass. As a result, seed yield increased significantly compared to control treatments. Arslanoğlu [65] demonstrated that the application of vermicompost and poultry manure stimulated root development and plant biomass and also increased soil organic matter content. Thus, the latter authors suggested that vermicompost could be successfully used without chemical fertilizers in soybean cultivation. However, other studies [66–68] have demonstrated that vermicompost is beneficial, but its effects are increased when supplemented with mineral fertilization.

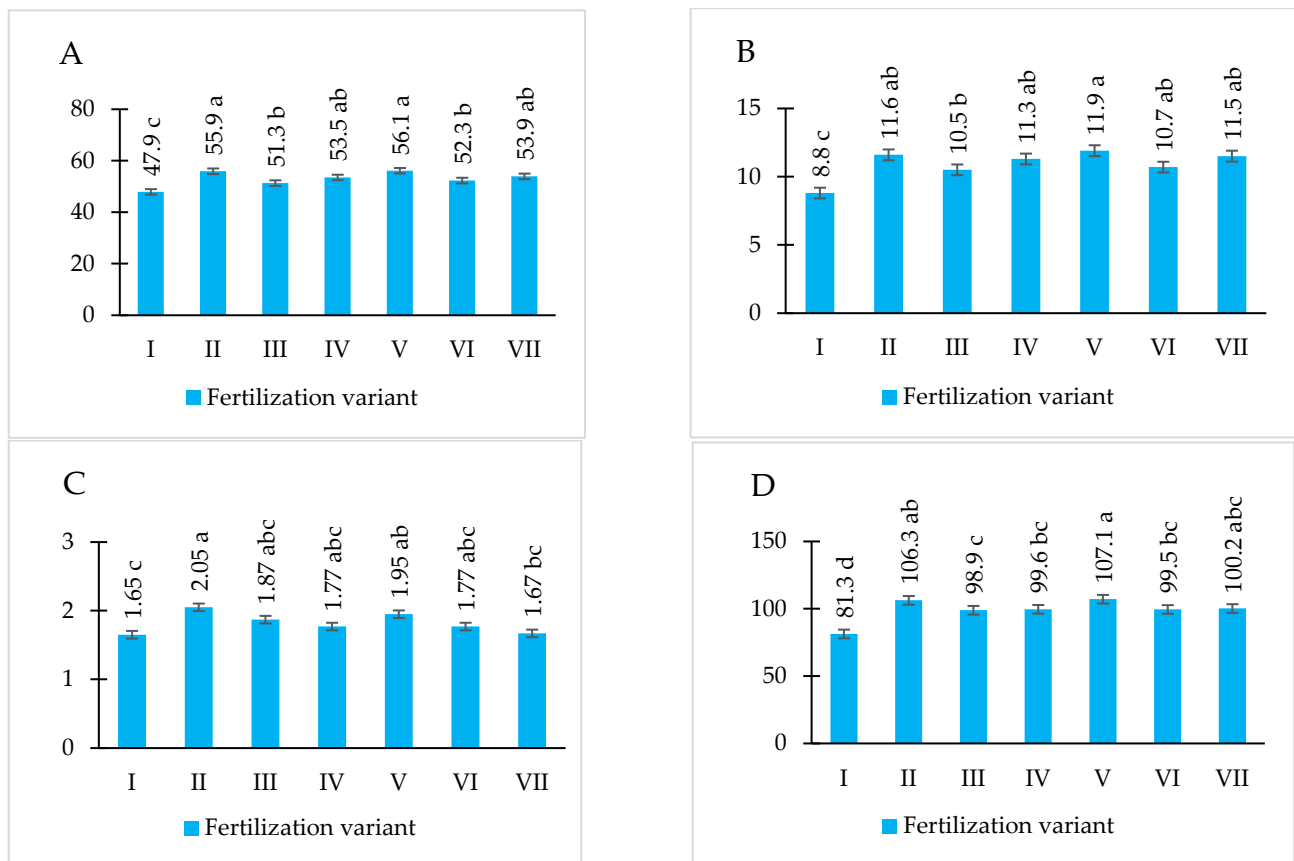


Figure 2. Biometric measurements and yield components. (A)—Plant height (cm), (B)—Number of pods per plant, (C)—Number of seeds per pod, (D)—Thousand seed weight (g). Different lowercase letters indicate significant differences ($p < 0.05$).

3.3. Nodulation, Seed Mass and Quality

Vermicomposts did not significantly alter the number of root nodules compared with the control. It was demonstrated, however, that the seed weight per plant was higher after organic fertilization, especially in variants II and V. The difference obtained, relative to the control, was 1.36 g and 1.31 g, respectively. Seed protein content was higher following the application of sewage sludge vermicompost than in the control.

The application of *Methylobacterium symbioticum* had a smaller effect on both seed weight per plant and seed protein content (Table 5).

Table 5. The influence of compost on quantitative and qualitative characteristics.

Fertilization Variant	Number of Nodules on the Root (pcs.)	Seed Weight Per Plant (g)	Protein Content in Seeds (% d.m.)
I	16.8 ± 0.53 a	1.17 ± 0.13 c	33.6 ± 1.17 c
II	14.9 ± 0.84 a	2.53 ± 0.18 a	37.4 ± 1.02 ab
III	16.2 ± 0.83 a	1.93 ± 0.13 b	34.8 ± 1.17 c
IV	15.9 ± 1.31 a	1.99 ± 0.11 b	35.3 ± 0.79 bc
V	14.4 ± 0.80 a	2.48 ± 0.15 a	37.8 ± 0.65 a
VI	15.6 ± 0.45 a	1.88 ± 0.14 b	35.2 ± 1.43 bc
VII	14.8 ± 1.12 a	1.92 ± 0.11 b	35.7 ± 0.71 abc

Different lowercase letters indicate significant differences ($p < 0.05$); ± standard deviation (SD).

Martolia et al. [69] demonstrated that vermicompost fertilization positively affected the number of root nodules, yield components, overall yield, and soybean seed quality

(protein and fat). However, some cultivars responded better to vermicompost than others. Mathenge et al. [70] reported that nodule formation and biological nitrogen fixation in low-fertility soils were not inhibited by vermicompost fertilization.

3.4. Correlation Between the Tested Parameters

A strong positive correlation was observed between the second SPAD measurement and plant height ($r = 0.92$), TSW ($r = 0.85$), and seed yield ($r = 0.91$). A strong correlation was also observed between SPAD values and seed protein content. These results allow us to conclude that SPAD measurement allows for the assessment of plant condition and optimization of fertilization, mainly nitrogen.

Moreover, the number of nodules was positively correlated with the number of pods ($r = 0.86$) and TSW ($r = 0.81$). Among the yield components, the number of pods per plant was most strongly correlated with yield, followed by TSW and the number of seeds per pod. Physiological measurements were positively correlated with the studied traits, and the weakest correlation was observed for the number of seeds per pod (Figure 3).

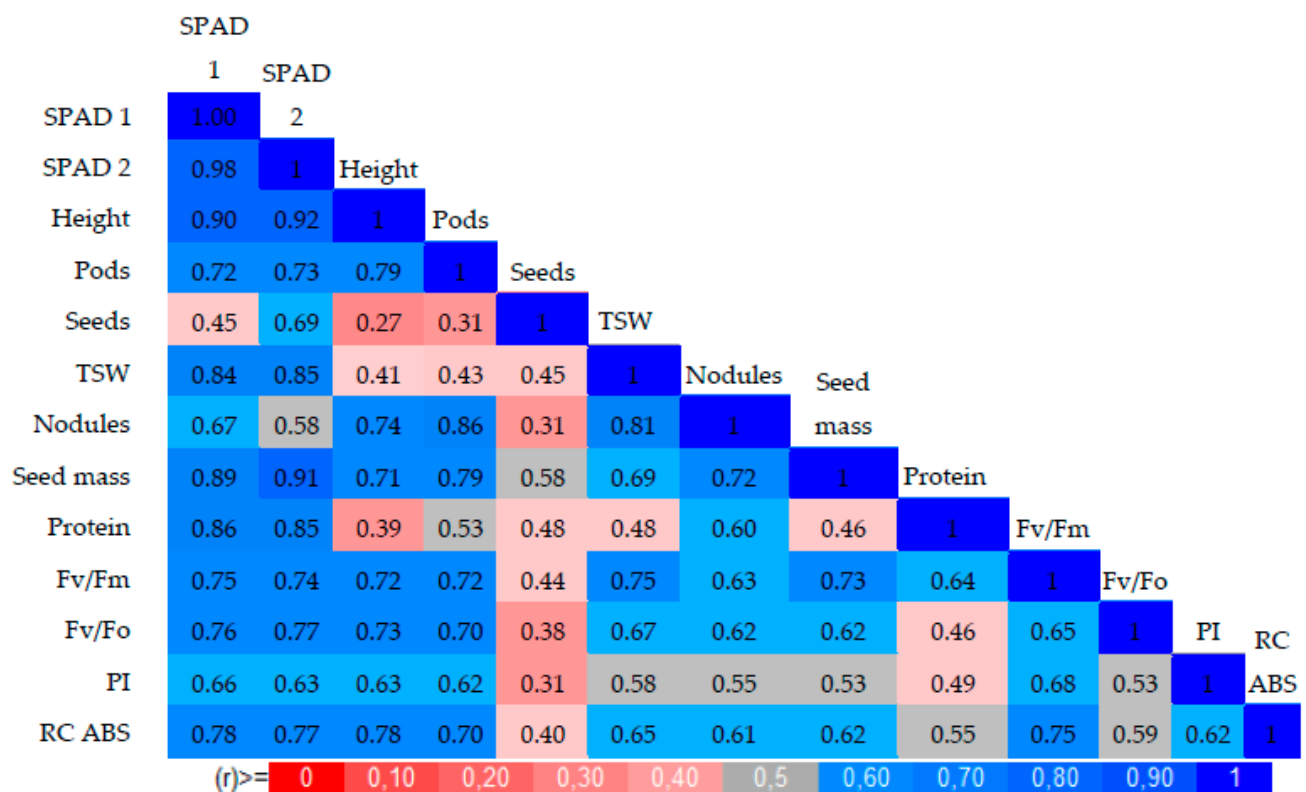


Figure 3. Colored map for correlation coefficients (r) between the tested parameters. The correlation coefficients are significant with $p < 0.05$.

Latifnia and Eisvand [71] showed that fertilizers reduced F_0/F_m measurements but increased F_v/F_m . They also observed a significant negative correlation between leaf N, P, Fe, and Mo content and F_m . Based on these results, they suggested that chlorophyll fluorescence measurements are an important non-destructive physiological indicator for monitoring plant nutritional status.

This area (Figure 4) showed the strongest positive correlations between plant height and pod number. However, plant height was weakly correlated with seed number per pod. This is important because both pod number and seed number directly affect soybean yield.

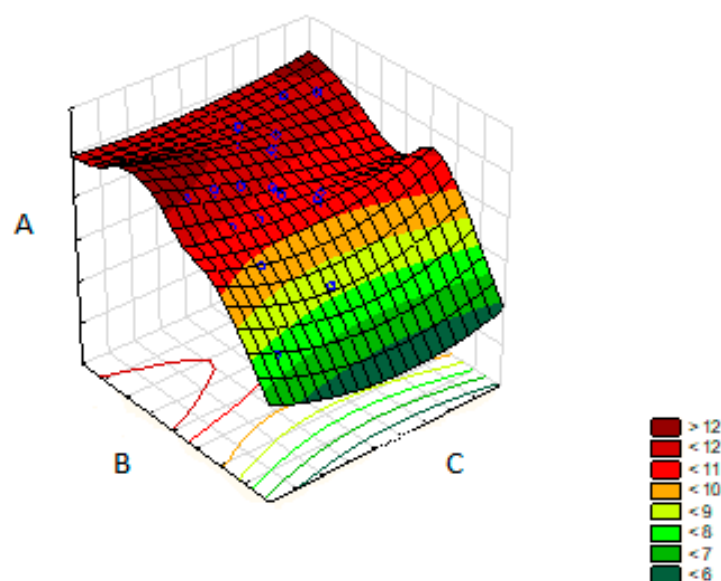


Figure 4. Correlation coefficients between the selected parameters of the analyzed traits: A—number of pods per plant, B—plant height (cm), C—number of seeds per pod. The correlation coefficients are considered significant at $p < 0.05$.

4. Conclusions

Fertilization of soybean with vermicompost from organic waste produced beneficial effects in the pot experiment, as confirmed by measurements of plant nutritional status (SPAD value). Sewage sludge vermicompost proved more effective than vermicompost from garden and park waste, which resulted from the former having a higher content of nitrogen, phosphorus and macroelements. Fertilization with vermicompost and the application of *Methylobacterium symbioticum* had no effect on the number of root nodules and thus did not inhibit nodulation. Measurements (F_v/F_m , F_v/F_0 , PI, RC/ABS) showed that the application of *Methylobacterium symbioticum* did not significantly modify the plant physiology and had a limited impact on final seed mass per plant. Seed protein content was primarily influenced by fertilization with sewage sludge vermicompost. However, this fertilization variant combined with foliar application of *Methylobacterium symbioticum* did not result in a synergistic interaction. Future studies should verify the obtained results under field conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

SPAD	Soil Plant Analysis Development
F_v/F_m	Maximal Photochemical Efficiency of PSII
F_v/F_0	Maximum Quantum Yield of Primary Photochemistry
PI	Performance Index
RC/ABS	Total Number of Active Reaction Center for Absorption
TSW	1000-seed weight

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