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Strain-specific and cultivation-dependent bioactivity of *Schizophyllum commune* ethanol extracts in promoting seedling performanceJovana Mišković^{1*}, Maja Karaman¹, Milena Rašeta², Nenad Krsmanović¹, Maja Ignjatov³, Gordana Tamindžić³¹ Faculty of Sciences, ProFungi Laboratory, Department of Biology and Ecology, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia² Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia³ Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, 21000 Novi Sad, Serbia*Corresponding author: jovana.maric@dbe.uns.ac.rs

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ABSTRACT

This study aimed to assess the strain-specific and cultivation-dependent bioactivity of *Schizophyllum commune* ethanol extracts in promoting seed germination and early seedling performance of garden pea (*Pisum sativum* L.). The work contributes to the search for sustainable, biologically based plant growth promoters. Two dikaryotic strains of *S. commune* originating from Serbia and Italy were cultivated under submerged conditions for 7–28 days. Ethanol extracts were prepared from fungal biomass and culture filtrates at concentrations of 10 mg/mL and 50 mg/mL. Seeds of *P. sativum* cv. Dunav were bioprimered for 6 h and tested under controlled laboratory conditions following ISTA procedures. Germination parameters, shoot and root growth, elongation rates, biomass accumulation, and seedling vigor index were determined. All extracts significantly enhanced pea seedling performance relative to the control ($p \leq 0.001$). The strongest effects were observed for the SRB strain, particularly filtrate and biomass extracts from 14- and 21-day cultures. Treatments 21F1 and 21F5 increased shoot elongation rate up to 7.6 mm day⁻¹ and seedling vigor index to 705, compared with 3.3 mm day⁻¹ and 412.5, respectively, in the control. The ITA strain showed delayed but notable stimulatory effects after longer cultivation (21–28 days). The results confirm that the biostimulant potential of *S. commune* depends on strain origin and cultivation period, identifying mid-cultivation SRB extracts as the most effective bioprimering agents for enhancing legume seedling vigor.

Keywords: *Schizophyllum commune*, bioprimering, seedling performance, *Pisum sativum***ИЗВОД**

Циљ ове студије је био да се испита биоактивност етанолних екстраката гљиве *Schizophyllum commune*, условљена пореклом соја и периодом култивације, у погледу њиховог утицаја на клијање семена и рани развој клијанаца баштенског грашка (*Pisum sativum* L.). Истраживање представља допринос развоју одрживих, биолошки заснованих стимулатора раста биљака. Два дикариотска соја *S. commune*, пореклом из Србије и Италије, гајена су у условима потопљене култивације у трајању од 7 до 28 дана. Етанолни екстракти су припремљени из гљивичне биомасе и филтрата у концентрацијама од 10 mg/mL и 50 mg/mL. Семена *P. sativum* cv. Дунав подвргнута су биопрајмингу у трајању од 6 часова и испитивана у контролисаним лабораторијским условима, у складу са препорукама ISTA. Анализирани су параметри клијања, раст надземног и кореновог система, брзина елонгације, акумулација биомасе, као и индекс виталности клијанаца. Сви примењени екстракти значајно су унапредили развој клијанаца грашка у односу на контролу ($p \leq 0,001$). Најизраженији ефекти су забележени код соја пореклом из Србије, нарочито код екстраката биомасе и филтрата из култура култивисаних 14 и 21 дан. Третмани 21F1 и 21F5 довели су до повећања брзине елонгације изданка до 7,6 mm дан⁻¹ и индекса виталности клијанаца на 705, у поређењу са 3,3 mm дан⁻¹ и 412,5 у контролној групи. Соја пореклом из Италије испољио је одложено, али јасно изражено стимулативно дејство након дужег периода култивације (21–28 дана). Добијени резултати потврђују да биостимулативни потенцијал гљиве *S. commune* у великој мери зависи од порекла соја и трајања култивације, при чему се екстракти српског соја из средње фазе култивације издвајају као најефикаснији агенси за биопрајминг и унапређење виталности клијанаца легуминоза

Кључне речи: *Schizophyllum commune*, биопрајминг, развој клијанаца, *Pisum sativum***1. Introduction**

The search for sustainable and eco-friendly strategies to enhance crop productivity has intensified

in recent years, largely driven by the need to reduce reliance on synthetic agrochemicals and mitigate their negative environmental impacts (Mišković et al., 2024;

Zhou and Achal, 2025). Biostimulants of microbial origin have emerged as promising alternatives due to their ability to improve seed germination, seedling vigor, stress tolerance, and overall plant performance (Zuma et al., 2023). Among the diverse microbial resources, fungi have gained particular attention as reservoirs of bioactive compounds that can be harnessed for agricultural applications (Muhammad et al., 2024; Selvakumar, 2025).

One of the most effective ways to utilize microbial biostimulants is through seed biopriming (Singh et al., 2023; Tamindžić et al., 2024a). Biopriming involves the controlled hydration of seeds in the presence of beneficial microorganisms or their metabolites, leading to physiological and biochemical changes that accelerate and synchronize seed germination, improve seedling establishment, and enhance tolerance to adverse conditions (Tamindžić et al., 2024b; Shil et al., 2025). Unlike conventional seed treatments, biopriming provides a sustained effect by triggering defense responses and metabolic readiness in seeds, offering a practical and eco-friendly tool for sustainable agriculture (Srivastava et al., 2024; Shil et al., 2025). Fungal extracts, in particular, represent an attractive biopriming agent, as they combine diverse secondary metabolites with potential signalling functions in plant development and stress resilience (Subba & Mathur, 2022; Mišković et al., 2024; Tiwari et al., 2025).

Schizophyllum commune Fr. is a cosmopolitan basidiomycete known for its versatile metabolism and production of diverse secondary metabolites, including polysaccharides, polyphenols, terpenoids, and other biologically active molecules (Mišković et al., 2021, 2023). Traditionally studied for its medicinal potential, *S. commune* is also increasingly recognized for its role in promoting plant growth and protecting plants against biotic and abiotic stresses (Mišković et al., 2024). However, despite its ubiquity and metabolic potential, the use of *S. commune* in crop biostimulation remains insufficiently explored.

Fungal cultivation conditions are known to strongly influence metabolite profiles and, consequently, the biological activity of resulting extracts (Elisashvili, 2012; Berikashvili et al., 2023). Submerged culture, in particular, enables controlled growth and separation of biomass and extracellular filtrates, each representing distinct sources of bioactive compounds (Elisashvili, 2012; Dudekula et al., 2020). Moreover, strain-specific variability plays an equally critical role, as different isolates of the same fungal species may exhibit contrasting metabolic capacities and bioactivities (Mišković et al., 2021). Understanding these factors is essential for optimizing the use of fungal extracts in agriculture.

In the present study, garden pea (*Pisum sativum* L.) was selected as a model plant for evaluating the biostimulant potential of fungal extracts. Garden pea is not only an important grain legume with high nutritional and agronomic value, but also a well-established experimental system for studying seed physiology and stress responses (Mihailović et al., 2019; Quilichini et al., 2022). The seeds of *P. sativum* L. are particularly responsive to seed biopriming, which can improve germination uniformity, early seedling vigor, and resilience to environmental constraints (Shil et al., 2025). Such variability in metabolite production highlights the need to test fungal extracts on relevant

crop models to assess their practical biostimulant effects.

This study focuses on the 80% ethanol (EtOH) extracts of *S. commune* derived from two geographically distinct strains (Serbian and Italian) cultivated under submerged conditions for varying time intervals (from 7 to 28 days). By evaluating their effects on seed germination and seedling performance in *P. sativum* L., we aimed to determine how strain origin and cultivation duration shape the biostimulant potential of *S. commune*. The findings provide novel insights into the strain-specific and cultivation-dependent bioactivity of this fungus and highlight its potential as a sustainable source of natural plant growth promoters.

2. Materials and methods

2.1. Fungal material and extract preparation

Two dikaryotic strains of *S. commune* were collected in Italy (2016) and Serbia (2012), identified based on macro- and micromorphological traits, and deposited in the ProFungi culture collection (FUNGICULT, University of Novi Sad) under accession numbers 0043 (SRB) and 0047 (ITA). As described previously (Mišković et al., 2021), mycelia were cultivated on malt agar and subsequently subjected to submerged fermentation under controlled conditions (26 °C, 120 rpm, light) for 7, 14, 21, and 28 days. Biomass (B) and culture filtrates (F) were lyophilized, ground, and extracted with 80% ethanol (EtOH), yielding standardized EtOH extracts of two concentration (10 and 50 mg/mL) used for further analyses.

2.2. Plant material and seed priming

Garden pea (*P. sativum* L.) cv. Dunav, developed at the Institute of Field and Vegetable Crops (Novi Sad, Serbia), was used in this study. Seeds produced at the experimental site Rimski Šančevi, Institute of Field and Vegetable Crops, Novi Sad (Serbia), in 2022 were surface sterilized with 5% NaClO and rinsed thoroughly before seed priming. Hydropriming was performed in distilled water, whereas seed biopriming involved immersion in EtOH extracts prepared at two different concentrations, 10 mg/mL and 50 mg/mL from SRB and ITA fungal strains for 6 h, after which all seeds were thoroughly rinsed with distilled water and air-dried to their initial weight, following established protocols (Arafa et al., 2020) as published in Mišković et al. (2024). Control seeds were left unprimed

2.3. Assessment of seed germination and initial seedling development

Seed germination was evaluated according to the International Rules for Seed Testing (ISTA 2025). The germination test included 400 seeds, arranged in four replicates of 100. Seeds were placed between moistened filter paper in Petri dishes and incubated at 20 °C under a 12-hour photoperiod for 8 days. Germination energy was determined on the 5th day by counting the number of normally developed seedlings, while the final germination percentage was recorded on the 8th day. In addition to final germination,

abnormal seedlings were identified and categorized according to ISTA criteria. These included seedlings with damaged or missing essential structures (e.g., decayed radicles, deformed hypocotyls, or missing cotyledons), as well as those exhibiting stunted or unbalanced growth (ISTA 2025).

For the purpose of assessing initial plant growth and development, 25 pea seeds were rolled in filter paper and placed in an incubator under the same conditions as the germination test. Shoot and root lengths were measured on the 5th and 8th day using a caliper, on ten randomly selected normal seedlings per replicate. On the 8th day, fresh shoot biomass was determined by weighing the same seedlings, followed by oven-drying at 80 °C for 24 hours to obtain dry biomass (Tamindžić et al., 2024b).

Seedling vigor index (SVI) was calculated according to the method described by Abdul-Baki and Anderson (1973), using the formula: $SVI = \text{Seed germination (\%)} \times \text{Seedling length (cm)}$, where mean seedling length represents the sum of shoot and root lengths measured on ten randomly selected normal seedlings per replicate.

Growth-related parameters, shoot elongation rate (SER) and root elongation rate (RER), were calculated based on the increase in shoot and root lengths, respectively, between the 5th and 8th day, using the formula: $SER/RER = (\text{Length on day 8} - \text{Length on day 5}) / \text{Number of days}$ (Channaoui et al., 2019).

2.4. Statistical analysis

Statistical analyses included one-way and two-way ANOVA, with mean differences evaluated by Tukey's HSD test at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics v22.0 (Windows) and Statistica v12.01 (StatSoft Inc., Tulsa, OK, USA). PCA analysis was performed with Past4Project software (version 4.03), while the spider plot was generated in Microsoft Excel (Microsoft Office 365).

3. Results

3.1. Yield results

Both strains exhibited a three-day lag phase with slower mycelia growth due to adaptation to submerged culture conditions (Figure S1). The ITA strain showed continuous mycelia growth until day 28, reaching the highest yield (1.78 g), whereas the SRB strain peaked at

day 14 (1.83 g), followed by a decline caused by nutrient depletion. Filtrate production also varied: the ITA strain reached its maximum at days 7 and 14, while the SRB strain peaked at the end of cultivation (28 days).

3.2. Biopriming data

Statistical analysis (Table 1) revealed that all evaluated parameters were significantly influenced by the fungal strain ($p \leq 0.001$), except for root length. The extract type exerted a significant effect on all assessed traits ($p \leq 0.001$). Furthermore, the interaction between fungal strain and extract type significantly affected all examined parameters, except for seed germination (SG) and the percentage of abnormal seedlings (AS).

As shown in Table 2, biopriming treatments with fungal extracts significantly influenced seed germination in garden pea. The extract type exerted a statistically significant effect on seed germination for both fungal strains, ITA and SRB ($p \leq 0.001$), indicating that seed germination was markedly affected by the origin and cultivation period of the fungal extract—whether derived from culture filtrate or fungal biomass collected at specific growth stages. The highest increase in seed germination was observed in 21B5 treatment and 7F5 treatment (83.3%) compared to the control (81.0%). Moreover, all applied treatments contributed to a reduction in the frequency of abnormal seedlings. However, these decreases did not reach statistical significance in the ITA strain. In contrast, the SRB strain showed a statistically significant decrease in abnormal seedling occurrence following biopriming with 7B5 and 21F1, where the incidence dropped to 3.3%, compared to 5.3% in the untreated control. In addition, all tested extract types derived from ITA and SRB fungal strains promoted an increase in garden pea shoot length relative to the control (Table 2). Among them, biopriming treatments with 21F1 and 21F5 demonstrated the highest efficacy in enhancing shoot growth. In contrast to shoot length, a distinct pattern was observed for root elongation. Although all treatments positively influenced pea root length, the most prominent effects were recorded following biopriming with 21B5, followed by 21F1 and 21F5 in the ITA fungal strain. Within the SRB fungal strains, biopriming with 21F5 exhibited the strongest effect, followed by 7B1, when compared to the untreated control.

Table 1.

Analysis of variance for garden pea parameters after applying ITA and SRB fungal strains of *S. commune* as biopriming agents.

Parameters	Fungal strain (F)	Extract type (ET)	F x E
Seed germination	0.0002***	0.0000***	0.5087 ^{ns}
Abnormal seedlings	0.0002***	0.0001***	0.1722 ^{ns}
Shoot length	0.0000***	0.0000***	0.0000***
Root length	0.3007 ^{ns}	0.0000***	0.0000***
Shoot elongation rate	0.0000***	0.0000***	0.0000***
Root elongation rate	0.0000***	0.0000***	0.0000***
Fresh seedling weight	0.0000***	0.0000***	0.0000***
Dry seedling weight	0.0000***	0.0000***	0.0000***
Seedling vigor index	0.0000***	0.0000***	0.0000***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns—not significant.

SER in *P. sativum* L. was significantly enhanced as a result of biopriming, with the extract type playing a decisive role across both ITA and SRB fungal strains. Treatments 21F1 and 21F5 demonstrated the highest efficacy. In ITA fungal strains, biopriming with 21F1 and 21F5 led to a more than twofold increase in shoot elongation rate, reaching 7.6 mm day⁻¹ and 7.5 mm

day⁻¹, respectively, compared to the control (3.3 mm day⁻¹). Similarly, in SRB strains, 21F1 and 21F5 achieved 6.3 mm day⁻¹ and 6.0 mm day⁻¹, respectively, clearly outperforming the control. When it comes to RER in garden pea, biopriming with SRB fungal strains yielded notably improved results (Table 3).

Table 2.

Effect of seed biopriming treatments with different fungal strains on seed germination and initial plant growth of garden pea

Fungal strain	Extract type	Seed germination (%)	Abnormal Seedlings (%)	Shoot length (mm)	Root length (mm)
ITA	7B5	82.3±0.6 ^{def}	5.0±1.0 ^d	19.3±0.2 ^{ghi}	54.1±0.4 ^c
	7B1	82.7±1.5 ^{cde}	4.7±0.6 ^{bcd}	18.2±0.4 ^{def}	55.9±0.8 ^b
	7F5	82.7±1.2 ^{cde}	4.7±1.2 ^{bcd}	20.6±0.3 ^{cd}	54.3±0.3 ^c
	7F1	82.3±1.2 ^f	4.3±0.6 ^{bc}	18.8±0.7 ^{bc}	54.3±0.3 ^e
	14B5	82.3±0.6 ^{def}	4.0±1.0 ^{bc}	20.0±0.8 ^{cd}	53.6±0.9 ^c
	14B1	82.7±1.2 ^{cde}	4.7±0.6 ^{ab}	21.8±0.3 ^b	54.6±0.5 ^e
	14F5	83.3±0.6 ^{bcde}	4.7±0.6 ^{bcd}	23.0±0.2 ^{efg}	56.3±0.5 ^c
	14F1	83.7±0.6 ^{abcde}	4.3±0.6 ^{bc}	22.5±0.7 ^{def}	57.0±0.3 ^c
	21B5	83.3±0.6 ^{ab}	4.3±0.6 ^{bcd}	22.5±0.5 ^{efg}	58.8±0.4 ^{ef}
	21B1	83±0.0 ^{abcde}	4.7±0.6 ^{bc}	22.9±0.4 ^{def}	56.1±0.6 ^f
	21F5	83.3±1.2 ^{abcd}	4.0±1.0 ^{cd}	26.8±0.2 ^a	57.8±0.4 ^a
	21F1	83.0±1.0 ^{abc}	3.7±0.6 ^d	24.2±0.3 ^a	58.0±1.6 ^c
	28B5	81.7±1.2 ^{def}	4.0±1.0 ^{bcd}	18.8±0.4 ^{hi}	54.6±0.4 ^{cd}
	28B1	82.3±0.6 ^{abcde}	4.0±1.0 ^{cd}	21.0±0.6 ^{ghi}	53.5±0.4 ^{de}
	28F5	82.7±1.2 ^{abcde}	5.0±0.0 ^{bcd}	19.8±0.3 ⁱ	54.4±0.3 ^{ef}
	28F1	82.3±0.6 ^{cde}	5.0±0.0 ^{bcd}	21.9±0.3 ^{cde}	55.0±3.1 ^e
	Control	81.0±1.0 ^{ef}	5.3±0.6 ^a	13.0±0.7 ^j	37.9±1.3 ^g
	HP	82.7±0.6 ^{abcd}	4.7±0.6 ^{ab}	18.8±1.3 ^{def}	54.0±0.3 ^e
	P val.		0.0002	0.0008	0.0000
SRB	7B5	81.3±0.6 ^{def}	3.3±0.6 ^d	18.0±1.0 ^{ghi}	56.7±0.3 ^c
	7B1	81.7±0.6 ^{cde}	4.0±0.0 ^{bcd}	18.9±0.3 ^{def}	58.4±0.4 ^b
	7F5	83.3±0.6 ^b	4.0±0.0 ^{bcd}	19.4±0.3 ^{cd}	56.2±0.3 ^c
	7F1	80.3±0.6 ^f	4.3±0.6 ^{bc}	20.3±0.3 ^{bc}	54.7±0.4 ^e
	14B5	81.3±0.6 ^{def}	4.3±0.6 ^{bc}	19.5±0.2 ^{cd}	56.0±0.4 ^c
	14B1	81.7±0.6 ^{cde}	4.7±0.6 ^{ab}	20.6±0.3 ^b	54.0±0.8 ^e
	14F5	82.0±1.0 ^{bcde}	4.0±0.0 ^{bcd}	18.5±0.5 ^{efg}	55.9±0.8 ^c
	14F1	82.3±0.6 ^{abcde}	4.3±0.6 ^{bc}	18.9±0.3 ^{def}	56.9±1.0 ^c
	21B5	83.3±0.6 ^{ab}	4.0±0.0 ^{bcd}	18.5±0.3 ^{efg}	53.8±0.4 ^{ef}
	21B1	82.3±0.6 ^{abcde}	4.3±0.6 ^{bc}	18.8±0.4 ^{def}	52.7±0.9 ^f
	21F5	82.7±1.2 ^{abcd}	3.7±0.6 ^{cd}	21.7±0.5 ^a	61.6±0.4 ^a
	21F1	83.0±1.0 ^{abc}	3.3±0.0 ^d	21.9±0.3 ^a	56.0±0.4 ^c
	28B5	81.3±0.6 ^{def}	4.0±0.0 ^{bcd}	17.8±0.4 ^{hi}	55.8±0.3 ^{cd}
	28B1	82.3±0.6 ^{abcde}	3.7±0.6 ^{cd}	18.2±0.3 ^{ghi}	54.8±0.4 ^e
	28F5	82.0±0.0 ^{abcde}	4.0±0.0 ^{bcd}	17.5±0.2 ⁱ	53.8±0.6 ^{ef}
	28F1	81.5±0.0 ^{cde}	4.0±0.0 ^{bcd}	19.4±0.4 ^{cde}	54.4±0.8 ^e
	Control	81.0±1.0 ^{ef}	5.3±0.6 ^a	14.9±4.0 ^j	43.4±4.1 ^g
	HP	82.7±0.6 ^{abcd}	4.7±0.6 ^{ab}	17.3±3.3 ^{def}	48.3±4.9 ^e
	P val.		0.0002	0.0008	0.0000

* Data are represented as mean ± SD (n = 3); Differences between treatments were analyzed using the Tukey's HSD test (p ≤ 0.05). Means within each trait followed by the same letters are not significantly different. HP – hydropriming, P val. – P values.

Treatments 21F1 and 21F5 reached 12.9 mm day⁻¹ and 12.4 mm day⁻¹, respectively, nearly doubling the control value of 6.5 mm day⁻¹. In contrast, the response to extract type within ITA fungal strains differed. The

highest RER was recorded in treatment 21B5 (13.9 mm day⁻¹), followed by 21F1 (13.0 mm day⁻¹), both substantially exceeding the control. As for biomass accumulation in garden pea (Table 3), the biopriming

treatment 21B5 within ITA fungal strains exhibited the most pronounced effect on both fresh and dry seedling weight, with increases of 117.7% and 69.7%, respectively, compared to the control. In contrast, within SRB fungal strains, the highest biomass enhancement was recorded in treatment 21F1, showing increases of 88.3% in fresh weight and 51.1% in dry weight relative to the control. These findings

underscore the strain-specific efficacy of biopriming agents in promoting seedling biomass accumulation. Moreover, analysis of the SVI revealed that all tested biopriming treatments within both ITA and SRB fungal strains had a positive effect on SVI compared to the control (Table 3). Among the evaluated treatments, 21F5, followed by 21F1, proved to be the most effective.

Table 3.

Effect of seed biopriming treatments with different fungal strains on germination- and growth-related parameters and biomass accumulation of garden pea

Fungal strain	Extract type	SER (mm day ⁻¹)	RER (mm day ⁻¹)	Fresh seedlings weight (g)	Dry Seedlings weight (g)	Seedling vigor index
ITA	7B5	4.82+0.23 ^{fgh}	10.42+0.11 ^{defg}	0.884+0.0053 ^k	0.087+0.0009 ^g	604.3+4.5 ^e
	7B1	4.58+0.02 ^{gh}	10.18+0.39 ^{efg}	0.882+0.0058 ^l	0.086+0.0009 ^{efg}	612.3+12.5 ^c
	7F5	5.00+0.03 ^{de}	9.62+0.10 ^h	0.899+0.0097 ^{fg}	0.090+0.0015 ^{cd}	619.2+12.0 ^c
	7F1	4.59+0.21 ^{def}	9.27+0.23 ^{gh}	0.897+0.0066 ^e	0.087+0.0005 ^{cd}	601.8+4.2 ^{ef}
	14B5	5.07+0.21 ^{efgh}	10.41+0.34 ^{defg}	1.016+0.158 ^d	0.100+0.0006 ^c	606.3+16.6 ^{de}
	14B1	5.37+0.09 ^{efg}	11.32+0.05 ^d	0.984+0.0070 ^b	0.094+0.0021 ^b	631.3+12.8 ^e
	14F5	6.40+0.06 ^{cd}	11.69+0.19 ^c	1.027+0.0022 ^b	0.111+0.0014 ^b	660.6+9.4 ^{de}
	14F1	6.62+0.21 ^c	12.94+0.05 ^b	1.056+0.0021 ^{bc}	0.106+0.0010 ^b	665.1+4.0 ^{cd}
	21B5	6.71+0.15 ^{de}	13.87+0.20 ⁱ	1.158+0.0017 ^{hij}	0.120+0.0021 ^{fg}	677.8+11.0 ^{ef}
	21B1	6.51+0.08 ^{defg}	12.31+0.13 ^{defg}	1.112+0.0013 ^{ij}	0.110+0.0031 ^g	656.3+7.9 ^{fg}
	21F5	7.50+0.16 ^b	12.72+0.02 ^b	1.063+0.0053 ^{cd}	0.111+0.0007 ^b	705.0+9.3 ^a
	21F1	7.60+0.14 ^a	12.99+0.57 ^a	1.039+0.0029 ^a	0.104+0.0019 ^a	682.1+20.4 ^b
	28B5	5.40+0.17 ^{de}	10.86+0.26 ^{def}	0.905+0.0077 ^{ghi}	0.090+0.0004 ^{efg}	599.5+10.6 ^{ef}
	28B1	5.80+0.30 ^{cde}	11.09+0.20 ^{fgh}	0.868+0.0113 ^{fgh}	0.090+0.0007 ^{efg}	631.1+10.3 ^{ef}
	28F5	5.80+0.12 ^{de}	11.73+0.03 ^{de}	0.905+0.0081 ^e	0.092+0.0009 ^{def}	613.4+8.2 ^g
	28F1	6.09+0.12 ^{de}	10.67+0.96 ^{efg}	0.898+0.0046 ^j	0.091+0.0008 ^{fg}	632.9+30.4 ^{ef}
	Control	3.26+0.13 ⁱ	6.49+0.53 ^j	0.532+0.0101 ^l	0.071+0.0015 ^h	412.5+4.4 ^h
	HP	4.46+0.33 ^h	8.61+0.13 ⁱ	0.875+0.0291 ^{ef}	0.088+0.0033 ^{cde}	601.9+116.2 ^{ef}
		P val.	0.0000	0.0000	0.0000	0.0000
SRB	7B5	4.63+0.28 ^{fgh}	10.08+0.18 ^{defg}	0.810+0.006 ^k	0.084+0.001 ^g	607.8+8.0 ^e
	7B1	4.56+0.07 ^{gh}	9.91+0.08 ^{efg}	0.833+0.007 ^j	0.085+0.001 ^{efg}	631.0+6.8 ^c
	7F5	4.94+0.07 ^{de}	9.33+0.18 ^h	0.865+0.013 ^{fg}	0.089+0.001 ^{cd}	630.3+2.5 ^c
	7F1	4.86+0.04 ^{def}	9.67+0.37 ^{gh}	0.885+0.003 ^e	0.089+0.001 ^{cd}	602.2+4.7 ^{ef}
	14B5	4.72+0.23 ^{efgh}	10.17+0.22 ^{defg}	0.912+0.003 ^d	0.090+0.001 ^c	614.1+6.7 ^{de}
	14B1	4.77+0.03 ^{efg}	10.42+0.22 ^d	0.943+0.005 ^b	0.096+0.002 ^b	609.0+6.9 ^e
	14F5	5.09+0.13 ^{cd}	11.14+0.32 ^c	0.954+0.006 ^b	0.096+0.001 ^b	610.1+9.0 ^{de}
	14F1	5.24+0.13 ^c	12.02+0.39 ^b	0.940+0.006 ^{bc}	0.096+0.001 ^b	624.1+10.9 ^{cd}
	21B5	4.89+0.05 ^{de}	8.87+0.07 ⁱ	0.846+0.008 ^{hij}	0.085+0.001 ^{fg}	602.2+8.8 ^{ef}
	21B1	4.83+0.07 ^{defg}	9.98+0.37 ^{defg}	0.843+0.012 ^{ij}	0.083+0.000 ^g	588.4+11.1 ^{fg}
	21F5	6.02+0.14 ^b	12.4+0.27 ^b	0.924+0.009 ^{cd}	0.096+0.001 ^b	688.3+10.5 ^a
	21F1	6.29+0.08 ^a	12.9+0.09 ^a	1.001+0.009 ^a	0.107+0.003 ^a	646.3+2.8 ^b
	28B5	4.93+0.20 ^{de}	10.26+0.14 ^{def}	0.853+0.007 ^{ghi}	0.086+0.001 ^{efg}	598.6+8.6 ^{ef}
	28B1	5.00+0.19 ^{cde}	9.77+0.23 ^{fgh}	0.863+0.007 ^{fgh}	0.086+0.002 ^{efg}	601.0+4.3 ^{ef}
	28F5	4.9+0.03 ^{de}	10.32+0.04 ^{de}	0.884+0.011 ^e	0.087+0.001 ^{def}	584.4+6.4 ^g
	28F1	4.81+0.12 ^{de}	9.97+0.42 ^{efg}	0.831+0.002 ^j	0.085+0.001 ^{fg}	550.6+66.7 ^{ef}
	Control	3.78+1.03 ⁱ	7.64+1.81 ^j	0.633+0.171 ^l	0.075+0.009 ^h	508.2+93.8 ^h
	HP	4.17+0.72 ^h	7.79+1.38 ⁱ	0.752+0.196 ^{ef}	0.084+0.010 ^{cde}	538.7+42.2 ^{ef}
		P val.	0.0000	0.0000	0.0000	0.0000

Data are represented as means (n = 3); Differences between treatments were analyzed using the Tukey's HSD test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different. HP – hydropriming; Con. – Control; P val. – P values.

Additionally, the correlation analysis confirmed that all evaluated parameters were interrelated and that biopriming with fungal extracts of different origins enhanced the development of garden pea seedlings (Table 4a). Within the ITA fungal strain, a consistent positive relationship was observed between seed germination and most growth-related traits, while

abnormal seedlings showed a negative association with shoot and root length, shoot and root elongation rates, fresh and dry biomass accumulation, and seedling vigor index. These findings suggest that, in untreated seeds, the presence of abnormal seedlings may reflect underlying physiological stress, whereas biopriming treatments effectively reduced their occurrence by

promoting seed repair and enhancing overall seedling performance.

The data further revealed that shoot length was positively correlated with root length, shoot and root elongation rates, biomass accumulation parameters, and seedling vigor index, indicating a coordinated growth response across above- and below-ground organs. Root length also showed strong associations with shoot and root elongation rates, fresh and dry seedling weight, and seedling vigor index, reinforcing its role as a central determinant of early seedling development. Similarly, shoot and root elongation rates were closely linked to biomass accumulation and seedling vigor index, while fresh and dry seedling weights were positively correlated with each other and with seedling vigor index. These interdependencies reflect a tightly integrated physiological response to biopriming, where improvements in one trait are mirrored across multiple developmental dimensions.

In SRB fungal strains, a positive interrelationship was observed between seed germination and shoot

elongation rate, fresh and dry biomass accumulation, and seedling vigor index (Table 4b). Abnormal seedlings, on the other hand, showed a negative correlation with shoot and root length, shoot and root elongation rates, biomass accumulation parameters, and seedling vigor index, indicating their inverse association with overall seedling performance. Growth-related traits, including shoot and root length as well as shoot and root elongation rates, were strongly associated with fresh and dry biomass accumulation and seedling vigor index, while fresh seedling weight was closely correlated with both dry seedling weight and seedling vigor index, reflecting a tightly integrated developmental response to biopriming. Taken together, these results demonstrate that biopriming with fungal extracts of different origins not only improves individual growth parameters in garden pea seedlings, but also reinforces their physiological coherence, highlighting the systemic nature of the developmental response induced by fungal-based treatments.

Table 4.

Pearson correlations between garden pea parameters. ITA fungal strain (a); SRB fungal strain (b)

ITA fungal strain (a)									
	SG	AS	SL	RL	SER	RER	FSW	DSW	SVI
SG	1.00	-0.15 ^{ns}	0.52 ^{***}	0.53 ^{***}	0.48 ^{***}	0.52 ^{***}	0.55 ^{***}	0.53 ^{***}	0.64
AS		1.00	-0.35 ^{**}	-0.34 [*]	-0.35 ^{**}	-0.34 [*]	-0.36 ^{**}	-0.34 [*]	-0.35 ^{**}
SL			1.00	0.79 ^{***}	0.93 ^{***}	0.83 ^{***}	0.82 ^{***}	0.83 ^{***}	0.92 ^{***}
RL				1.00	0.72 ^{***}	0.81 ^{***}	0.86 ^{***}	0.72 ^{***}	0.96 ^{***}
SER					1.00	0.90 ^{***}	0.78 ^{***}	0.84 ^{***}	0.85 ^{***}
RER						1.00	0.86 ^{***}	0.86 ^{***}	0.87 ^{***}
FSW							1.00	0.90 ^{***}	0.89 ^{***}
DSW								1.00	0.81 ^{***}
SVI									1.00
SRB fungal strain (b)									
	SG	AS	SL	RL	SER	RER	FSW	DSW	SVI
SG	1.00	-0.07 ^{ns}	0.27 ^{ns}	0.23 ^{ns}	0.41 ^{**}	0.19 ^{ns}	0.28 [*]	0.27 [*]	0.38 ^{**}
AS		1.00	-0.39 ^{**}	-0.56 ^{***}	-0.54 ^{***}	-0.50 ^{***}	-0.42 ^{**}	-0.39 ^{**}	-0.52 ^{***}
SL			1.00	0.78 ^{***}	0.83 ^{***}	0.73 ^{***}	0.83 ^{***}	0.80 ^{***}	0.88 ^{***}
RL				1.00	0.73 ^{***}	0.75 ^{***}	0.81 ^{***}	0.64 ^{***}	0.97 ^{***}
SER					1.00	0.89 ^{***}	0.80 ^{***}	0.83 ^{***}	0.82 ^{***}
RER						1.00	0.82 ^{**}	0.86 ^{***}	0.78 ^{***}
FSW							1.00	0.89 ^{***}	0.85 ^{***}
DSW								1.00	0.73 ^{***}
SVI									1.00

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns—not significant. SG – seed germination; AS – abnormal seedlings; SL – shoot length; RL – root length; SER – shoot elongation rate; RER – root elongation rate; FSW – fresh seedling weight; DSW – dry seedling weight; SVI – seedling vigor index.

3.2. Mycochemical characterization of phenolics

As published in our previous study (Mišković et al., 2021), in the EtOH extracts, four compounds were quantified using LC-MS/MS analysis (Table S1), including three hydroxybenzoic acids (*p*-hydroxybenzoic, protocatechuic, and gallic acid) and

quinic (a cyclohexane carboxylic acid). Among these, gallic acid was present at levels below the limit of quantification (LoQ), with the exception of SRB F14 (75.77 $\mu\text{g g}^{-1}$ d.w.). Overall, EtOH extracts contained most of the analyzed phenolic acids compared to polysaccharide-rich extracts, with F samples showing a higher total phenolic content (167.55 $\mu\text{g g}^{-1}$) than B samples. The most abundant compound was gallic acid, particularly in the SRB F extract (75.77 $\mu\text{g g}^{-1}$), while *p*-hydroxybenzoic acid was consistently present in all

EtOH extracts, with higher levels detected in the ITA strain (64.92 $\mu\text{g g}^{-1}$ in total). Strain-specific variability was evident, as the SRB strain reached secondary metabolism earlier (14 days), producing high concentrations of gallic acid, which corresponded with strong DPPH and FRAP activities, whereas the ITA strain expressed critical metabolic points later (21 and 28 days) with different phenolics detected.

3.3. PCA analysis

Principal component analysis (PCA) was applied to the LC–MS/MS profiles of EtOH extracts obtained from *S. commune* cultures of SRB and ITA strains, which were published in our earlier study (Mišković et al. 2021). The first two principal components explained 95.23% of the total variance (PC1 = 83.55%, PC2 = 11.68%) (Figure 1).

The score plot demonstrated clear separation of extracts according to both strain origin and type of material extracted. SRB extracts were distributed predominantly on the negative axis of PC1, while ITA extracts were positioned on the positive side, reflecting strain-specific metabolite differences. Within each strain, F and B extracts also showed clear separation, indicating distinct chemical signatures, as previously confirmed by FTIR and LC–MS/MS analyses (Mišković et al., 2021).

Also, it is important to note that cultivation time contributed to further structuring of the data. Early-stage extracts (7 and 14 days) clustered more closely, whereas extracts obtained after longer incubation (21 and 28 days) shifted along both PC1 and PC2, suggesting dynamic changes in metabolite accumulation during submerged culture (Figure 1).

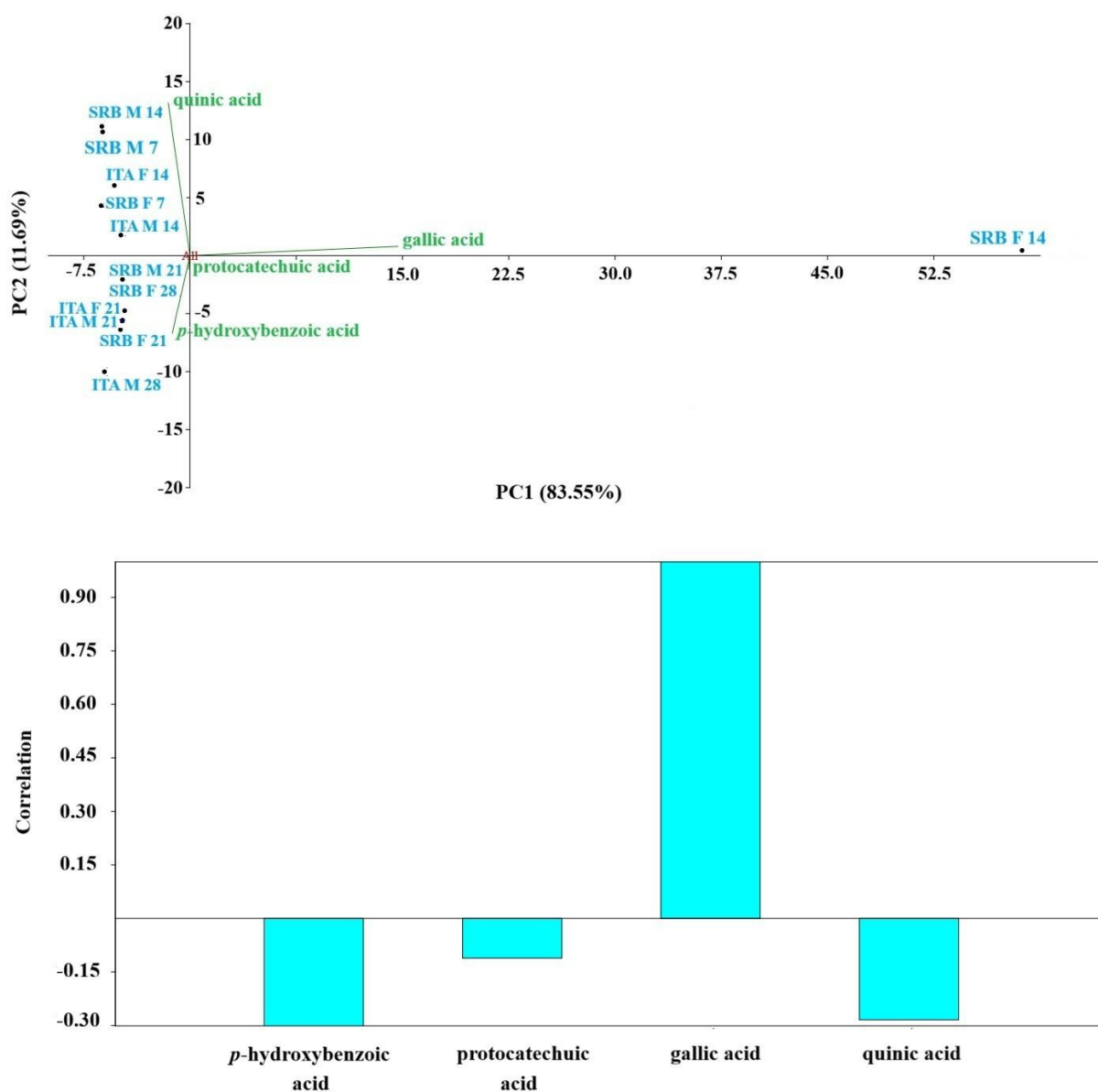


Figure 1. PCA analysis of LC–MS/MS profiles of *S. commune* SRB and ITA EtOH extracts. Distribution of variables in (above) the score plot and (below) the loading plot of the first two principal components. The following abbreviations are used for the examined parameters: B—biomass; F – filtrate; SRB – Serbian strain; ITA – Italian strain

4. Discussion

Yield results suggest that the log phase ended earlier in the ITA strain (by day 14), whereas the SRB strain maintained it longer, leading to later filtrate accumulation. The sustained growth of the ITA strain despite early entry into the stationary phase may be attributed to the utilization of schizophyllan as an alternative carbon source through β -glucanase activity (Reyes et al., 2009). The enhanced performance of extracts from intermediate to longer cultivation periods (14 to 28 days) is consistent with the progressive accumulation of secondary metabolites during the stationary phase of fungal growth (Calvo et al., 2002; Wadhwa et al., 2024). Conversely, the weaker or non-significant effects observed for certain parameters in the ITA strain (e.g., SG and AS) could indicate strain-specific differences in metabolic profiles or in the timing of metabolite synthesis and release. Overall, the results highlight that both the origin of the fungal strain and the choice of cultivation and extraction parameters are critical for maximizing the bioactivity of *S. commune* EtOH extracts. Although both strains produced extracts that significantly enhanced seedling performance compared to the control, the SRB strain exhibited more consistent and pronounced effects across all measured traits. The ITA strain showed selective improvements, with strong effects on vigor and biomass traits but limited influence on SG and AS. B and F extracts from intermediate to longer cultivation periods (14 to 28 days) tended to deliver the greatest enhancements, suggesting that both fungal strain and extraction timing are key determinants of biological performance. This aligns with our earlier findings, where the fungal strain and cultivation duration of the same species differentially influenced the antioxidant and anti-acetylcholinesterase activities of the examined extracts (Mišković et al., 2021).

Environmental factors including climate, altitude, and ecosystem diversity shape fungal survival, reproduction, metabolism, and stress responses, ultimately influencing both the yield and composition of bioactive compounds of different strains (Trung et al., 2024). Furthermore, local biodiversity and soil composition affect nutrient acquisition and activate specific strain metabolic pathways of fungi, resulting in distinctive bioactive compound profiles (Zeb et al., 2024). This, in combination with different genetic factors, may explain the observed differences in extract efficacy between strains (Mišković et al., 2021), since the stronger and more consistent effect of the SRB strain across all traits suggests a higher or more diverse production of growth-promoting compounds under the tested culture conditions. These findings align closely with the results obtained using polysaccharide fractions of *S. commune* cultivated for 14 days under submerged conditions, where the SRB strain again demonstrated the most pronounced stimulatory effects under stress conditions (Mišković et al., 2024). Specifically, SRB-derived polysaccharides led to substantial increases in SL under drought (up to +45% compared to the control), while also enhancing SVI values under optimal conditions by nearly 25%. Conversely, polysaccharides from the ITA strain exerted its strongest influence on SG, but its effects on subsequent growth traits were less pronounced than those of SRB-derived treatments (Mišković et al., 2024).

The production of biologically active compounds can be influenced by various factors, such as pH, temperature, aeration, and others (Elisashvili, 2012). In the present study, both extract type and cultivation period contributed to variations in the production and release of bioactive metabolites by *S. commune*. Fungal metabolites are small molecules classified either as primary, which are essential for growth and metabolism, or as secondary, which are non-essential byproducts often involved in defense. Secondary metabolites, in particular, enhance stress tolerance and ecological adaptability, as demonstrated in several studies (Conrado et al., 2022; Rodríguez-Berríos et al., 2023; Sharma et al., 2023). For example, B EtOH extracts may contain a higher proportion of intracellular compounds, such as polysaccharides, phenolics, and low-molecular-weight secondary metabolites (Shih et al., 2007; Berikashvili et al., 2023), which can directly stimulate seed germination and early growth. The findings of Elisashvili (2012) indicate that mycelia tend to accumulate intracellular polysaccharides at lower temperatures. In contrast, extracellular polysaccharide production increased with increasing culture temperature, reaching its maximum at 25 °C (Elisashvili, 2012). Since the temperature of submerged cultivation in this study was constant at 26 °C, it is suggested that biomass extract mostly contained phenolics and other secondary metabolites. This is also in accordance with our previous study, where we examined the antioxidant and anti-AChE activity of these EtOH extracts, and determined total phenolic content (TPC) together with LC/MS–MS profile (Mišković et al., 2021). Findings revealed that the EtOH biomass extracts after 14 days of incubation in both strains exhibited TPC values (ITA: 76.65 \pm 1.30 mg GAE/g d.w.; SRB: 82.62 \pm 0.99 mg GAE/g d.w.) comparable to those of the filtrate extracts (ITA: 75.23 \pm 1.04 mg GAE/g d.w.; SRB: 81.93 \pm 0.81 mg GAE/g d.w.), with no significant differences detected. Phenolic compound levels in *S. commune* biomass extracts, isolated from Georgia, were high as well, but liquid culture contained higher phenolic levels (Berikashvili et al., 2023). Filtrate extracts probably primarily capture extracellular metabolites secreted into the culture medium (Pinu & Villas-Boas, 2017), some of which may be more diffusible and readily absorbed by seeds such as exopolysaccharides and phenolics. Furthermore, the mushroom's extracellular enzymes can degrade lignocellulose in the substrate, releasing free phenolics and potentially generating novel and higher levels of secondary bioactive compounds, which may enhance the biological activity of the extracts as well (Berikashvili et al., 2023). It is also plausible that biochemical interactions lead to the formation of complex compounds, such as polyphenol-polysaccharide conjugates, consistent with our previous findings (Mišković et al., 2021).

The loading plot presented in Figure 1 indicated that gallic and quinic acids contributed most strongly to the positioning of filtrate samples, whereas protocatechuic and *p*-hydroxybenzoic acids were associated with biomass extracts. Given that these phenolic acids have reported growth-promoting and antioxidant effects (Vafiadis & Bakalbassis, 2003; Khan et al., 2015; Makaure et al., 2023; Da Silva et al., 2025), their higher abundance in certain SRB extracts could explain the stronger and more consistent biological performance observed in this study.

SRB strains SRB 14F and SRB 21B/F occupy similar chemical space, consistent with their comparable high bioactivity in our germination and vigor assays. This observation may be related to the fact that fungal secondary metabolite production is typically most pronounced after two to three weeks of fungal cultivation (Wadhwa et al., 2024). This is because secondary metabolites are generally produced during the stationary phase, after the primary rapid growth phase (exponential phase) has declined (Demain, 2014; Wadhwa et al., 2024). The production is linked to environmental stimuli, nutrient depletion, and stress conditions that trigger specific biosynthetic gene clusters responsible for these metabolites (Gaude & Jalmi, 2025). This timing aligns with the transition from primary growth to secondary metabolism, where fungi shift their metabolic focus from biomass accumulation to producing bioactive compounds, such as antibiotics, mycotoxins, pigments, and phenolics, among other secondary metabolites (Goyal et al., 2017).

When comparing biomass and filtrate extracts in the PCA space, some overlap is observed; however, the strongest PC1 loadings appear to be driven more by strain and cultivation time than by extract type (Figure 1). This supports the notion that both biomass and filtrate can exhibit high bioactivity when harvested during mid-late culture, although SRB consistently provides superior mycochemical profiles and biological performance.

Conclusion

This study demonstrates that the biostimulant potential of *S. commune* ethanol extracts is both strain- and cultivation-dependent. EtOH extracts from the SRB and ITA strains significantly enhanced pea seedling performance, but their effects varied with extract type and cultivation period. The SRB strain consistently produced superior results, with biomass- and filtrate-derived extracts harvested after 14 and 21 days markedly improving seedling length, root development, vigor indices, and biomass accumulation. In contrast, the ITA strain showed a delayed yet notable stimulatory effect, with extracts from 21 and 28 days exerting the strongest influence on growth and physiological traits.

These findings reveal a clear functional divergence between strains: SRB extracts are more effective in promoting seedling growth and vigor, particularly under stressful conditions, while ITA extracts favor seed germination but require extended cultivation for optimal activity. Moreover, both sets of results emphasize that extract origin and preparation method play a pivotal role in determining bioactivity, with mid-cultivation ethanol filtrates and SRB-derived polysaccharides emerging as the most promising treatments for improving seedling establishment and stress tolerance. Overall, this work highlights *S. commune* as a valuable, sustainable source of natural biostimulants and provides new insights into how strain variability and cultivation timing can be harnessed to optimize fungal extracts for agricultural applications.

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Declaration of competing interests

The authors have declare that no competing interests exist.

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