

Mitigation of *Solidago canadensis* invasion using natural substances and selected endophytes

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Abstract

Aims

The uncontrolled spread of invasive plant species is a major driver of biodiversity loss in plant communities. We evaluated a recently proposed biological method for invasive plant control, based on the application of bioherbicide containing L-arginine developed by White et al., Rutgers University, USA. This approach exploits the ability of microorganisms to synthesize ethylene from arginine supplied by plants, which, at high concentrations, induces excessive production of reactive oxygen species (ROS), leading to plant death while leaving no chemical residues in the treated soil.

Methods

The bioherbicide was tested to control *Solidago canadensis* colonizing areas in Krakow, Poland. To enhance the effectiveness of the bioherbicide, endophytic microbes were isolated from *Solidago canadensis*, molecularly identified, and multiplied. The influence of the bioherbicide on both above- and below-ground plant organs in the presence of endophytes was examined under field conditions. Photosynthetic efficiency and mycorrhizal diversity were assessed before and after application of the bioherbicide.

Results

One year after treatment, *S. canadensis* exhibited reduced photosynthetic performance, rhizome degradation, and a significant decline in shoot number, including generative shoots. Mycorrhizal colonization of remaining plants from treated plots remained unaffected.

Conclusions

These findings highlight the potential of L-arginine-based bioherbicides as an environmentally safe alternative to chemical herbicides for invasive plant management, particularly under conditions of climate change and ongoing species introductions.

Introduction

Solidago canadensis (goldenrod) was introduced from North America to Europe, likely as an ornamental plant, in the mid-17th century (Dubovik et al. 2019). It spread rapidly during the 19th and 20th centuries (Lambdon et al. 2008; Dubovik et al. 2019) and is now considered one of the most aggressive weeds in non-native regions (Perera et al. 2021). Its expansion has been facilitated by agricultural land abandonment, certain farming practices (Bartha et al. 2014; Fenesi et al. 2015), favourable environmental conditions, human-mediated dispersal, and inherent biological traits (Perera et al. 2021).

Goldenrod's success is linked to its clonal growth via vigorous, black rhizomes that are highly resistant to mechanical removal, as well as its release of root exudates that alter soil structure, change nutrient composition, and reduce functional biodiversity. These effects inhibit the growth, germination, and survival of native species, giving goldenrod a competitive advantage (Bartha et al. 2014; Fenesi et al. 2015; Pal et al. 2015; König et al. 2016). Ecosystems with high biodiversity, however, tend to be less susceptible to invasion.

Despite its ecological risks, goldenrod has recognised benefits for humans. It contains biologically active compounds, including flavonoids and terpenoids, which exhibit anti-inflammatory, analgesic, diuretic, wound-healing, and antioxidant properties (Poljuha et al. 2024). Extracts have been shown to inhibit the growth of cancer cells *in vitro* and to yield essential oils with anticancer potential. Goldenrod also plays a role in apiculture, providing pollen that supports honeybee immunity and reduces mortality from viral infections (Leonard et al. 2020).

Given its invasive potential, effective control measures are essential. Conventional chemical herbicides negatively affect the environment and native vegetation, leading to restrictions on their use (Szymura et al. 2019; Weidlich et al. 2020; Schulz et al. 2021; Weisskopf et al. 2021). Alternative strategies include biocontrol with the pathogen *Sclerotium rolfsii* combined with mechanical methods, which has achieved over 90% elimination of *S. canadensis* ramets (Tang et al. 2010), as well as the use of specific essential oils. Current management practices also include mowing, grazing, flooding, or combinations of these approaches (Nagy et al. 2020).

A novel biological control method developed at Rutgers University (New Jersey, USA) and the United States Geological Survey uses a bioherbicide containing L-arginine, citrus oil, and sucrose to eliminate unwanted vegetation and suppress invasive plant spread without leaving chemical residues. The formulation is rapidly biodegradable, enabling the reintroduction of native plants displaced by invasives. This approach exploits interactions between microbes (fungi and bacteria) and plant cells (Chang et al. 2021). Under natural conditions, microbes produce ethylene using arginine supplied by the host. Plants regulate ethylene levels by controlling arginine availability. Excess external arginine disrupts this balance, causing overproduction of reactive oxygen species (ROS), which leads to oxidative damage, chlorophyll degradation, uncontrolled microbial proliferation within plant cells, and ultimately plant death. Microscopic studies have documented ROS accumulation and chloroplast damage under these conditions (White and Torres 2010; White et al. 2014; White et al. 2018).

Citrus oil in the formulation dissolves the waxy cuticle on leaves, enhancing tissue penetration and increasing stress (Baker 1982). Sucrose stimulates microbial vitamin production outside the plant (Chang et al. 2021). Additional organic acids and salts inhibit plant metabolism (Lanzagorta et al. 1988; Tramontano and Scanlon 1996; Qiu et al. 2017), causing root branching, browning, and increased bacterial and fungal colonisation. Introducing native endosymbiotic microbes further elevates ethylene production, intensifying stress and accelerating tissue death. Studies indicate that bioherbicide

effectiveness varies by species, with annual plants responding more rapidly than perennials, particularly rhizomatous ones (Imaizumi and Fujimori 1997; Jones and Eastwood 2019).

This study aimed to evaluate the effectiveness of L-arginine-based substances in controlling the growth of *S. canadensis*. The impact of treatment was assessed based on photosynthetic efficiency, using chlorophyll a fluorescence measurement. While this method is well established, it is applied here for the first time to characterise the effects of a bioherbicide on *S. canadensis*. The herbicide was tested for the first time in Europe on *Solidago* spp. in a three-year field experiment. The impact of the treatment on mycorrhizae was also assessed in control and treated plots. Additionally, for the first time, putative endophytic fungi were isolated from *S. canadensis*, and their viability was tested *in vitro*.

Materials and Methods

The field experiment was conducted over three years, starting in 2023, in the area adjacent to the Jagiellonian University Campus in Krakow, Poland, near an area protected under the Natura 2000 designation. In this area, wet meadows of *Phragmites australis* were dominating until the middle of the 20th century, and were assisted by two protected species, *Sanquisorba officinalis* L., and *Betonica officinalis* L. The area was also inhabited by *Cirsium arvense* (L.) Scop., *C. oleraceum* (L.) Scop., *Artemisia vulgaris* L., *Galeopsis bifida* (Hudson) K. Koch, *Symphytum officinale* L., *Taraxacum officinale* F.H. Wigg., *Calamagrostis epigejos* (L.) Roth, *Symphytum officinale* L., *Taraxacum officinale* F.H. Wigg., *Calamagrostis epigejos* (L.) Roth, *Geranium pratense* L., *Polygonum persicaria* L., *Tanacetum vulgare* L., *Glechoma hederacea* (L.) M. Bieb., *Rubus caesius* L., *Galium aparine* L., *Galium mollugo* L., *Calystegia sepium* (L.) Brummitt, *Phragmites australis* L., and *Hypericum perforatum* L. At the time of the experiment that started in 2023 most of these plants were absent because of invasion of *Solidago canadensis* L. and less common *Solidago gigantea* Aiton.

Isolation of putative endophytes

Endophytic fungi were isolated from shoots and roots of *S. canadensis*. Before isolation, plants were surface sterilized with 8% sodium hypochlorite for 5 min, followed by 96% ethanol for 1 min and 75% ethanol for 3 min and washed 5 times with sterile deionized water. The plant after the last rinsing was placed onto sterile medium for 30 sec in order to confirm sterility. After surface sterilization, plants were cut into small segments (app. 3 × 3 mm) and placed onto Gel Gro (MP Biomedicals, USA) droplets supplemented with 0.03% MgSO₄ (Silvani et al., 2008) and antibiotics: streptomycin (40 mg·L⁻¹), (40 mg·L⁻¹), and Terramycin (20 mg·L⁻¹). Antibiotics were dissolved in sterile deionized water, filter sterilized using a 0.22 µm syringe filter and added to medium. Samples were incubated in the darkness at 27°C and inspected every day for 4 weeks. Cultures of emerging fungi were transferred onto potato dextrose agar (PDA) medium and incubated in darkness at 27°C.

Identification of endophytic fungi

Pure cultures of endophytic fungi were sorted based on morphological features, followed by identification according to ITS sequence data. DNA was extracted with DNeasy Plant Mini Kit (QIAGEN, DE) according to the manufacturer's instruction. The ITS rDNA region was amplified with ITS1F (Gardes and Bruns, 1993) and ITS4 primers (White et al., 1990), LSU region with LR7 and LR0R primers and β -tubulin region Bt2a and Bt2b primers (Glass and Donaldson, 1995). PCR was performed in 25 μ L reaction mixtures containing 1 μ L DNA sample; 9.5 μ L of nuclease-free water; 12.5 μ L of Dream Taq 2x Green MasterMix (Thermo Scientific), and 1 μ L of each of the primers at 10 pmol concentration for each sample.

For ITS region PCR conditions included: 1) initial denaturation at 95 °C for 3 min; 2) 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 45 s; 3) final elongation at 72°C for 5 min. For LSU region PCR conditions included: 1) initial denaturation at 95°C for 1 min; 2) 35 cycles of denaturation at 95°C for 45 s, annealing at 52°C for 40 s, and elongation at 72°C 150 s; 3) final elongation at 72°C for 10 min.

For β -tubulin region PCR condition included: 1) initial denaturation at 95°C for 8 min; 2) 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 60 s; 3) final elongation at 72°C for 5 min. The presence of PCR products was visualized in 1.25% gel stained with SimplySafe™ (EURx). Gel/PCR Mini Kit (Syngen) were used for PCR products. The PCR products were sequenced by Eurofins Genomics (DE). The sequences were edited with Chromas software and BioEdit and subsequently compared with sequences published in the NCBI database by BLASTn algorithm. Fungi species were identified if at least 96% sequence similarity of ITS region matched reference sequences. Sequence data were deposited in the NCBI database under accession numbers given in Table 1.

Seed germination

Seeds of *S. canadensis* were washed and the tufts of whitish hairs (pappus) were removed. surface sterilized in 8% sodium hypochlorite for 5 min, followed by 96% ethanol for 1 min and 75% ethanol for 3 min and washed 5 times with sterile deionized water and then germinated on water agar. The surface sterility of the seeds was verified by spreading the last wash water on PDA medium and monitored for microbial growth. The seedlings were carefully checked daily for sterility.

Inoculation of seedlings with endophytes and verification of plant colonization

Ten-day-old seedlings were transferred to Petri dishes (three plants per plate, with five repetitions of plates containing individual fungal strains and 10 for the control) containing the Strullu-Romand (MSR) medium (Cranenbrouck et al., 2005). The medium was modified as follows: the concentration of GelGro™ (MP Biomedicals, France) was increased to 11.0 g L⁻¹ (from 3.0 g L⁻¹), and sucrose was omitted. After

solidification, half of the agar disk was removed from the dish to prevent direct contact between the green plant parts and the agar, while still allowing them to grow under sterile conditions. Inoculated and non-inoculated seedlings were prepared. Non-inoculated plants were compared to seedlings that were inoculated with a single strain of fungal endophytes.

To inoculate the seedlings, small pieces of agar (ca. 2 x 2 mm) with young endophyte mycelium were placed close to the roots of the seedlings. After closing the dishes with parafilm, half of the dish containing the roots was covered with black tissue paper. Plants were cultivated in vitro for five weeks to check the growth promotion/inhibition. Plants were grown in growth chambers at 21°C, with a 12/12 h photoperiod, 60% humidity, and a light intensity of approximately $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR (Photosynthetically Active Radiation).

After finishing the experiment plants were harvested and stained. Plants from in-vitro and pot cultures (N = 5) were stained with and Sudan IV, according to modified procedure)(Barrow, 2003). As a modification a vacuum was used instead of autoclave. The colonization was verified by observation with an Olympus compound microscope.

Field experiment description

The experimental plots were established in June 2023. Fifteen 25 m² plots were randomly selected in areas uniformly covered with *Solidago* spp. Ten plots were treated two times (five control plots were left untreated for comparison) at weekly intervals starting on June 9 with bioherbicides (as described in U.S. patent application no. 17/266,489) containing: 5% L-arginine (ARG05.25 BioShoop, Canada) and 10% lemon oil, 10% sucrose per 1 litre of water (basic mixture). The third treatment included additionally glycerol, hexanoic, oxalic and citric acids (each at 3%). Cultures of endophytic organisms isolated from *S. canadensis* (*Epicoccum nigrum* B29, *Fusarium* sp. B30, and *Alternaria alternata* B37) from the study area were grown on PDB medium (liquid culture) for 10 days, filtered on a sieve, washed with water, homogenised with a blender, and added to a container fitted with a spray nozzle. One litre of bioherbicide was applied to each plot, ensuring even spraying of all plants. Because plants treated twice with the basic mixture showed no visible changes, a third mixture was additionally supplemented with hexanoic acid, oxalic acid, citric acid, and glycerol (each at a 3% concentration). One year after the treatments, the number of vegetative and flowering shoots, as well as the number of plant species appearing in the plots, were measured. Root samples were also collected from control plots and the mixture-treated substrate. Root samples were prepared according to a modification of the method of (Phillips and Hayman, 1970). Briefly, after washing in tap water, the roots were softened in 10% KOH for 24 hours, then rinsed in tap water, acidified in 5% lactic acid for 1 hour at room temperature, and stained with 0.01% aniline blue in pure lactic acid for 24 hours. After staining, roots were stored in pure lactic acid and then cut into 1-cm sections, mounted on microscope slides in lactoglycerin, and analysed. Absolute mycorrhizal colonisation (m) and arbuscule richness (a) were assessed according to the method of Trouvelot et al. (1986). Only the thinnest roots were included in the assessment.

Measurement of plants' photosynthetic efficiency

Photosynthetic efficiency of plants was estimated by the use of chlorophyll *a* fluorescence measurements using Handy PEA fluorimeter (Hansatech Instruments Ltd., UK). Measurements were performed on fully developed leaves collected from the plots and leaves obtained from the *in vitro* experiment. During a one-second measurement, red light (peak at 650 nm) with an intensity of 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was applied to excite chlorophyll in PSII, thereby inducing photosynthetic electron transport and variable fluorescence signals. Data acquisition was carried out at intervals of 10 μs (from 10 to 300 μs), 0.1 ms (0.3 to 3 ms), 1 ms (3 to 30 ms), 10 ms (30 to 300 ms), and 100 ms (300 ms to 1 s). The measurements were conducted on intact leaves (8–15 replicates for each endophyte in every treatment) that was dark-adapted for a minimum of 30 min before measurement. For each treatment, the average Chlorophyll *a* fluorescence OJIP transients were analysed according to the JIP-test (Bueno et al., 2004; Strasser et al., 2004). Three groups of parameters were chosen to be calculated (all parameters refer to onset of fluorescence induction at time zero): i) ET_0/RC - electron transport flux (further than Q_{A}^-) per RC; ii) specific fluxes: ABS/CS - absorption flux per cross section (CS); TR_0/RC - trapped energy flux per RC; ET_0/RC - electron transport flux per reaction center (RC); DI_0/RC - dissipated energy flux per active reaction center; iii) vitality indexes: maximum yield of primary photochemistry ($\phi_{\text{E}_0}/1-\phi_{\text{E}_0}$); electron transport beyond Q_{A}^- (primary quinone acceptor) ($\Psi_{\text{E}_0}/1-\Psi_{\text{E}_0}$); fraction of reaction center chlorophyll per chlorophyll of the antennae (RC/ABS) and performance index on absorption basis (PI_{ABS}) which combines the three above mentioned indexes. For a detailed and analytical description, see Strasser et al. (2004). For updated formulae and a glossary of terms used in the JIP test, refer to Gururani et al. (2017), Tsimilli-Michael (2020) and Kalaji et al. (2014, 2017).

Statistical analyses

Photosynthetic parameters and fungal data obtained under *in vitro* conditions were analyzed using the Kruskal–Wallis test. Prior to applying subsequent statistical analyses, data normality and homogeneity were evaluated. When data deviated from normality, log or square root transformations were applied to improve distribution and reduce heteroscedasticity. All analyses were performed in STATISTICA version 13 (StatSoft), with significance levels set at $P < 0.05$.

Results

Fungal endophytes isolated from *S. canadensis*

From the 250 fungal endophyte colonies isolated from *S. canadensis*, 65 morphotypes were selected for molecular analysis. According to Index Fungorum (CABI) and (Tedersoo et al., 2018) except for *Mucor moelleri* (member of Mucoromycetes, Mucoromycota) and *Mortierella alpina* isolated only from roots, all the remaining strains were identified as members of Ascomycota (Table 1). The most common isolates from roots were members of genera: *Fusarium*, *Cadophora*, *Paraphoma* and *Umbelopsis*. From

aboveground stems most common isolates belonged to *Cadophora*, *Ilyonectria*, *Pseudopithomyces*. Dominating genera isolated from seeds of healthy plants were *Epicoccum*, *Fusarium* and *Alternaria* while *Ilyonectria*, *Umbelopsis*, *Bipolaris*, *Xylaria* (identification based on stromata formed in culture) and *Diaporthe* were less common. Seeds of unhealthy plants were colonized by members of *Fusarium* and *Alternaria* only while their stems gave isolates of *Plectosphaerella* and *Aspergillus*.

Effect of fungal isolates on seedling performance and chlorophyll a fluorescence

The largest *in vitro* seedlings were obtained for strains *Xylaria* B53, *Tricladium* B10, *Plectosphaerella plurivora* B1, and *Aspergillus sydowii* B9 (Fig. 1). In most cases, however, by the end of the observation period, when conditions in the plates were no longer optimal for plant growth, signs of mycelial growth were visible on the surface, although the leaves remained green. In the case of strains *Alternaria*, *Paraphoma*, *Dothideomyces*, *Fusarium*, and *Diaporthe*, the seedlings died at the initial stage of their development, with the entire seedling covered in mycelium and accompanied by plant death. The remaining strains did not cause rapid seedling death, but the mycelium visibly grew on their surface. Plants with a sufficiently large surface area were subjected to chlorophyll and fluorescence measurements to assess the physiological status of the photosynthetic apparatus. All measured samples exhibited maximal PSII efficiency (Fv/Fm) values close to 0.8 relative units. .

In the case of *Exophiala* and *Ilyonectria* (Fig. 2A), statistical analysis showed significantly higher PI_{ABS} against control plants. In the first case, this was correlated with a relatively higher number of reaction centres per leaf section (RC/CS_o) and lower energy dissipation per reaction centre (DI_o/RC). In this second case, the changes were not significant. Three instances of higher driving forces (DF) were found. In addition to the two mentioned before, DF was also higher than in the case of control plants inoculated with *Xylaria* sp. (Fig. 2A). In this case, however, PI_{ABS} was significantly lower, although these plants had visibly bigger leaves in comparison to other plants *in vitro*

Plants possible to measure but showing reduced effects of inoculation of leaf surface area and lower survival % of seedlings, had mostly much lower PI_{ABS} (except for *Alternaria*) and mostly higher energy dissipation (e.g., DI_o/RC) (Fig. 2B).

Effect of bioherbicide on *S. canadensis* of experimental plots

Plants from the experimental plots (Fig. 3) that were treated only with the basic substances (no organic acids and glycerol) showed few differences visually in comparison to the control plants. On the contrary the third treatment supplemented with organic acids and glycerol showed stronger differences such as leaf wilting (10 min after the treatment), turning brown (30 min) and drying (Fig. 3). The chlorophyll a fluorescence showed significant changes in increased heat dissipation (DI_o/RC) and decreased efficiency indices (PI_{ABS} , PSI_{total} , and PI_{CS}) resulting from the forces (D.F) between the two photosystems,

as well as reduced electron transport (ET_o/CS), a reduction in active reaction centres calculated on both absorption level and cross section of the samples (10RC/ABS and RC/ CS_m), and a significant decrease in maximum PSII efficiency (ϕ_{E0}). These differences persisted until the end of the growing season; however, it is worth noting that plants from individual plots did not respond uniformly to the treatments. In some cases, the differences were not statistically significant, although trends were visible.

One year after the field experiment, differences were visible especially after three treatments where fewer generative shoots were found (Fig. 5). Generative shoots from treated areas were longer after one treatment while those after three treatments were significantly shorter (Fig. 6), but no differences in the number of vegetative shoots were observed. In the treated areas, specimens of *Sanquisorba officinalis* and *Betonica officinalis* were found among the thinned *Solidago* spp. shoots. The situation returned to the drastic dominance of *Solidago* spp. in the third year.

Bioherbicide treatment did not significantly affect the frequency and colonization of mycorrhizal roots (Fig. 7). However, the difference was significant in the abundance of arbuscules, which were statistically significantly more abundant after bioherbicide treatment compared to the control.

Discussion

Effective control of invasive plants is essential for maintaining biodiversity. However, management strategies often ignore the use of natural substances and the specific biological and ecological characteristics of individual species. The experiment was conducted as part of the research discussed in this paper involved a trial application of a bioherbicide developed as a result of the pioneering research of Rutgers University group on the phenomenon of "rhizophagy" (White and Torres 2010; White et al. 2014; White et al. 2018). Observations of several plant species have shown that their endophytic microorganisms produce ethylene using arginine supplied by plants. To control the balance, the microorganisms receive a sufficient amount of arginine from plants, and microbial ethylene then acts as a plant growth hormone. However, when arginine levels (used as a bioherbicide ingredient) are too high, the plant rapidly produces excess ROS, leading to plant suicide and ultimately shifting the status of endophytic microorganisms to saprophytes that degrade plant tissues. Therefore, this innovation exploits natural processes in the soil, causing a short-term disturbance of the habitat, enabling recovery to the pre-invasion state. To our knowledge, this is the first time that the endophytic fungi from *Solidago canadensis* have been isolated and their effects on plant seedlings have been tested.

S. canadensis is a plant species characterized by effective wind-mediated seed dispersal. Moreover, once established, the plant spreads through numerous underground rhizomes that are difficult to eradicate. It also releases allelopathic compounds that inhibit the growth of other plant species (Kato-Noguchi and Kato, 2022). *S. canadensis* colonizes both relatively undisturbed habitats and heavily polluted sites, including industrial waste dumps with high concentrations of lead, cadmium, copper, and zinc (e.g., Dambiec et al., 2022). The traits mentioned do not close the list of the traits used by *S. canadensis*. As shown presently, a diverse community of fungal endophytes assists the plant, and during

the plant's growth, they are considered beneficial, although their effects depend on the host species. According to the literature, it can also rely on developmental stage, and environmental stressors such as potentially toxic metals (Domka et al. 2019; Zhao et al. 2025). The endophyte strains obtained could have value in strengthening the growth of other plants, particularly those cultivated under extreme conditions, such as those using industrial wastes. *In vitro* assays confirmed that obtained isolates are different concerning plant growth stimulation and improvement of specific photosynthetic efficiency parameters of the young seedlings. However, results obtained from very young plants should be interpreted with caution, as changes in test conditions, such as culture medium desiccation or nutrient depletion, can affect outcomes.

Among the endophytes isolated from *S. canadensis* plants, *Exophiala*, *Ilyonectria*, and *Xylaria* were the most effective in growth stimulation. Those of *Exophiala* and *Ilyonectria* had particularly beneficial effects, increasing assimilation area and enhancing photosynthesis. The driving force (DF) parameter best reflected their endophytic potential, while energy dissipation (energy loss) remained comparable to that of control plants. The highest performance index (PI_{ABS}) that was recorded for *Exophiala oligosperma*, a species not previously reported from plants but known to degrade organic pollutants such as styrene (Braun-Lüllemann et al. 1997; Rene et al. 2012), suggests its potential applications in environmental remediation. Other *Exophiala* strains described in literature were also recognised for promoting plant growth, enhancing stress tolerance, and providing pest protection (Thitla et al. 2022).

Similarly, *Aspergillus sydowii* and *Epicoccum nigrum* have been reported as multifunctional endophytes that stimulate plant growth, increase pathogen resistance, and contribute to agricultural ecosystem balance. *Cadophora luteo-olivacea*, another species isolated from *S. canadensis*, is known to enhance disease resistance, influence pathogen population dynamics, contribute to nutrient cycling, and potentially shape plant community composition through interactions with host plants and herbivores. *Alternaria alternata*, a cosmopolitan fungus with a broad host range, can be either beneficial or harmful depending on the context and interacts with pathogens to regulate plant defence mechanisms (DeMers, 2022). It is often seed-transmitted. *In vitro* interactions between *A. alternata* strains and *S. canadensis* in the present study varied, with some strains showing positive effects and others detrimental ones. Some species, such as *Paraphoma*, reduced seedling viability but are not necessarily effective biocontrol agents. To date, *Sclerotium rolfsii* remains the only species known to parasitise *Solidago* spp. and effectively control them (Tang et al. 2010), although it was not detected in the present study.

Although endophytes are important during plant growth, after the plant's death they all act as saprophytes, as is the case here following the use of the bioherbicide. The change in endophyte role is typical for all endophytes due to their former presence in living plant tissues; thus, they are the first to access previously accumulated nutritional resources. When the plant commits suicide by producing excessive amounts of ROS, it is the endophytic fungi and bacteria that become the beneficiaries of the situation. One may ask why naturally occurring soil microbes are not sufficient to meet these demands. Our unpublished data indicated that the degradation of underground plant parts was much more efficient in plots treated with the bioherbicide containing additional microbes than in plots without them.

However, in all other respects, these plots did not differ from those described in the present paper. This was the reason that isolated fungal strains were incorporated into bioherbicide treatments applied in field plots. On the Jagiellonian University campus, this approach limited the growth of *S. canadensis*, although the study's small plot size means it should be considered a pilot experiment. Herbicide treated plants showed reduced photosynthetic activity in leaves and, in the following year, a decrease in the number of generative shoots, reducing seed production. Rhizome and root rot, similar to root rot reported for *Phragmites australis* in the United States (personal information), was also observed in the second year of the study. In the present case, the trial was constrained by plot size, possible uneven spray distribution due to wind variability during application, and others.

The bioherbicide treatment effects were most evident in the second year after application. Chlorophyll *a* fluorescence analysis indicated that L-arginine application induced significant stress in the photosynthetic apparatus, reducing light energy conversion efficacy and impairing the performance of both PSII and PSI. The observed increase in heat dissipation (DI_o/RC parameter) reflected greater energy loss, while decreases in PI_{ABS} , PSI_{total} , and PI_{CS} indicated weakened photochemical activity of plants' photosynthetic apparatus. The reduction in active reaction centres (RC/ABS and RC/CS_m) and lower DF between the two photosystems, along with reduced electron transport (ET_o/CS), suggest a serious disruptions in the electron transport chain, limiting ATP and NADPH production. The significant decline in the maximum efficiency of PSII (Φ_{E_o}) further confirms the negative impact of bioherbicide on photosynthetic performance.

Previous studies have shown that *S. canadensis* forms arbuscular mycorrhizae (Vallino et al. 2006; Majewska et al. 2015; Zubek et al. 2020; Řezáčová et al. 2021), which under certain conditions can enhance its growth and competitive ability (Genre et al. 2020; Dong et al. 2021; Yu and He, 2022). However, the species can also survive without them, indicating limited dependence on this symbiosis. Mycorrhizae may be replaced or complemented by endophytes, which spread more easily and can be transmitted through seeds. Significantly, the bioherbicide treatment did not reduce mycorrhizal abundance; in fact, arbuscule richness in roots even increased in the second year following the treatment. This suggests that such treatments may facilitate the re-establishment of native mycorrhizal plant species if propagules remain in the soil. For invasive plant control, the choice of bioherbicide composition is therefore critical. By the third year, after bioherbicide degradation, rhizomes from outside the plots recolonised and dominated the area, again highlighting the need for larger-scale trials.

The potential applications of bioherbicides extend beyond invasive plant control. They may also be used in pre- and catch crops, such as *Medicago* spp. and *Trifolium* spp., to improve soil fertility while eliminating the need for tillage. In such cases, the entire treated plant can contribute to the soil nutrient base while preserving the natural mycorrhizal network for subsequent crops. Because plant responses vary, bioherbicide applications require species-specific testing. Unpublished data from our research indicate that *Plantago lanceolata* is eliminated after a single bioherbicide application, while mycorrhizal fungi remain unaffected.

Conclusions

The findings of this study indicate that, under the tested conditions, bioherbicide application modifies plant physiological processes in ways that contribute to limiting invasion. To our knowledge, this is the first report in which endophytic fungi from *Solidago canadensis* have been isolated and their effects on plant seedlings evaluated. The observed reduction in photosynthetic efficiency in treated plants was accompanied by decreases in growth parameters, including the number and height of generative shoots. As a method based on natural compounds, this approach represents a promising alternative to synthetic herbicides, with reduced risks to soil organisms as well as to human and animal health.

Declarations

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Author Contributions

Conceptualization (JW, KT), methodology (MB, PB-K, HK, KT), investigation (MB, PB-K, HK), data curation (PB-K, MB), formal analysis (PB-K, KT), resources (JW), writing – original draft (KT), visualization (MB, PB-K, HK), supervision (KT), funding acquisition (MB, JW). All authors approved the final version of the manuscript.

Financial interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

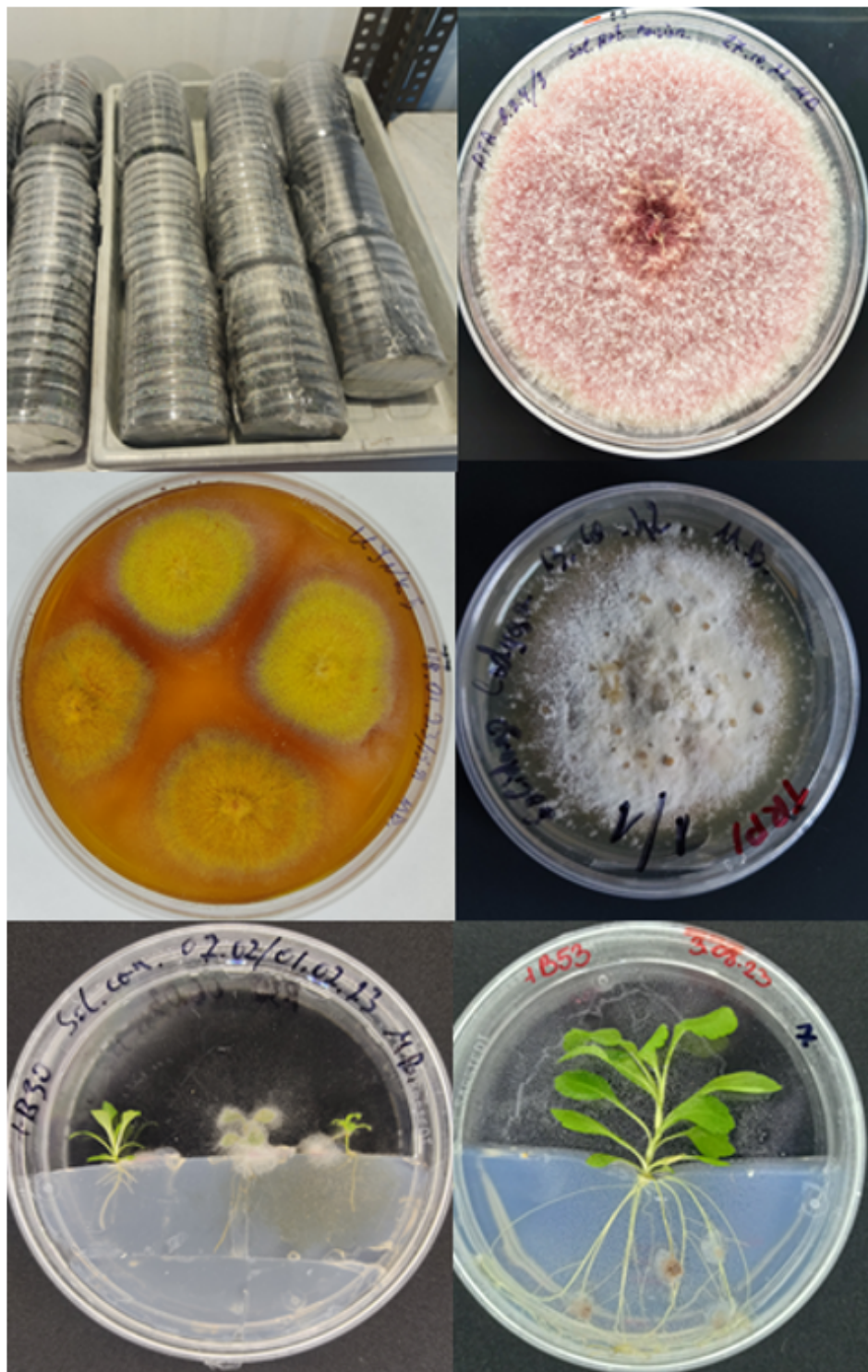


Figure 1

In vitro cultures of *S. canadensis*: A – culturing system in Petri dishes; B – culture of *Fusarium* sp; C – *Epicoccum*; D – *Xylaria* sp; E – plants inoculated with *Fusarium oxysporum*; F – plants inoculated with *Xylaria*

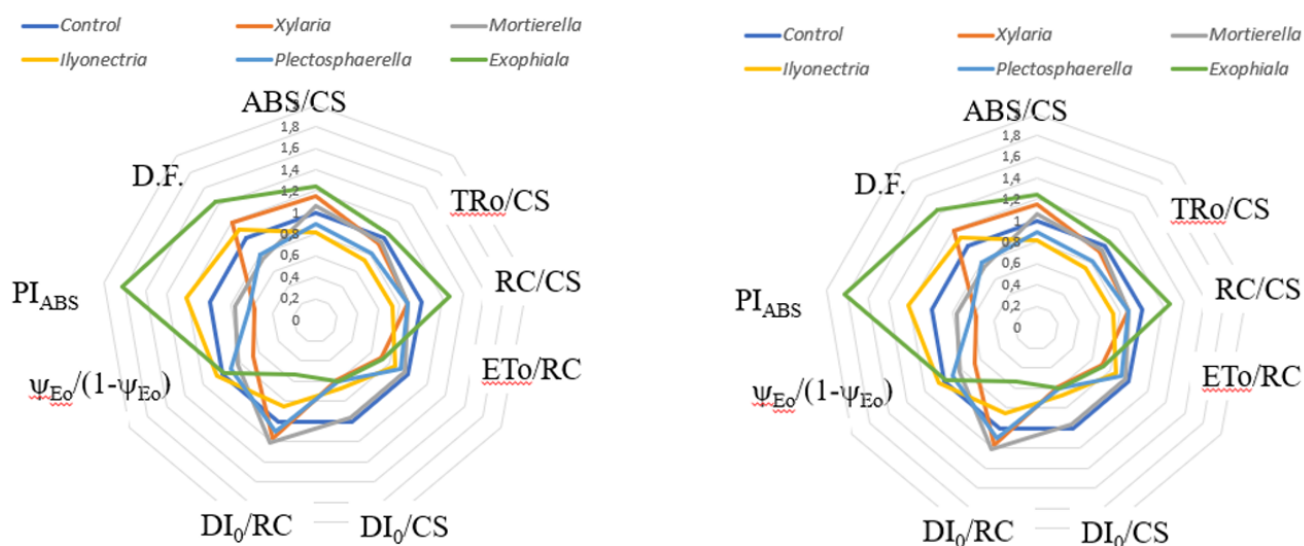


Figure 2

A. Parameters of chlorophyll *a* fluorescence for plants inoculated with the most effective fungal strains; abbreviations: ABS/CS - absorption flux (exciting PSII antenna Chl *a* molecules) per cross section (CS), TRo/CS, trapped energy flux (leading to QA⁻ reduction) per CS, RC/CS- fraction of reaction center chlorophyll per CS, ET₀/RC - electron transport flux per RC, DI₀/RC dissipated energy flux per - active RC, DI₀/CS - dissipated energy flux per CS, $\Psi_{E0}/(1-\Psi_{E0})$ - electron transport beyond QA (primary quinone acceptor), PI_{ABS} - performance index on absorption basis, , DF – driving force (=log PI_{total}).

B. Parameters of chlorophyll *a* fluorescence for plants inoculated with the less effective fungal strains, although fungi rarely killed plants (compare to Fig. 2A); abbreviations: ABS/CS - absorption flux (exciting PSII antenna Chl *a* molecules) per cross section (CS), TRo/CS, trapped energy flux (leading to QA reduction) per CS, RC/CS - fraction of reaction center chlorophyll per CS, ET₀/RC - electron transport flux per RC, DI₀/RC dissipated energy flux per - active RC, DI₀/CS - dissipated energy flux per CS, $\Psi_{E0}/(1-\Psi_{E0})$ - electron transport beyond QA (primary quinone acceptor), PI_{ABS} - performance index on absorption basis, D.F. – driving force (=log PI_{total}).

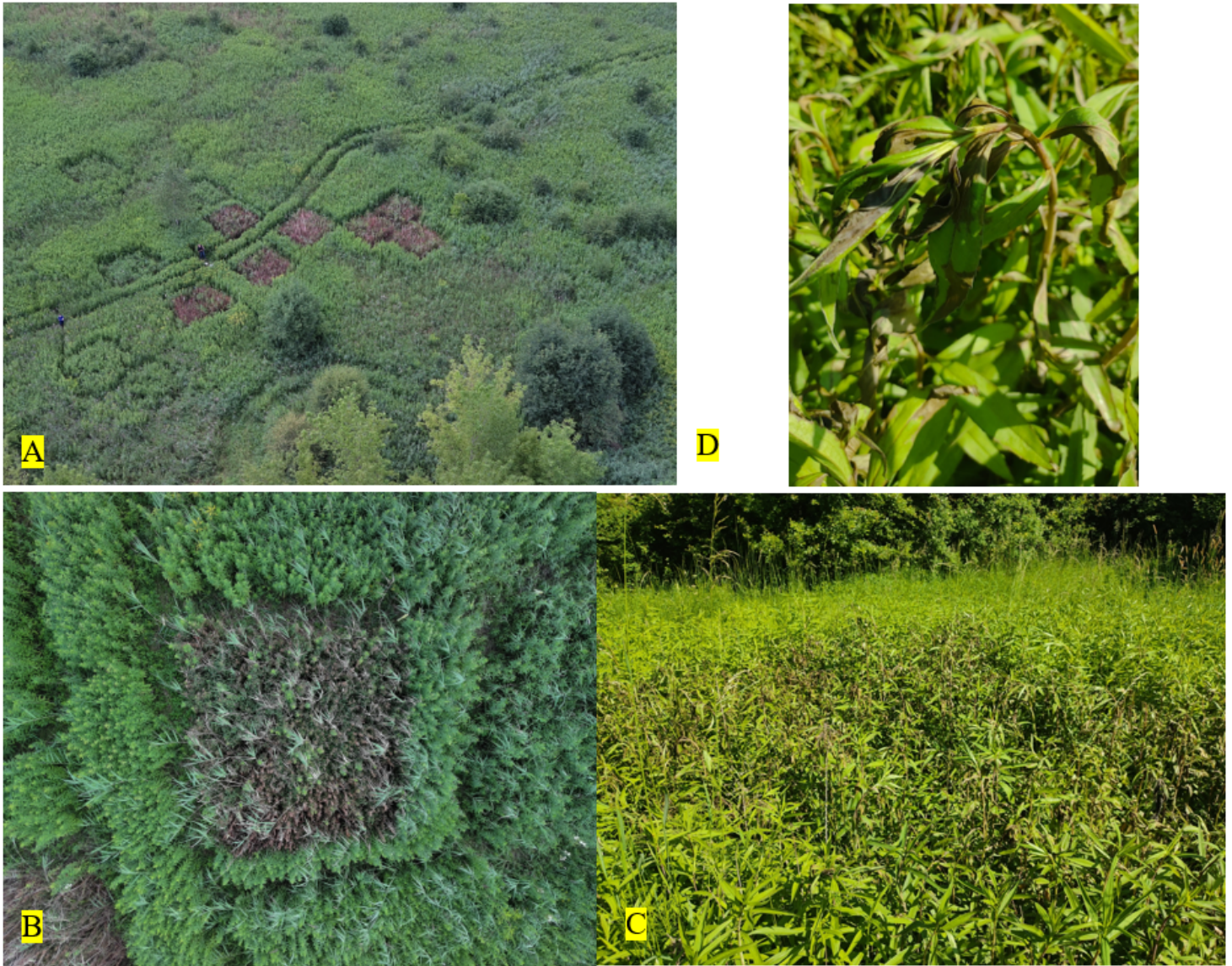


Figure 3

Experimental plots (5x5 m) treated with differently composed bioherbicide: A and B (below A) - plots visible from above; C – closer view of treated plot after the first treatment; D - close view of the individual plant after the third treatment

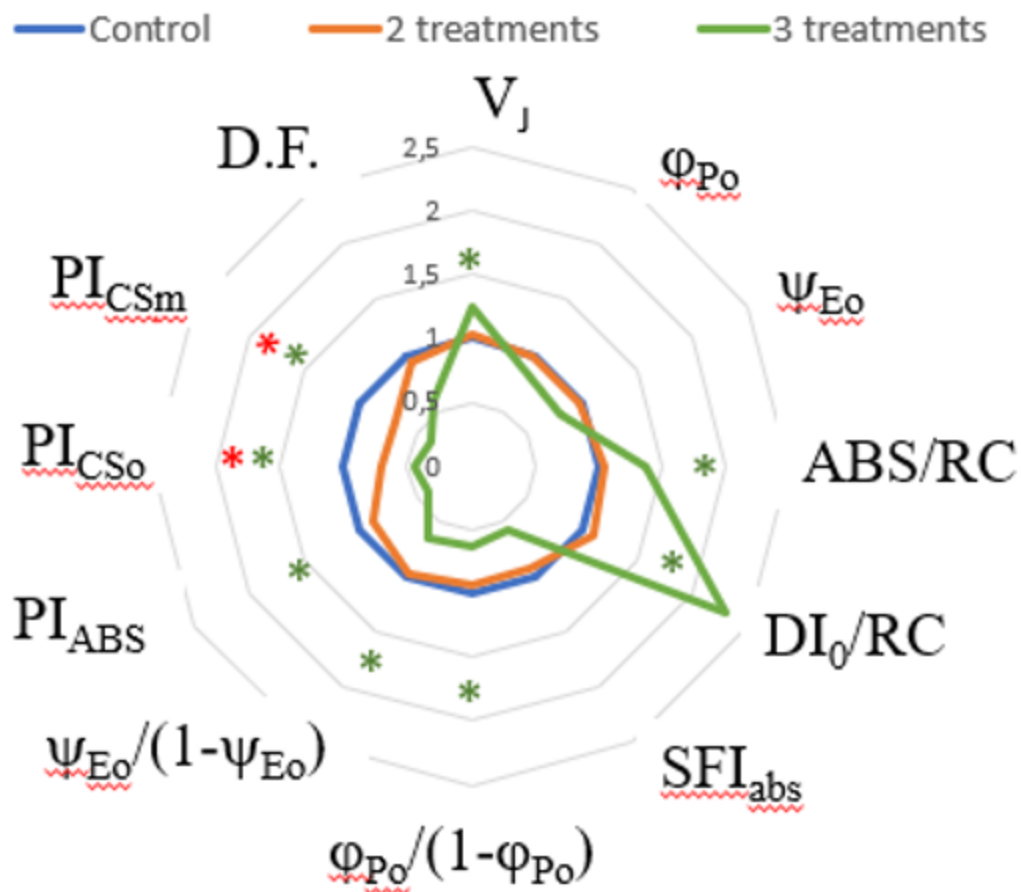


Figure 4

Comparison of parameters of chlorophyll *a* fluorescence for *S. canadensis* treated twice (without organic acid and glycerol and three times with the bioherbicide (including organic acids) and non-treated (control) during the field experiment; green asterisk means statistical difference between control and plots treated 3 times and red asterisk – between control and two treatments; abbreviations: V_j - relative variable fluorescence at the J-step, ϕ_{Po} - Maximum quantum yield of primary photochemistry (at $t = 0$), ψ_{Eo} - Probability (at $t = 0$) that a trapped exciton moves an electron into the electron transport chain beyond QA^- ABS/RC - absorption flux (exciting PSII antenna Chl *a* molecules) per active reaction Centre (CS), DI_0/RC - dissipated energy flux per CS TRO/CS, trapped energy flux (leading to QA reduction) per RC, SFI_{abs} - structure function index (combining structural and functional criteria of PSII, $\psi_{Eo}/1-\psi_{Eo}$ - electron transport beyond QA (primary quinone acceptor), $\phi_{Eo}/1-\phi_{Eo}$ - maximum yield of primary photochemistry, PI_{ABS} - performance index on absorption basis, PI_{CS} - performance index on CS, PI_{CS} - performance index on a cross section basis, DF – driving force ($=\log PI_{total}$).

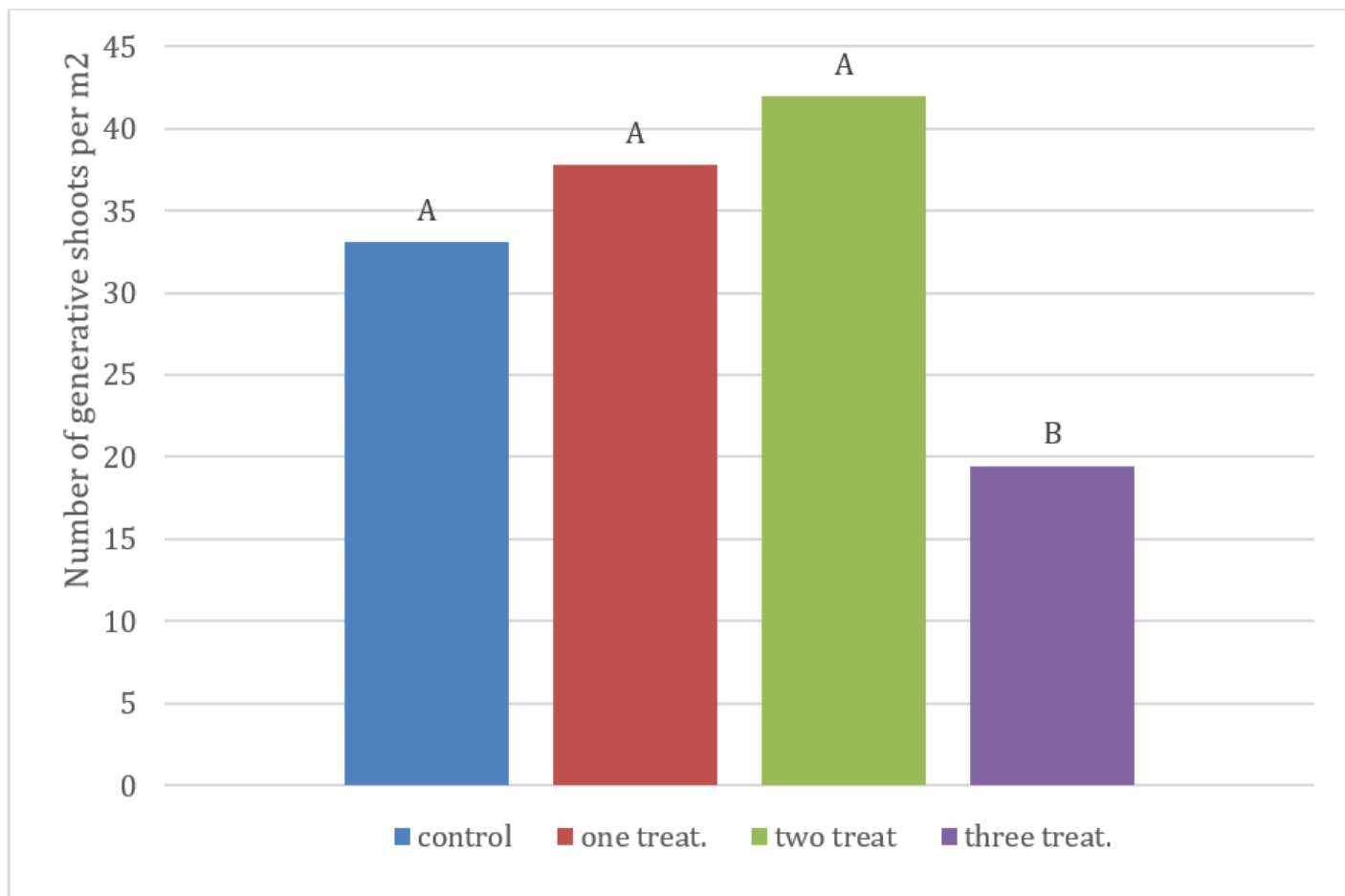


Figure 5

Number of generative shoots in control and treated plots on control and bioherbicide treated plots (1st and 2nd treatment with L-arginine, sucrose and citric acid; 3rd treatment with addition of oxalic acid) as observed in the second year of the field experiment.

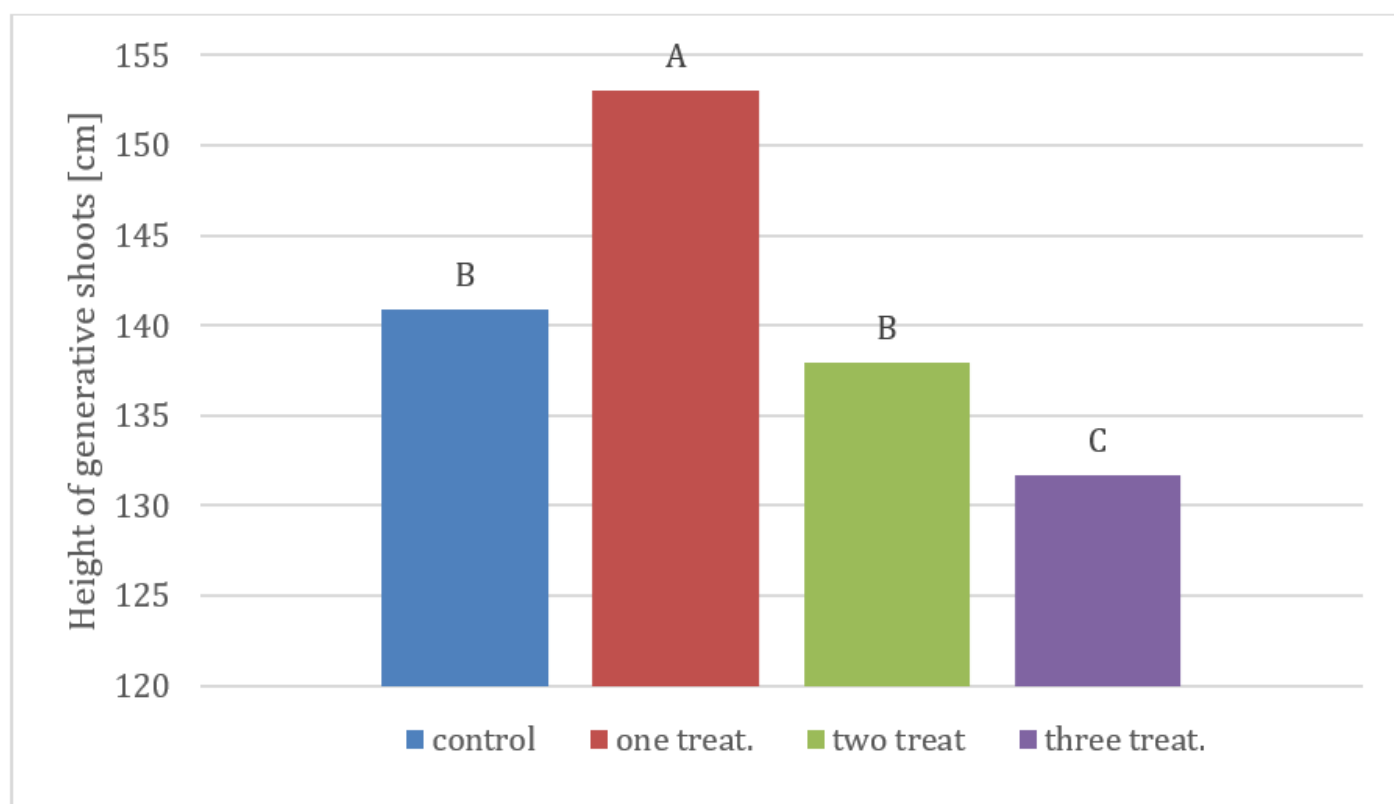


Figure 6

Height of generative shoots on control and bioherbicide treated plots (1st and 2nd treatment with L-arginine, sucrose and citric acid; 3rd treatment with addition of oxalic acid) as observed in the second year of the field experiment.

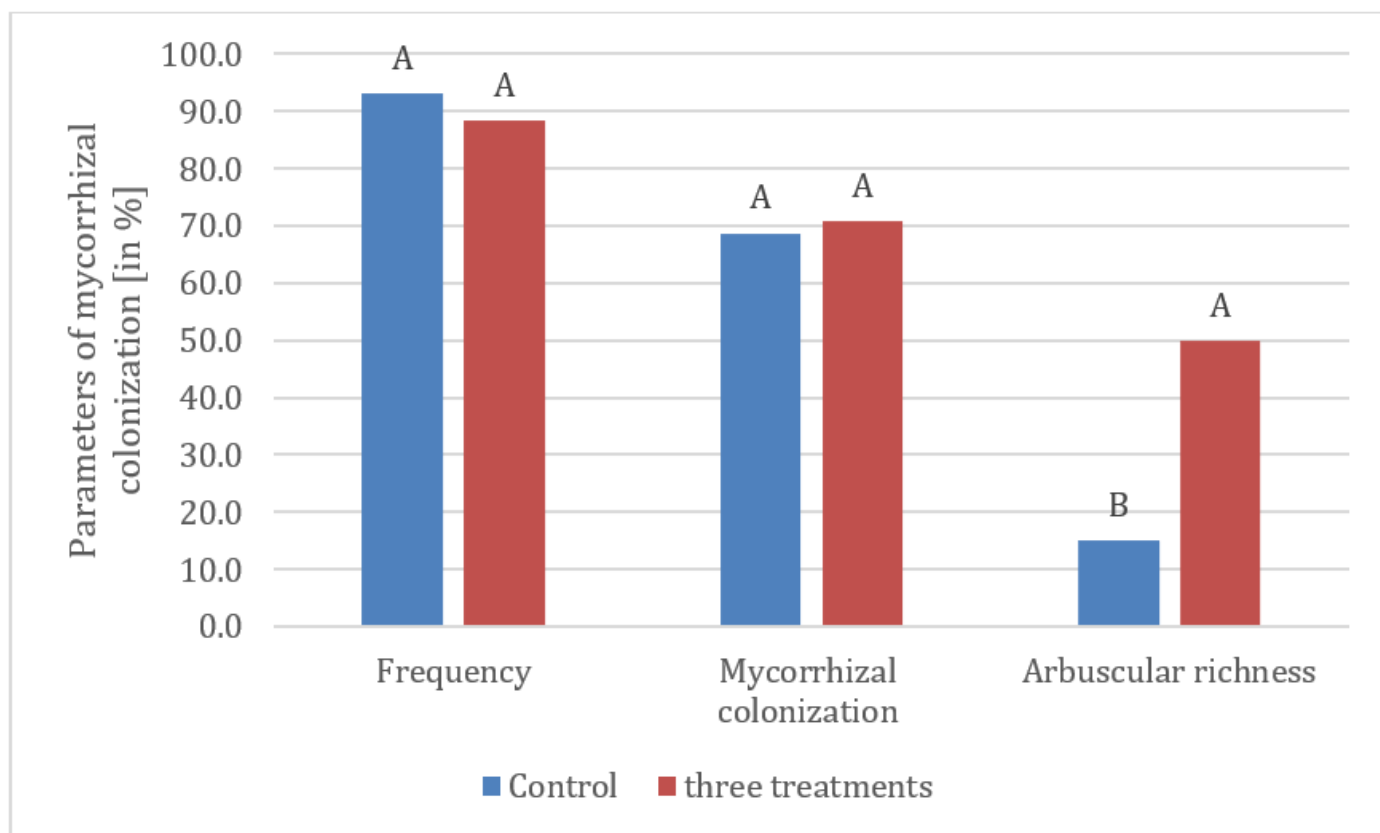


Figure 7

Comparison of mycorrhizal parameters in control and bioherbicide treated plots; different letters above bars mean presence of significant differences.

Supplementary Files

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- [Table1.docx](#)