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The Use of Coherent Laser Stimulation of Seeds and a Fungal Inoculum to Increase the Productivity and Health of Soybean Plants

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Abstract: The laser stimulation of seeds is regarded as a modern method of seed enhancement. Our study evaluated the productivity and health of soybean plants resulting from the coherent irradiation of seeds and irradiation of an arbuscular mycorrhizal fungi (AMF) inoculum. The two-factor pot experiment took into account (1) the type of irradiated biological material (seeds, AMF inoculum, and seeds and inoculum) and (2) the means of irradiation (red laser—LR, blue laser—LB, red and blue laser—LR + LB, and control). Seed weight per plant, pod number per plant, root weight, the Fv/Fm fluorescence parameters, and the health status of the aboveground and underground parts of the plants were assessed. Stimulation with a laser light was shown to have a positive effect on the productivity and health of soybean plants. Significantly better effects can be obtained by stimulating the seeds alone. The stimulation of seeds treated with AMF inoculum slightly reduced the productivity of the plants. However, with regards to the conditions of plants, the treatment of seeds with AMF inoculum and laser irradiation was shown to reduce the incidence of Septoria brown spots.

Keywords: mycorrhizal inoculum; soybean; laser stimulation

1. Introduction

Soybeans are one of the most important crops worldwide. Soybean production amounted to 265 million metric tons (MMT) in 2010 and increased to 333.7 MMT in 2019. A very large increase in soybean production was also recorded in Central Europe—from 249 MMT in 2010 to 15,540 MMT in 2019 [1]. Nonetheless, there are a number of important environmental constraints that threaten soybean production by directly reducing the seed yield and seed quality. The most dangerous for soybean growth and development are pathogenic diseases. [2]. Each year, soybean plants are susceptible to various diseases (root rot, ascochytosis, anthracnose, septoriosis—brown spot, fusariosis, cercospora leaf blight, purple seed stain, and *Fusarium* browning of pods) throughout their growing season. Fungal diseases can decrease the soybean productivity to 50%, while bacterial diseases caused yield losses between 15% and 60%. The degree of economic damage due to plant diseases depends upon the type of pathogen, environmental conditions, plant tissue affected, severity of diseases, host plant resistance, and plant stress level. Reducing the impact of pathogens is a challenge in all agricultural production systems [3,4].

There are various methods of disease prevention, but new methods of plant protection are constantly being sought. Under the new EU Green Deal strategy, only environmentally friendly bioprotection methods will be developed and implemented into agronomic practice. Arbuscular Mycorhizal Fungi (AMF) plays a key role in plant protection against environmental stresses and can be used as potential tool in disease management. The establishment of AMF in the plant root has been shown to reduce the damage caused by soil-borne plant pathogens, such as the species of *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinium*, *Verticillium*, and *Thielaviopsis*, with the enhancement of resistance in mycorrhizal plants [5,6]. A positive aspect of AMF was noted in soybean protection. It was proven that the inoculation of soybeans with AMF enhances the abiotic stress tolerance, disease resistance, overall growth, and soil carbon sequestration [7,8]. An AMF inoculation increases the nutrient uptake, such as phosphorus and nitrogen, in inoculated plants [9]. In addition, the mycorrhizal inoculation increases the morphological parameters of legumes, e.g., plant height, stem girth, number of leaves, and leaf area, which significantly contribute to the yield [10].

The use of laser irradiation to control plant health is a modern trend combining technological intensification with ecological requirements. Laser stimulation does not cause harmful changes in the environment, which is of great importance in organic and integrated agriculture [11–13]. Following experimental confirmation of the positive influence of laser light on the condition of soybean seeds [12], an attempt was made to use it to enhance the resistance of plants to fungal pathogens. *Fusarium oxysporum* caused *Fusarium* root rot, and *Septoria glycines* caused *Septoria* brown spot. It has not yet been verified experimentally whether the positive effect of laser stimulation is primarily associated with the irradiation of seeds or whether it can be enhanced by additionally stimulating the fungal inocula applied to seeds.

The aim of the study was to verify the research hypothesis that the type of laser stimulation and type of irradiated biological material affect the morphological parameters of soybean plants and their health status. The study included an analysis of the selected biometric traits, assessment of the photochemical activity of the photosynthetic apparatus (Fv/Fm parameter), and assessment of the health condition of plants.

2. Materials and Methods

2.1. Biological Materials

Certified, uniform soybean seeds of the Augusta cultivar, from the company KWS, were used for testing. The seeds were treated with AMF inoculum. The commercial preparation Mycoflorin (Company Mycoflor, Końskowola, Poland) was used for seed inoculation. The preparation contains spores and dormant mycelia of fungi: *Glomus aggregatum*, *G. intraradices*, *G. etunicatum*, *G. mosseae*, *G. caledomium*, and *Gigaspora margarita.* The spore density of AMF was 50 per 1 g of preparation.

2.2. Experimental Design

The irradiation was carried out at the Department of Environmental Biotechnology and Ecology, AGH University of Science and Technology in Krakow. The soybean seeds used were of high quality, with no discoloration, mycelium deposits, or insect or mechanical damage. The surfaces of the soybean seeds were disinfected with 0.1% sodium hypochlorite for 1 min and then rinsed three times in distilled water. A two-factor experiment was conducted twice in greenhouse conditions in the randomized block design in the four replications. The first factor in the experiment was the type of laser irradiation, and the second factor was the irradiated material. The first factor included 4 variants: (1) red laser light (LR) applied three times, 9 s each, with 9-s intervals between laser exposure; (2) blue laser (LB) irradiation applied 3 × 3 s at 3-s intervals; (3) LB applied 3 × 1 s, followed by LR 3 × 3 s, at 1- and 3-s intervals, respectively; and (4) the control, with no irradiation. The second factor included three variants of the irradiated materials: (1) seeds and arbuscular mycorrhizal fungi (AMF) inoculum, (2) the AMF inoculum alone, and (3) the seeds alone. The seeds and AMF inoculum were exposed to two types of lasers: helium-neon (He-Ne, referred to here as LR) comprising of red light with a wavelength of 632.8 nm and density of irradiation of 2 W m^{-2} and an argon laser (Ar, here referred to as LB) with blue light, a wavelength of 514 nm and a density of irradiation of 5 W m^{-2}. The time duration was controlled by a device of our own design. The device is autonomous and includes drivers that can be programmed for a specific running time and programmable drivers activating the appropriate laser at the correct time.

A pot experiment was set up with a completely randomized design with four replications per treatment—for one independent repetition: four pots and five plants per pot. Each pot was filled with 8 kg of sterilized medium-grain loamy sand taken from the humus layer. The soil contained available forms of phosphorus and potassium in the amounts of 3.39-mg P_2O_5 kg DW and 33.81-mg K_2O kg DW. Throughout the growing period, the moisture level of the substrate (slightly loamy sand) in the pots was monitored daily and kept at a level of 70–75% of available water capacity (AWC). The plants were fertilized with nitrogen (1.6-g N per pot in the form of NH_4NO_3), phosphorus (0.64-kg P per pot in the form of KH_2PO_4), potassium (1.92-g K per pot in the form of K_2SO_4), and magnesium (0.5-g Mg per pot in the form of $MgSO_4$). The fertilizers were mixed with the soil as the pots were being filled. The fertilizers were applied again, in the same amounts, as the top dressing at the start of flowering. The soybean seeds were planted in the first 10 days of May. The pots were placed in a vegetation hall. The analysis of the disease symptoms was performed in the 5th week after infection. The experiment was conducted twice in order to receive the outcomes.

At the end of the experiment, the roots from each treatment with AMF were taken to examine its mycorrhizal colonization. It was evaluated microscopically after clearing the roots in 10% KOH and staining with 0.05% tryptan blue in lactophenol [14].

2.3. Plant Measurements

The fluorescence of chlorophyll a was measured in randomly selected leaves set in the middle of the main stem (third leaf from the top) using a portable Handy PEA fluorimeter at the end of the pod and seed development phase in BBCH 79. Within each treatment, two leaves from each replicate were taken for measurements. The maximum quantum efficiency of photosystem II, expressed as the Fv/Fm ratio, is presented in the results. The chlorophyll fluorescence was measured in order to diagnose the abiotic and biotic stresses during the growth and development of the plants [15].

Biometric measurements were made after the plants were harvested. The analysis included selected characteristics that directly defined the productivity of plants, i.e., seed dry weight per plant, pod number per plant, and the dry weight of the root system.

2.4. Assessment of the Incidence of Fusarium and Septoria Diseases in Soybean Plants

The plants were infected naturally with pathogens whose spores were in the environment (air inside the seeds). The plants were infected with *Fusarium oxysporum* causing *Fusarium* root rot and *Septoria glicynes* causing *Septoria* brown spot. Foliar and root diseases were identified in the vegetation hall based on visual symptoms, as described in the literature, and by microscopic observations of causal pathogens. Symptoms of Septoria brown spot on the leaves were assessed as small, dark brown spots that can merge to form larger, irregularly shaped blotches. The lesions caused by *F. oxysporum* appeared on the roots and lower stems as dark brown necrotic spots [16]. The health status of the soybean plants was evaluated at the end of the pod and seed development phase in BBCH stage 79. Fungal infection was determined in each plant. The plants were visually evaluated for disease severity and rated on a scale of 0 to 4: 0 = no symptoms or lesions, 1 = some minor lesions present on 1–10% of the leaf/root, 2 = lesions present on 11–25% of the leaf/root, 3 = 26–50% of leaf/root, and 4 = 51% or more of the leaf/root covered with lesions and withered and dead leaf/root. The disease index (DI) was calculated for the diseases as a percentage, where DI (%) = [sum (class frequency × score of rating class)]/[(total number of plants) × (maximum scale value)] × 100.

Visual observations of the disease infection were made, and infected leaf/root tissue samples were taken to the laboratory, and the pathogens were identified under a microscope (Nikon Eclipse E-200 MV, Tokyo, Japan) optical microscope with 200× magnification and computer image analysis) using aqueous mounting media [17]. The species of fungi were identified on the basis of the mycological keys and monographs [17,18]. The identification of each isolate was conducted twice.

2.5. Statistical Analysis

The results from the obtained data were analyzed statistically using Statistica version 13.3 TIBCO software Inc., Kraków, Poland. To investigate the main effects of the experimental factors, an ANOVA was conducted. The significance of the treatment differences was evaluated on the basis of a multiple Tukey's test at $p \leq 0.05$.

A factor analysis (FA) was used to determine the relationship between the analyzed parameters. A FA is a principal component method deployed to analyze a dataset containing both quantitative and qualitative variables. A FA makes to analyze the similarities between individuals by taking into account mixed-variable types. Statistica version 13.3 TIBCO software was used for the FA analysis.

3. Results

The statistical analysis showed that the means of irradiation and the type of irradiated biological material had varied effects on the morphological parameters (Table 1) and the Fv/Fm fluorescence parameter (Table 2). The weight of the seeds depends on the means of irradiation of the material and on the irradiated material. The use of blue laser light with or without red laser light significantly increased the seed weight per plant relative to the control (Table 1). Irrespective of the type of stimulation, a significantly higher seed weight per plant was obtained following the combined stimulation of both the seeds and the mycorrhizal inoculum compared to the stimulation of seeds alone (Table 1). An in-depth statistical analysis confirmed significant interactions of the experimental factors for two parameters: seed dry weight and pod number per plant. A significantly lower seed weight per plant was noted following the stimulation of the AMF inoculum with LB or LR + LB laser light compared to the combined stimulation of both the seeds and inoculum (Figure 1).

Table 1. Effect of irradiation stimulation on the seed dry weight, number of pods per plant, and root dry weight.

Factor	Seed Dry Weight (g/Plant)	Pod Number per Plant (pcs)	Root Dry Weight (g/Plant)
	Irradiated Material (IM)		
Seeds	1.99	9.0 ^{a}	0.266 ^{a}
AMF	1.74	8.1 ^{b}	0.226 ^{b}
Seeds + AMF	1.95	8.6 ^{ab}	0.202 ^{b}
p-value	ns	0.03 *	0.01 *

Table 1. *Cont.*

Factor	Seed Dry Weight (g/Plant)	Pod Number per Plant (pcs)	Root Dry Weight (g/Plant)
	Irradiation Type (IT)		
Control	0.89 ^{b}	5.67 ^{c}	0.245
LR	1.92 ^{a}	9.08 ^{b}	0.243
LB	2.56 ^{a}	10.2 ^{a}	0.233
LR + LB	2.21 ^{a}	9.25 ^{ab}	0.203
p-value	0.001 **	0.001 **	ns
IM × IT	0.002 **	0.001 **	ns

Different letters indicate the significant differences at $p < 0.05$. * *p*-value significant at level < 0.05, ** *p*-value significant at level < 0.01.

Table 2. Effect of irradiation stimulation on the fluorescence parameter.

Factor	Fluorescence (Fm/Fo)	Fluorescence (Fv/Fo)	Fluorescence (Fv/Fm)
	Irradiated Material (IM)		
Seeds	4.69	3.69	0.767
AMF	4.80	3.80	0.770
Seeds + AMF	4.48	3.48	0.787
p-value	ns	ns	ns
	Irradiation Type (IT)		
control	4.77	3.77	0.705 ^{b}
LR	4.80	3.80	0.799 ^{a}
LB	4.72	3.72	0.810 ^{a}
LR + LB	4.34	3.34	0.784 ^{ab}
p-value	ns	ns	0.02 *
IM × IT	ns	ns	ns

Different letters indicate the significant differences at $p < 0.05$. * *p*-value significant at level < 0.05.

The number of pods per plant was significantly dependent on both the type of laser used and the type of irradiated biological material (Table 1). The best production effects were obtained by stimulating the seeds compared to stimulation of the AMF inoculum or of both the seeds and inoculum. A significantly higher seed number was obtained following the stimulation of soybean seeds with blue laser light than in the remaining treatments (Table 1 and Figure 2). Irradiation of the AMF inoculum with red laser light resulted in a significant increase in the number of pods per plant relative to the control. The use of combined irradiation with two lasers led to a significant increase in the number of seeds only in the treatments with seed stimulation. The combined stimulation of the seeds and AMF inoculum significantly reduced the productivity of the plants compared to the irradiation of seeds alone (Figure 2).

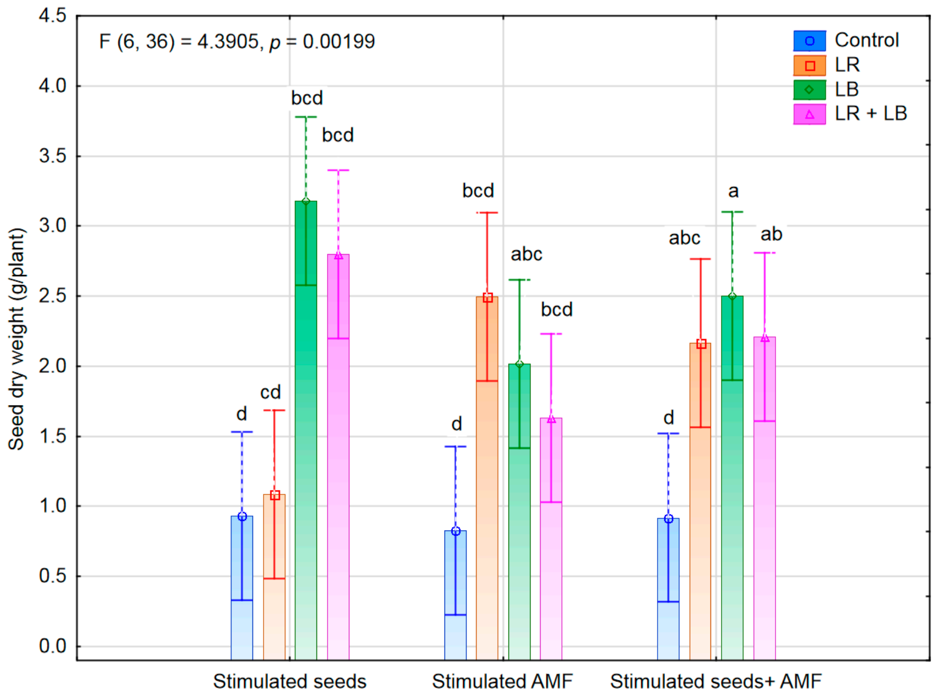

Figure 1. Effect of the interactions of the factors on the seed dry weight per plant. Different letters indicate the significant differences at $p < 0.05$.

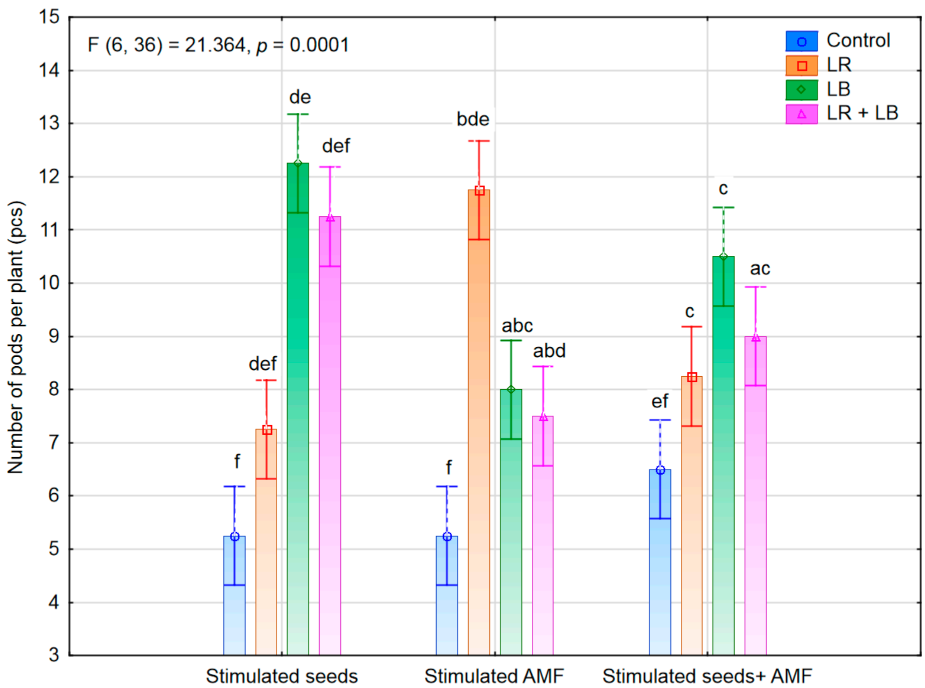

Figure 2. Effect of the interactions of the factors on the pod number per plant. Different letters indicate the significant differences at $p < 0.05$.

The root weight was significantly influenced only by the type of irradiated biological material but not by the type of laser used (Table 1). A significantly higher root weight was obtained following the irradiation of seeds in comparison to the remaining treatments. No significant effect of the interactions of the experimental factors was observed (Figure 3).

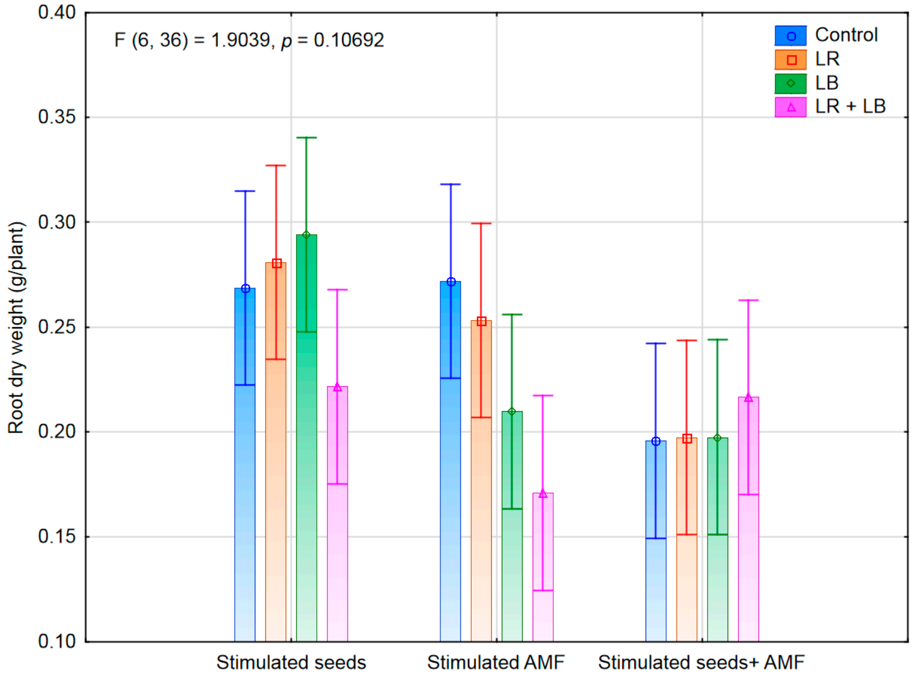

Figure 3. Effect of the interactions of the factors on the root weight.

The Fv/Fm parameter is considered a reliable measure of photochemical activity of the photosynthetic apparatus. In optimal conditions for plant growth, it should be about 0.85 relative units. We showed that the Fv/Fm ratio varied depending on the type of laser used (Table 2). In the treatments in which the blue laser was used, the values were closer to the optimum value than in the case of the control. Lower values were noted in the treatments with stimulation of the seeds compared to treatments with a combined stimulation of both the seeds and AMF inoculum (Table 2 and Figures 4 and 5).

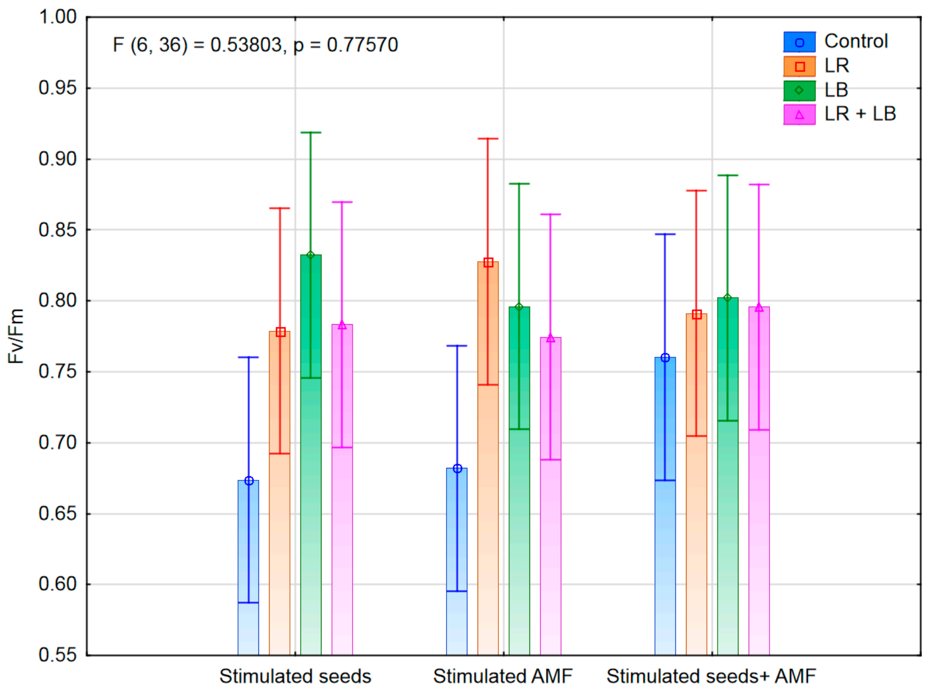

Figure 4. Effect of the interactions of the factors on the Fv/Fm parameters.

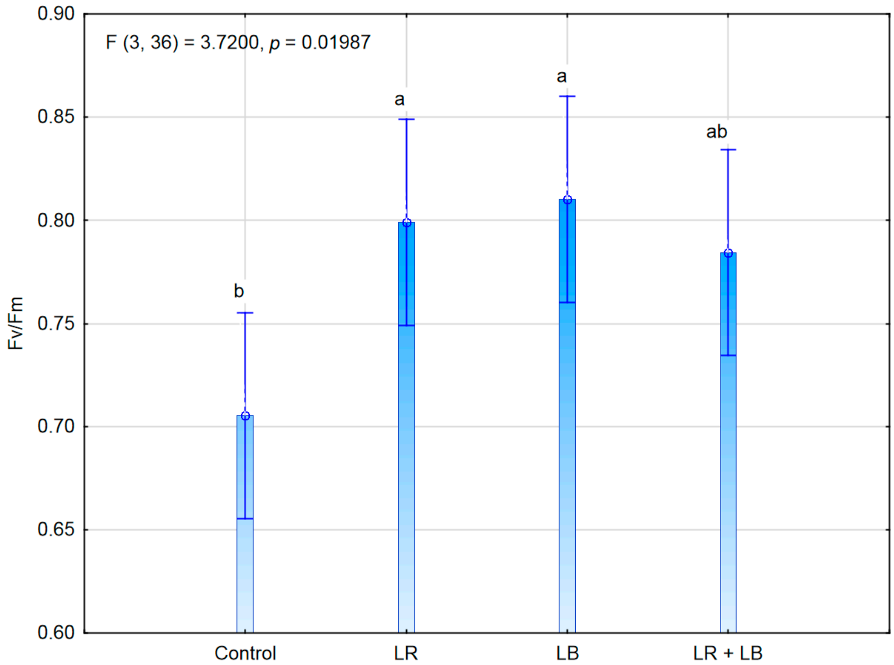

Figure 5. Effect of the irradiation type on the Fv/Fm parameters. Different letters indicate the significant differences at $p < 0.05$.

3.1. Fungal Diseases on the Plant

The diseases *Fusarium* root rot and *Septoria* brown spot were observed on the plants (Table 3 and Figures 6 and 7).

Table 3. Effect of irradiation stimulation on the diseases index.

Factor	Fusarium Root Rot (*Fusarium oxysporum*)	Septoria Brown Spot (*Septoria glycines*)
	Irradiated Material (IM)	
Seeds	10.5 [a]	20.9 [a]
AMF	22.5 [b]	14.7 [b]
Seeds + AMF	28.6 [c]	21.3 [a]
p-value	<0.001	<0.001
	Irradiation Type (IT)	
control	17.6 [a]	45.1 [a]
LR	15.0 [a]	12.1 [b]
LB	17.1 [a]	8.54 [b]
LR + LB	32.5 [b]	10.2 [b]
p-value	<0.001 **	<0.001 **
IM × IT	<0.001 **	<0.001 **

Different letters indicate the significant differences at $p < 0.05$. ** *p*-value significant at level < 0.01.

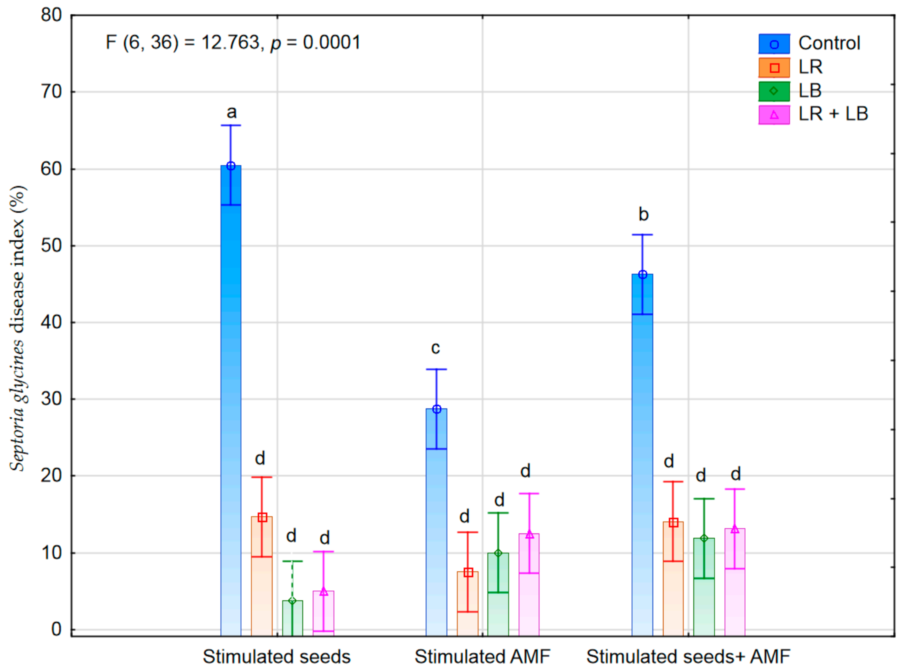

Figure 6. *Septoria glycines* disease index (%) on the aboveground parts of soybean plants grown from seeds treated with arbuscular mycorrhizal fungi (AMF) and LR—helium-neon laser (red light), LB—argon laser (blue light), and LB + LR—Ar laser, followed by He-Ne laser. Different letters indicate the significant differences at $p < 0.05$.

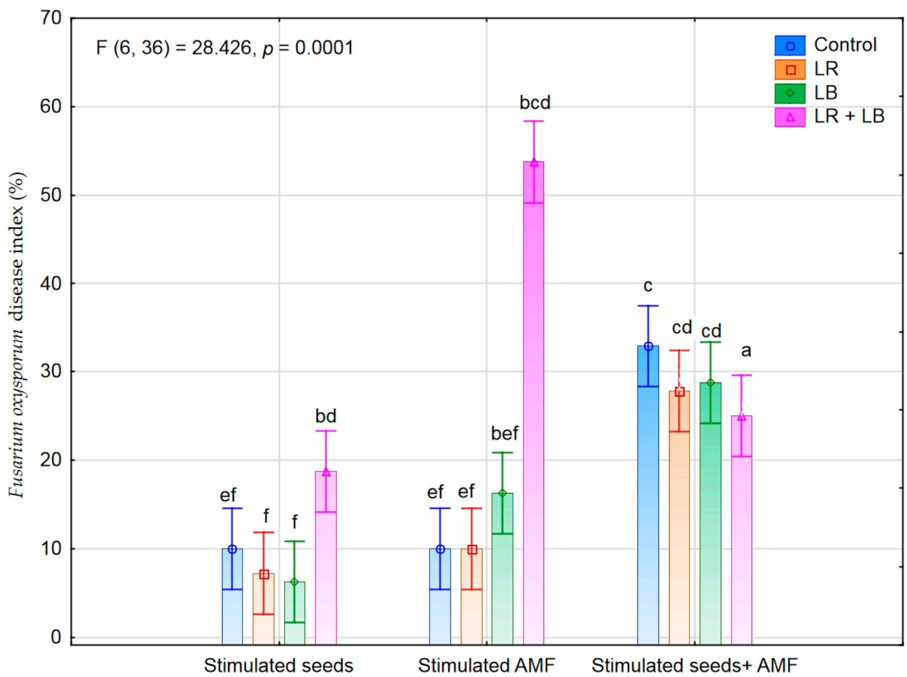

Figure 7. *Fusarium oxysporum* disease index (%) on the underground parts of soybean plants (roots) grown from seeds treated with arbuscular mycorrhizal fungi (AMF) and LR—helium-neon laser (red light), LB—argon laser (blue light), and LB + LR—Ar laser, followed by He-Ne laser. Different letters indicate the significant differences at $p < 0.05$.

Based on the statistical analysis, it was found that the type of laser and the type of irradiated material affect the disease index (Table 3). The effect of biostimulation is diversified in relation to pathogenic fungi. The least infestation with *F. oxysporum* was recorded in the combination in which only the seeds were irradiated. On the other hand, the lowest occurrence of brown spot on the leaves was in the combination in which only AMF was irradiated. The use of irradiation with each type of laser significantly improved the health of soybean leaves.

The degree of infection in the plants was varied. The average *Septoria glycines* disease index for the soybean leaves of the control plants was 45% (Figure 6). Each type of laser stimulation was found to significantly reduce the occurrence of Septoria brown spot. The best plant health was noted where the seeds alone were treated with LB and LR + LB layers (Figure 6).

The average *Fusarium oxysporum* disease index in the control plants was 17% (Figure 7). The plants in which LR and LB lasers were used to stimulate seeds alone were the least-infected by this pathogen. For the fungus *F. oxysporum*, a combined LR + LB irradiation of the seeds alone or inoculum alone increased the degree of infection of the plants. The combined stimulation of the seeds and AMF inoculum, irrespective of the type of laser light, caused a significant increase in infection by *F. oxysporum* (Figure 7).

3.2. FA Analyses

The FA generally indicated different effects of laser stimulation (of seeds, AMF, and seeds with AMF) on the biological parameters—variables (Table 4). The factor analysis was based on a two-factor solution. The obtained correlations between seven variables and two main factors, which were extracted by default, are presented in the table below.

Table 4. Component matrix of the variables.

Variable	Factor Analysis					
	Stimulated Seeds		Stimulated AMF		Stimulated Seeds + AMF	
	PC1	PC2	PC1	PC2	PC1	PC2
Irradiation type	−0.952	0.187	−0.974	−0.129	0.863	0.091
Fusarium	−0.346	0.825	−0.787	−0.436	−0.518	0.561
Septoria	0.881	0.161	0.634	−0.555	−0.940	−0.026
Root dry mass	0.183	−0.737	0.845	0.315	0.009	0.916
Number of pods	−0.945	−0.146	−0.262	0.848	0.870	−0.001
Seed weight per plant	−0.858	−0.051	−0.359	0.823	0.871	0.028
Fv/Fm	−0.601	−0.458	0.279	0.485	0.347	0.448
Cumulative variance %	54.7	21.7	42.3	32.1	50.5	19.5

The FA analysis was conducted in three groups of main experimental factor (irradiated material by laser).

In the present experiment, irradiated seeds by laser constituted the most important experimental factor, explaining more than 50% of the biological parameters named variables.

In the first analyzed group (stimulated seeds), PC1 explained 54.7% of the variables. PC1 included the following variables negatively correlated: irradiation type, seed dry weight, number of pods per plant, and variable *Septoria* positively correlated with it.

PC2 explained two variables at 21.7%. PC2 included variables negatively correlated with the root dry mass and the *Fusarium* variable positively correlated with it.

The presented analysis was validated by the outcomes, since the weight of the seeds and number of pods per plant depended on the means of irradiation of the materials. Each type of laser stimulation was found to significantly reduce the occurrence of *Septoria*, whereas, for the fungus *Fusarium*, the combined LR + LB irradiation of seeds alone increased the degree of infection of the plant roots.

In the second analyzed group (stimulated AMF), PC1 explained 42.3% of the variables. PC1 included the following variables negatively correlated with the irradiation type and Fusarium, and the mass of the roots was positively correlated with it. PC2 explained two variables at 32.1%. PC2 included the following positively correlated variables: the number of pods and the weight of seeds per plant.

The presented analysis confirmed our outcomes. The irradiation of the AMF inoculum with red or blue laser light resulted in a reduction of *Fusarium* infestation on the plant roots. The irradiation of the AMF inoculum with red laser light resulted in a significant increase in the number of pods per plant relative to the control.

In the third analyzed group (stimulated seeds and AMF), PC1 accounted for 50.5% of the variables. PC1 included the variable *Septoria* negatively correlated with it and the variables positively correlated with the irradiation type, seed dry weight, and number of pods per plant. PC2 explained two variables at 19.5%. PC2 mainly included the dry root mass positively correlated with it. The results had confirmation in our findings, since the combined irradiation of the seeds with AMF significantly affected the weight of the seeds and number of pods per plant. Irrespective of the type of stimulation, a significantly higher seed weight per plant was obtained following the combined stimulation of both the seeds and the AMF compared to the stimulation of the seeds alone.

4. Discussion

The research showed that the laser stimulation of seeds prior to sowing significantly influenced the biometric and physiological characteristics of the soybean plants. Both the type of stimulation and the type of irradiated material were significant factors. As regards the biological material (seeds or AMF inoculum), the study clearly showed that the best production effects are obtained by laser stimulation of the seeds alone. It is worth noting, however, that the use of the combined stimulation of seeds inoculated with AMF inoculum had only a slight negative effect on the biometric traits of the plants.

The research indicates that LR, LB and LR + LB laser irradiation decreased the disease index of *S. glycines* and *F. oxysporum*. However, irradiation with LR + LB increased the severity of *Fusarium* root rot. The pre-sowing irradiation treatment of seeds can be beneficial to plant health, because it modifies the biochemical and physiological processes in seeds, thereby accelerating seedling emergence and improving plant development [19]. The pre-sowing irradiation of seeds greatly protected soybean stands against *F. solani* [20]. The reduction in disease incidence was accompanied by an accumulation of high proline and phenol levels in the infected root tissues, suggesting that these compounds have a role in the prevention of disease development. Podleśna et al. [21] reported that the pre-sowing treatment of pea seeds with He-Ne laser light increased the concentrations of amylolytic enzymes and the content of indole-3-acetic acid (IAA) in the seeds and seedlings. The exposure of seeds to He-Ne laser light improved the germination rate and uniformity and modified growth stages, thus accelerating the flowering and ripening of the pea plants [21]. This is important, because plants grown from seeds with high viability have higher growth potential and are more resistant to environmental stressors and less susceptible to disease, reducing the need for chemical protection. Laser light irradiation can be useful for improving the rapeseed yield in field conditions, especially in regions where blackleg disease occurs. Depending on the laser power and exposure time, the treatment could be stimulating or destructive. The positive influence of laser light on the seed condition has been confirmed experimentally, and it has been used to enhance the winter rapeseed resistance to the most dangerous fungal pathogen—*Phoma lingam*. The best results for seedling health were obtained using irradiation at a wavelength of 632 nm with a helium-neon laser at 30–90-min exposure time [22].

In the present study, the commercial mycorrhizal inoculum had a protective effect against *Septoria glycine* and *Fusarium oxysporum*. However, the effect of AMF on *Fusarium* root rot was weaker than on Septoria brown spot. The two diseases attack the aerial or the belowground part of soybeans. The mode of action of AMFs for the protection of the plant against an aerial or root disease may be different.

It is considered that AMF reduces the damage caused by soil-borne pathogens. Many studies revealed a reduction of the incidence and/or severity of diseases as root rot or wilting caused by diverse fungi such as *Fusarium, Rhizoctonia, Macrophomina*, or *Verticillium* and oomycetes such as *Phytophthora, Pythium*, and *Aphanomyces* [23,24]. Reduced damage in mycorrhizal plants may be due to changes in the root growth and morphology, histopathological changes in the host root, physiological and biochemical changes within the plant, changes in the host nutrition, mycorrhizosphere effects that modify microbial populations, the competition for colonization sites and photosynthates, and the activation of defense mechanisms [25].

In interactions of aboveground pathogens and mycorrhizal plants, two main mechanisms may be operative. One is the potential changes in the nutrient levels of the host plant that may affect the suitability of the plant for leaf and shoot pathogens. The other is the modification of the plant defense mechanisms [23]. During mycorrhiza formation, modulation of the plant defense responses occurs, potentially through cross-talk between the salicylic acid and jasmonate-dependent signaling pathways [5]. The type of pathogen may also play a role in these interactions. Biotrophic pathogens, such as powdery mildew and rust (*Blumeria*, *Oidium,* and *Uromyces*) thrive better in mycorrhizal plants [26]. Barley with mycorrhizal AMF was more susceptible to the obligate biotrophic leaf pathogen *Erysiphe graminis* f. sp. *hordei*. On the leaves of mycorrhizal plants, the sporulation rate of the mildew fungus was more than twice that on the control plants. However, in mycorrhizal barley, leaf disease did not impair either the quantity or quality of the grain yield [26]. Concerning hemi-biotrophs, the impact of the symbiosis varies from no effect to a reduction of the disease on shoots and leaves. AMF are root-associated organisms and are effective against necrotrophic pathogens. Tomato plants colonized with the AMF *Rhizophagus irregularis* display enhanced resistance against the necrotrophic foliar pathogen *Botrytis cinerea*. Leaves from AMF plants develop smaller necrotic lesions. AMF plants react by accumulating higher levels of the vitamins folic acid and riboflavin, indolic derivatives, and phenolic compounds such as ferulic acid and chlorogenic acid. AMF have beneficial roles in crop protection by promoting an induced resistance not only under optimal nutritional conditions but also buffering the susceptibility triggered by transient N depletion [27]. Mycorrhizal tomato plants had significantly less necrosis and chlorosis of *Alternaria solani* on all leaves than non-mycorrhizal plants, but neither the plant growth nor phosphate uptake was enhanced by mycorrhizas [28].

The AMF *Glomus clarum* acts, to some extent, as a biological control agent against *Rhizoctonia solani*, the causal agent of root rot disease in cowpea plants. Cowpea root necrosis and the number of sclerotia in the rhizosphere produced by the pathogen were significantly reduced by the AM fungal inoculum. The research suggests that increasing the disease resistance in mycorrhizal plants may be due to the ability of mycorrhizal roots to absorb phosphorus and other nutrients, as well as due to the competition for infection sites and antagonistic reactions between the mycorrhizal fungus and the pathogen [29]. In a greenhouse experiment, at the flowering and maturity stages of soybean plant growth, the fresh and dry biomass, P and N content, and the number of nodules were significantly higher in plants inoculated with the fungus *Glomus mosseae* than in the uninoculated control [30]. Zambolim and Schenck [31] reported a reduction in the effects of pathogenic fungi on soybeans by the mycorrhizal fungus *Glomus mosseae*. The results of their research indicated that *G. mosseae* has a vital role in inhibiting the invasion of the common bean (*Phaseolus vulgaris*) by root pathogens (*Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium solani*). Inoculation with *G. mosseae*, in addition to decreasing the propagule number of *Fusarium solani* in the rhizosphere, decreased the pathogenic root rot by 34–77%. Furthermore, *G. mosseae*-inoculated plants were more tolerant of the fungal root pathogen. The assessment of the effect of the AMF *G. mosseae* on the infection of soybeans by *Macrophomina phaseolina*, *Rhizoctonia solani*, and *F. solani* determined that, although the incidence of infection by the pathogens was about the same in mycorrhizal and non-mycorrhizal plants, plants colonized by *G. mosseae* tolerated this infection better than non-mycorrhizal plants [31].

Jamiołkowska et al. [32] estimated the effect of the commercial product Mycoflorin containing mycorrhizal fungi on the photosynthetic activity, growth, and health status of tomato seedlings ('Pelikan F1') infected with the pathogenic fungus *Colletotrichum coccodes*. The experiment showed that the commercial mycorrhizal inoculum had varied effects on the health status of tomato seedlings depending on the pathogenic fungus isolate. The authors assumed that not only the pathogen but, also, the mycorrhiza is a stress factor for the seedlings and affects the plant's disease index. Mycoflorin has also been used in other studies to control root rot caused by various isolates of *Fusarium oxysporum* in sweet pepper seedlings. The mycorrhization of pepper seedlings contributed to the inhibition of the disease, as the disease index of mycorrhized seedlings infected with *F. oxysporum* was lower than for non-mycorrhized seedlings [33]. Similar results were obtained by Al-Hmoud and Al-Momany [34], who demonstrated that commercial products with *Glomus intraradices* reduced a *F. oxysporum* infection by increasing the plant height and reducing the root infection.

In soybean cultivation, the relationships between arbuscular mycorrhizal fungi (AMF) and pathogens can be modified by abiotic environmental factors, such as fertilization. Phosphorus application may reduce the soybean disease severity but can simultaneously partially reduce the protection by AMF *Rhizophagus intraradices* against the pathogen *Macrophomina phaseolina* (charcoal root rot) [35]. Nitrogen fertilization could increase the risk of *M. phaseolina* disease in soybean, but the mycorrhiza *R. intraradices* could help to control soybean charcoal root rot even if the crop is under N fertilization [36]. These effects should be considered in integrated agricultural management practices in soybean cultivation.

Given the increasing cost of inorganic fertilizers and the environmental and public health hazards associated with pesticides and pathogens resistant to chemical pesticides, AMF and laser irradiation may provide a more suitable and environmentally acceptable alternative for sustainable agriculture.

5. Conclusions

Laser stimulation significantly reduced Septoria brown spot. Every type of laser stimulation significantly reduced the incidence of this disease, with the best health observed in the treatments in which seeds alone were stimulated with LB and LR + LB lasers. On the other hand, laser stimulation reduced the incidence of *Fusarium* root rot to a limited degree. The best effect was obtained when the seeds alone were stimulated with LR and LB lasers. The average *Septoria glycines* disease index in the leaves of the control plants was 45%, while the disease index for *Fusarium oxysporum* was 17%. The stimulation of the seeds and AMF inoculum reduced the incidence of Septoria brown spot but only slightly reduced the occurrence of *Fusarium* root rot.

The use of the AMF inoculum (irradiated or not) as a factor improving the condition of plants had a negative effect on plant productivity. A slight decrease in seed weight per plant and pod number per plant was observed.

For plant health, a stimulation of seeds coated with AMF inoculum is recommended as a method of seed enhancement.

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