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Upcycling Residues from *Salvia rosmarinus* Distillation and Agroforestry Processes into a Dual-Function Bioagrochemical with Biostimulant and Antifungal Properties

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Abstract

There is a growing demand for sustainable crop strategies that reduce the use of agrochemicals while improving productivity. This study investigates distillation residues of *Salvia rosmarinus* to develop a novel biostimulant with enhanced antifungal activity, together with natural additives that ensure homogeneity and water dispersibility. Accordingly, several residual by-products released from agroforestry processes and regarded as "plant protection products" were evaluated in phytotoxicity tests in seeds of *Lactuca sativa* and *Lolium perenne*. The tested substances included solvents (ethanol, glycerol, propylene glycol, and DMSO) and adjuvants (soy lecithin, polysorbate-20, acetic acid, and ascorbic acid). Those showing the lowest adverse effects were combined with the extract following a 3^2 factorial design. Most formulations exhibited good water dispersibility and significantly enhanced the germination index of *Lactuca sativa*, while simultaneously reducing the growth of *Lolium perenne* and the fungus *Aspergillus flavus*, all in a clear dose–response manner, as suggested by the four-parameter log-logistic ($\log_{10}(x)$) models. These results indicate that both stimulatory and inhibitory effects were strongly influenced by concentrations, highlighting the importance of optimizing application doses. Among the evaluated carriers, the one lacking glycerol and containing high ratios of polysorbate-20 and soy lecithin demonstrated the most balanced overall performance in terms of physical stability, dispersibility, and biological activity, a response that can be attributed to the combined contribution of the extract and the selected carrier components. Overall, this study demonstrates that bioagrochemicals derived from agroforestry by-products can provide dual-function agricultural applications (biostimulant/antifungal or herbicide/antifungal), while supporting the framework of the circular bioeconomy.

Keywords: circular economy; bioagrochemicals; waste valorization; crop protection; aromatic plant distillates

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1. Introduction

Current challenges in crop protection, public health, and environmental sustainability, together with the growing resistance of pests and pathogens, have prompted the search for new ecologically active substances. A major goal in global agriculture is to enhance

crop productivity while reducing the consumption of land, agrochemicals or water. This objective can be achieved through bioengineering approaches or by developing plant biostimulants, as outlined by Calvo et al. (2014) [1]. These innovative products include microbial inoculants that increase nutrient fixation, improve plant tolerance to biotic and abiotic stresses, and promote growth through the addition of organic compounds, such as humic or fulvic acids, proteins and amino acids, polysaccharides and a wide variety of plant-derived metabolites [2], including phenolic compounds, as reviewed by Kisiriko et al. (2021) [3]. Within this context, growing interest has been devoted to valorizing agroforestry and urban residues as sustainable sources of bioactive compounds with plant stimulant potential, aligning with the principles of the circular bioeconomy [4–7]. In particular, the residual biomass of aromatic plants after essential oil (EO) distillation has demonstrated biofertilizer functions for alkaline soils and growth-promoting potential in the rooting of model seeds such as *Lactuca sativa*, *Lolium perenne*, and *Solanum lycopersicum* [8–12].

Ensuring the safe and effective use of these materials as carriers, it is necessary to evaluate them according to multiple agricultural application criteria, including: (1) low environmental toxicity, (2) high biodegradability, (3) safety for human use, and (4) regulatory compliance. Additionally, solvents should be considered for (5) potential phytotoxicity, (6) formulation homogeneity, and (7) water solubility. Effective formulations also require adjuvants (or additives) that enhance (8) dispersion, penetration and wettability, as well as (9) preservatives that prolong stability and efficacy, and (10) ingredient compatibility. In addition, there are other technical and economic considerations, including cost-effectiveness and large-scale suitability to ensure practical and sustainable implementation.

Different components reported in Table 1, including solvents and adjuvants derived from agroforestry residues, are regarded by EU regulations (ECHA) for their safety and suitability for plant protection uses.

Ethanol stands out as a safe solvent capable of dissolving a wide range of organic compounds. It is typically produced from sugarcane or other energy crops through sugar and lignocellulosic biomass fermentation followed by distillation. Glycerol, a highly polar and hygroscopic solvent commonly generated as a by-product of biodiesel production, enhances humectancy and viscosity in formulations. Likewise, propylene glycol (PG), which also functions as a solvent, dispersant and humectant, can be sustainably produced from glycerol via hydrogenation [13] or by fermentative processes using lactic acid bacteria [14]. Both glycerol and PG are employed as agricultural additives, improving foliar retention, viscosity, droplet adhesion and emulsion stability. They may also serve as co-solvents in liquid and concentrate formulations and as feed additives with known health benefits [15]. Dimethyl sulfoxide (DMSO) is obtained by oxidizing dimethyl sulfide (DMS), which is a by-product of the pulp and paper industry. While DMS is moderately toxic, DMSO is a highly polar, non-toxic solvent with outstanding penetration capacity. Owing to these properties, DMSO has been proposed as an additive in agrochemical formulations to enhance the cuticular cell penetration of active ingredients [16].

Soy lecithin and polysorbate 20 are widely used in the food and beverage industry because of their excellent dispersibility in both water-in-oil (W/O) and oil-in-water (O/W) emulsions. Soy lecithin is a natural emulsifier that acts as a wetting agent, dispersant, and surfactant, contributing to emulsion stability. Industrially, soy lecithin is obtained as a by-product of soybean processing, either through extraction from defatted flour or enzymatic recovery from crude oil. Polysorbate 20 (Tween-20) has been described as a low-toxicity surfactant suitable for foliar applications in agriculture [17] and can be produced sustainably by reacting ethylene oxide (derived from ethanol) with sorbitan esters obtained from residual corn and fatty acids of oils (such as palm, coconut or rapeseed). Finally, acetic acid and L-ascorbic acid provide strong preservative and acidifying effects and are

legally approved for use in organic food and farming [18]. Acetic acid is a key component of wood vinegar generated during the fermentation of sugar-rich materials from forestry or agricultural waste, whereas L-ascorbic acid, abundant in orange pulp [19], can be recovered from juice industry by-products and other residues of vitamin C-rich fruits and vegetables, such as red pepper.

Table 1. List of pre-selected ingredients commonly used in agriculture and identified as promising candidates for the formulations.

Ingredient/ EC No. [1]/ LD_{50} [2]	**Agrochemical Applications**	**Recovery of Compounds of Biological Origin from Agroforestry By-Products**
Ethanol 200-578-6 10.5	- Solubilizes hydrophobic organic actives. - Enhances leaf surface wetting, penetration, and spreadability.	Fermentation of released sugars and lignocellulosic biomass (second-generation ethanol).
Glycerol 200-289-5 27.2	- Increases foliar retention and reduces evaporation. - Improves formulation viscosity and droplet adhesion. - Serves as a co-solvent in liquid formulations.	Hydrolysis or transesterification of vegetable oils and animal fats during biodiesel production.
Propylene Glycol (PG) 200-338-0 22.0	- Improves emulsion stability and dispersion of actives. - Used as co-solvent in emulsifiable concentrates and suspension concentrates.	Catalytic hydrogenation of glycerol derived from biodiesel production [13]. Fermentation using lactic acid bacteria [14].
Dimethyl Sulfoxide (DMSO) 200-664-3 28.3	- Enhances cuticular penetration of active ingredients. - Serves as solvent and carrier for agrochemicals. - Provides preservative activity against microbial growth.	Controlled oxidation of secondary stream dimethyl sulfide (DMS) resulting from a waste product of the pulp and paper industry.
Soy lecithin 232-307-2 n.d.	- Improves emulsion stability and wetting. - Reduces phytotoxicity of active ingredients. - Acts as a natural adjuvant and Inhibiting agent.	Extraction from defatted soybean or enzymatic recovery from crude soybean oil.
Polysorbate-20 500-018-3 38.9	- Enhances wetting and dispersion of hydrophobic actives. - Promotes uniform leaf coverage and formulation stability.	Ethoxylation and esterification of lauric acid derived from plant oils (e.g., coconut or palm).
Acetic acid 200-580-7 3.3	- Regulates pH in formulations to improve stability. - Used as a natural inhibitor and preservative. - Inhibits microbial growth.	Fermentation of sugars derived from biomass (acetic fermentation), resulting in wood vinegar.
Ascorbic acid 200-066-2 11.9	- Protects active ingredients from oxidation and degradation. - Enhances product shelf life and stability.	Solvent extraction from citrus peel residues.

Note. [1] European Community (EC) number assigned by the European Chemicals Agency (ECHA) in relation to its potential use as a "plant protection product"; [2] Lethal doses (g/kg) causing 50% of deaths in rats according to the Regulation (EC) No. 1907/2006 (REACH).

This study aims to develop a novel bioagrochemical based on ethanolic extracts of distilled residues from a previously selected domesticated population of *Salvia rosmarinus* Spenn. syn. *Rosmarinus officinalis* L., commonly known as rosemary, within the framework

of circular bioeconomy. This plant was selected for its previously described biostimulant and antifungal properties [12]. To achieve this, different ingredients must be selected to develop an extraction vehicle that enhances the effects of the extracts and improves their water dispersibility. Consequently, the research is structured in three main phases: (1) evaluation and selection of ingredients based on minimal toxicity to the model seeds, (2) development of the extraction vehicle and formulation of the biostimulant product, and (3) in vitro evaluation of the resulting formulations as potential biostimulant and antifungal agents.

2. Materials and Methods

2.1. Ingredients of Residual Agroforestry Origin

Ethanol, glycerol, polysorbate-20 (Tween-20), and L-ascorbic acid were obtained from Sigma-Aldrich (Merck KGaA, Burlington, MA, USA), while propylene glycol (PG), glacial acetic acid, and dimethyl sulfoxide (DMSO) were supplied by Panreac (AppliChem ITW Reagents, Barcelona, Spain). Organic soy lecithin was obtained from El Granero (Biogran S.L., Madrid, Spain). All reagents were of analytical, food or pharmaceutical grade. In general, the solvents and co-solvents ethanol, glycerol, PG and DMSO were selected for the development of the solvent system (S), while the adjuvants soy lecithin and polysorbate-20 were incorporated as dispersants/emulsifiers, and acetic acid and ascorbic acid as preservatives for the formation of the aqueous matrix (A). The ingredients were considered to allow systematic evaluation of their individual and synergistic effects and to ensure formulation stability and optimal biological performance.

2.2. Ethanolic Extraction of Solid Distilled By-Products of S. rosmarinus (SrEE)

In a previous study, Ortiz de Elguea-Culebras et al. (2024) [12] characterized the ethanolic extracts of distilled biomass from different domesticated populations of *Salvia rosmarinus*. As a result, a population, originally sourced from Pina de Ebro (Zaragoza, Spain), was selected for exhibiting strong biostimulant effects, antifungal activities and high content of carnosic acid. As reported, the vegetal material was collected in 2020 and subjected to hydrodistillation. The resulting biomass was subsequently used for extract preparation and subjected to Soxhlet extraction for 16 h using 96% ethanol as extraction solvent. Following extraction, the ethanolic solution was filtered to remove residual impurities and concentrated under reduced pressure using an R-100 rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) at 50 °C and 175 mbar. The obtained crude extract was then oven-dried at 45 °C for 48 h to ensure complete solvent removal and subsequently finely ground to obtain a homogeneous powder. The resulting ethanolic extract of *Salvia rosmarinus* (SrEE) was finally evaluated for its biological capabilities and its phenolic profile [12].

2.3. Evaluation of Selected Ingredients on Test Model Seeds

All ingredients were assessed for allelopathic effects to identify those with growth-promoting potential or reduced phytotoxic effects. The experimental assays were conducted using model test seeds of *Lactuca sativa* L. var. Carrascoy (dicotyledonous) and certified *Lolium perenne* L. var. (2n) (monocotyledonous), both acquired from Semillas Fitó (Barcelona, Spain). The solvents and co-solvents ethanol, PG and glycerol were evaluated at concentrations of 0 to 5% in water, while DMSO was analyzed at concentrations of 0 to 0.2%. The adjuvants soy lecithin and polysorbate-20 were evaluated at concentrations of 0 to 0.5%, while the preservatives acetic and ascorbic acids were evaluated at concentrations of 0 to 0.2%. The tests were performed in 12-well plates containing 2.5 cm Ø paper filters, each loaded with 10 seeds of *L. sativa* or 1-day-hydrated seeds of *L. perenne*. Subsequently,

700 µL of each ingredient at the different concentrations was added to the wells, with water serving as control. The plates were then sealed in zip-lock bags and incubated in a growth chamber at 22 °C under a 16:8 h (L:D) photoperiod. Germination was recorded every 24 h for a total of 7 (*L. sativa*) and 10 days (*L. perenne*). Finally, root and/or shoot lengths of 25 randomly selected seedlings were measured using ImageJ software (Version 1.37r, 2010; https://imagej.nih.gov).

2.4. Product Formulation

Those ingredients (solvents and adjuvants) with growth-promoting potential or reduced phytotoxic effects were selected as potential carriers for the incorporation of the ethanolic extract of *S. rosmarinus* (SrEE). This was first dissolved at a concentration of 10% (*w*/*w*) in three selected solvent systems (S) and heated at 60 °C for 2 h with constant stirring and homogenized at 10,000 rpm for 4 min using a PT3000 Polytron homogenizer (K.H. Brinkmann GmbH & Co. KG, Werdohl, Germany), following the procedure described by Ortiz de Elguea-Culebras et al. (2019) [20]. Subsequently, the slurries were mixed in a 1:4 ratio in three different aqueous matrices (A) containing the remaining adjuvants (including surfactants, emulsifiers, and/or preservatives). The mixtures were then reheated at 60 °C for 1 h and homogenized at 20,000 rpm for 5 min. Therefore, following a 3^2 factorial design, nine formulations were prepared with a final SrEE concentration of 2.5% *w*/*w*. Similarly, nine carriers were also obtained by replacing the plant extract with an equivalent amount of water. Additionally, as a positive control, SrEE was dissolved directly in 96% ethanol at 25 mg/mL, also serving this solvent as the carrier. Finally, the twenty samples were stored at 4 °C before characterization.

2.5. pH and Water Dispersibility Capacity of the Tested Formulations

The pH of the formulations was measured using a Micro 2001 pH meter (Crison Instruments, S.A., Barcelona, Spain) equipped with a 12 mm × 130 mm electrode (Hach Co., Loveland, CO, USA). To evaluate the dispersibility of each treatment in water, 96-well plates were filled in triplicate with the analyzed formulations at concentrations ranging from 1.25 to 10 µL/mL. The maximum absorbance wavelength of formulations was determined from the scanning spectral data in a BioTek Epoch 2 Microplate Spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA) and fixed in 670 nm. Optical density (OD) was monitored over a 10 h period at 10 min intervals and 25 °C to monitor decreases in absorbance associated with precipitation of the SrEE extract. OD values at 5 h (OD_{5h}) were determined from a linear regression of OD vs. Time, and solubility (%) was calculated as (OD_{5h}/OD_{0h}) × 100. Finally, a lineal regression model was performed to correlate the estimated solubility at 5 h to the tested concentrations, allowing estimation of the highest concentration that preserved solubility above 90% (S_{90}) only for formulations that show strong model fit (R^2 > 90%).

2.6. Biostimulant Evaluation of the Products

The samples were subjected to allelopathic bioassays to identify those exhibiting greater biostimulant activity and to reject the most phytotoxic ones using the model seed species *Lactuca sativa* and *Lolium perenne*. The experiments were conducted under the same conditions described in Section 2.2, with minor modifications. Prior to the assays, all formulations and their corresponding carriers were dissolved in distilled water at concentrations ranging from 12.50 to 100 µL/mL (*w*/*v*) and 700 µL of each solution was carefully dispensed into the wells. Each treatment was performed in quadruplicate to account for experimental variability and repeated twice. Subsequently, the plates were incubated under controlled temperature and light conditions, as described in Section 2.2. Seed germination and early seedling growth were similarly monitored throughout the

experimental period (7 days for *L. sativa* and 10 days for *L. perenne*), allowing a quantitative comparison of the biostimulant effects of the different formulations.

The germination index (GI) was calculated as an integrated parameter combining final germination (relative germination, RG) and seedling growth. For *Lactuca sativa*, seedling growth was expressed as relative root growth (RRG), whereas for *Lolium perenne* both relative root (RRG) and shoot (RSG) growth were considered. GI values > 100% indicate positive growth responses with increasing biostimulatory capabilities, while values ranging from 100% to 0% reflect progressively increasing phytotoxic effects.

$$\mathrm{RG}\ (\%) = \left(\frac{\text{number of seeds germinated in samples}}{\text{number of seeds germinated in control } (H_2O)} - 1 \right) \times 100$$

$$\mathrm{RRG}\ (\%) = \left(\frac{\text{root length in samples}}{\text{root lenght in control } (H_2O)} - 1 \right) \times 100$$

$$\mathrm{RSG}\ (\%) = \left(\frac{\text{shoot length in treatments}}{\text{shoot lenght in control } (H_2O)} - 1 \right) \times 100$$

$$\mathrm{GI}\ (\%) = \frac{\mathrm{RG} \times \mathrm{RRG} \times \mathrm{RSG}}{100}$$

2.7. Antifungal Capacities of the Products

The assessment of the fungicidal activity was targeted on *Aspergillus flavus*, a fungus commonly reported as a pathogen in crops such as corn, peanuts or cotton. All samples were firstly dissolved in distilled water at concentrations ranging from 12.5 to 100 µL/mL, and 475 µL was added in triplicate (and repeated twice) to sterile 55 mm Ø Petri plates, previously filled with 10–15 mL of potato dextrose agar (PDA). After solvent evaporation (>1 h), a 0.6 cm Ø well was made at the center of the plate and added with 10 µL of the inoculum at a concentration of around 1×10^4 CFU/mL (Colony Forming Units). This was previously obtained through a 1:100 dilution of an initial concentration of 1×10^6 CFU/mL, determined by regression analysis correlating the optical density of the culture medium (OD, λ = 530 nm) with the logarithmic colony count after 48 h of incubation ($y = 1.335x + 5.922$, $R^2 = 0.943$). Subsequently, the plates were kept in darkness and maintained at a controlled temperature of 26 ± 2 °C. Systematically scanning was carried out every 24 h up to 7 days to determine the growth area of fungi (mm^2) using Image J Version 1.37r, 2010 software. Growth halos at 120 h were estimated using a linear regression test so that inhibition capacities (%) were calculated as $[(C_{120} - T_{120})/C_{120} \times 100]$, where C and T represent the growth areas of the control (untreated plate) and treated plates.

2.8. Statistical Analysis of Dose–Response Models

Spearman's correlation analysis ($p < 0.05$) was performed to determine positive (growth-promoting) or negative (inhibiting) relationships between treatments and target organisms. Subsequently, dose–response curves were generated for each treatment through four-parameter log-logistic (4PL) models based on log(x)-transformed concentrations. Only models that showed adequate fit, defined by coefficients of determination (R^2) greater than 0.90, were considered for further analysis. In addition, this led to establishing three levels of confidence similarly based on the R^2 values: *** for $R^2 > 0.99$, ** for $R^2 > 0.95$, and * for $R^2 > 0.90$. Finally, only samples meeting this criterion were considered for the calculation of reference values, whether growth-promoting or inhibitory. These include GI_{+25} (the concentration required to stimulate or inhibit 25% of the germination index) in the model seeds, and EC_{50} (the concentration that inhibits 50% of fungal growth) in Aspergillus flavus. All analyses were conducted using R software (version 4.5.2).

3. Results

3.1. Selection of Ingredients and Product Formulation

The analysis revealed clear marked differences among the evaluated ingredients in their effects on the growth of *Lactuca sativa* and *Lolium perenne* seedlings (Figure 1).

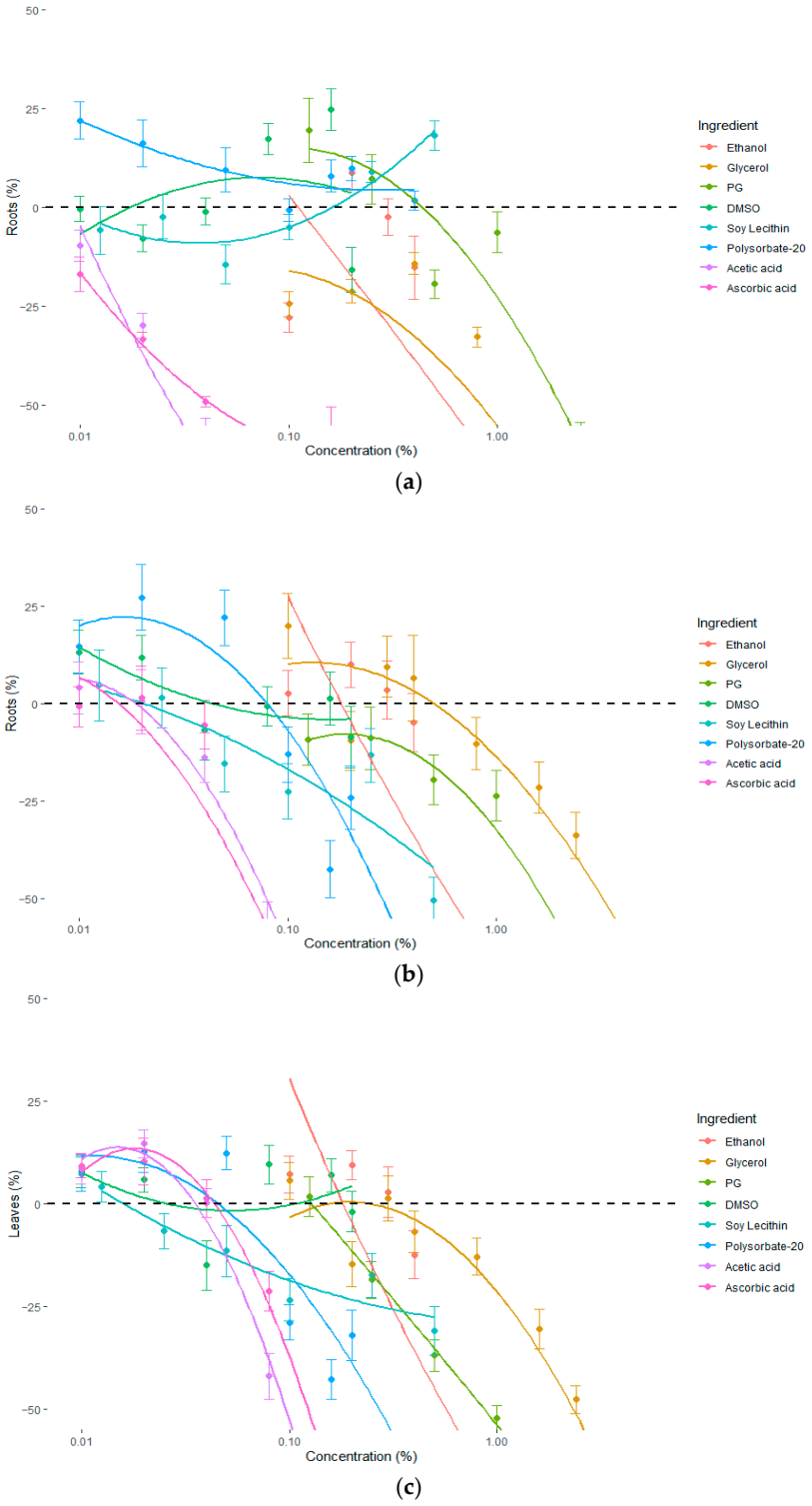

Figure 1. Effects of the selected ingredients on model seeds. The results (mean ± SE) represent seedling elongation (%) after 7 days for *Lactuca sativa* roots (**a**) and 10 days for *Lolium perenne* roots (**b**) and leaves (**c**). Dashed lines indicate the boundary between stimulatory and phytotoxic effects.

Most compounds exhibited dose–response-type patterns, encompassing both stimulatory and inhibitory effects (depending on the concentration). In particular, DMSO, soy lecithin, and polysorbate-20 showed favorable performance, maintaining seedling length above control values across most of the evaluated concentration range, indicating some stimulation under experimental conditions. In this line, *L. sativa* seedlings exhibited enhanced growth in response to soy lecithin and DMSO at concentrations above 0.1%, whereas *L. perenne* demonstrated growth stimulation at lower concentrations of polysorbate-20 and DMSO, but a pronounced growth sensitivity to higher levels of DMSO. The solvents glycerol and PG exerted moderately adverse effects on seeds development, with the exception of *L. sativa*, whose germination was completely inhibited at concentrations above 1.5% glycerol. By contrast, ethanol and acetic acid markedly inhibited root and leaf elongation, with phytotoxic effects intensifying at increasing concentrations and resulting in complete growth inhibition at ratios below 1% and 0.1%, respectively. Furthermore, although ascorbic acid showed less inhibitory effects than acetic acid at low concentrations, the roots of *L. perenne* were especially sensitive to concentrations above 0.05%, where root growth was substantially suppressed.

Based on these findings, and prioritizing compounds that exhibited low phytotoxic (or even stimulating) responses in the evaluated concentration ranges, a 3^2 factorial matrix design (3 solvent systems × 3 aqueous matrices) was established (Table 2).

Table 2. Final composition of the different formulations/treatments.

Ingredient (%)		Samples									
		Control + [1]	**S1 × Aa [2]**	**S1 × Ab [2]**	**S1 × Ac [2]**	**S2 × Aa [2]**	**S2 × Ab [2]**	**S2 × Ac [2]**	**S3 × Aa [2]**	**S3 × Ab [2]**	**S3 × Ac [2]**
Active Substance	*S. rosmarinus* EE	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Solvent System (S)	Ethanol	97.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Glycerol	0.00	0.00	0.00	0.00	4.50	4.50	4.50	9.00	9.00	9.00
	Propylene glycol	0.00	20.25	20.25	20.25	15.75	15.75	15.75	11.25	11.25	11.25
	DMSO	0.00	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Aqueous Matrix (A)	Soy lecithin	0.00	1.88	3.75	5.63	1.88	3.75	5.63	1.88	3.75	5.63
	Polysorbate-20	0.00	1.88	3.75	5.63	1.88	3.75	5.63	1.88	3.75	5.63
	Acetic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Ascorbic acid	0.00	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
	H_2O	0.00	71.11	67.37	63.61	71.11	67.37	63.61	71.11	67.37	63.61

Note. [1] Positive control (SrEE at 25 mg/mL in EtOH); [2] The carriers were prepared by replacing SrEE with H_2O (2.5% *w*/*w*).

Among the candidate ingredients to be considered within the formulations, ethanol and acetic acid were excluded due to the pronounced phytotoxic effects observed in the bioassays. However, ethanol was specifically employed to ensure the complete solubilization of SrEE, so it was considered as a positive control. Consequently, the solvent system was limited to glycerol (0–9.0%), PG (11.25–20.25%) and DMSO (2.25%), which allowed for modulating the dissolution and improving biological compatibility. Regarding the aqueous matrices, soy lecithin and polysorbate-20 were selected and incorporated into the final formulations at varying concentrations from 1.88 to 3.75% to assess their contribution to formulation stability and overall biological performance, while ascorbic acid was included at a fixed concentration of 0.13% in all formulations, acting as an antioxidant.

3.2. Physical Properties

As shown in Table 3, the pH of the formulations had a mean value close to 4.2, with certain differences between them, while the positive control had a more basic pH of approximately 6.1.

Table 3. pH and solubility (±SD) of formulations/treatments at different concentrations (5 h after preparation).

Sample	pH	Solubility (5 h)						
		1.25 µL/mL	2.5 µL/mL	5 µL/mL	10 µL/mL	Lineal Model Equation [1]	R^2	S_{90} [2]
EtOH [3]	6.13 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	99.96 ± 0.0	Total solubility		
S1 × Aa	3.95 ± 0.0	n.a.	n.a.	n.a.	n.a.	Poorly soluble		
S1 × Ab	4.19 ± 0.0	95.40 ± 1.4	95.66 ± 1.9	92.55 ± 1.0	89.77 ± 2.5	y = −0.698x + 96.613	0.953 **	9.48
S1 × Ac	4.40 ± 0.0	98.19 ± 0.1	96.34 ± 0.6	94.01 ± 0.0	92.08 ± 2.3	y = −0.663x + 98.277	0.917 *	12.48
S2 × Aa	3.89 ± 0.0	n.a.	n.a.	n.a.	n.a.	Poorly soluble		
S2 × Ab	4.14 ± 0.0	98.72 ± 0.1	95.97 ± 0.8	93.32 ± 1.5	89.27 ± 1.0	y = −1.021x + 99.107	0.964 **	8.92
S2 × Ac	4.39 ± 0.0	96.01 ± 1.5	96.11 ± 0.9	95.23 ± 0.9	91.34 ± 2.7	y = −0.563x + 97.312	0.930 *	13.00
S3 × Aa	4.00 ± 0.0	n.a.	n.a.	n.a.	n.a.	Poorly soluble		
S3 × Ab	4.28 ± 0.0	90.81 ± 3.1	92.43 ± 0.5	93.22 ± 1.8	87.78 ± 6.6	y = −0.420x + 93.035	0.456	n.d.
S3 × Ac	4.46 ± 0.0	94.03 ± 1.8	91.97 ± 0.5	90.80 ± 1.2	89.75 ± 2.7	y = −0.429x + 93.651	0.815	n.d

Note. [1] Lineal model regression for the solubility after 5 h of treatment preparation with the coefficient of determination; [2] S_{90} (%): Concentration estimated from lineal regression that permits a solubility > 90% after 5 h; [3] Positive control (SrEE at 25 mg/mL in EtOH or EtOH). **: R^2 > 95%. *: R^2 > 90%; n.a.: not assayed; n.d.: not determined (R^2 < 90%).

The presence of ascorbic acid, as expected, contributed to the increase in acidity in the samples. Among the formulations, S3 × Ac exhibited the highest pH (4.5), while S2 × Aa showed a value around 4.0, demonstrating also the role of the other additives in modulating the acidity of the final formulations. This effect is particularly pronounced regarding the aqueous matrices: samples with lower concentrations of soy lecithin and polysorbate-20 had pH values around 4.0, while those with higher concentrations reached pH values above 4.4.

Regarding solubility, the greatest aqueous dispersion was achieved, as expected, with formulations with higher proportions of dispersants, but in the absence of glycerol, as observed between samples containing S1 and S3 systems. Specifically, formulations S1 × Ab, S1 × Ac, S2 × Ab, S2 × Ac, and S3 × Ac maintained a solubility of around 90% at concentrations of 10 µL/mL and after 5 h of preparation. In addition, most formulations also exhibited a pronounced dose-dependent behavior, with two samples (S1 × Ab and S2 × Ab) having coefficients of determination (R^2) exceeding 95%. This strong trend allowed for a reliable estimation of the maximum concentration, maintaining a solubility of 90% after 5 h. Among them, S2 × Ac and S1 × Ac showed the most favorable performance, with an estimated S_{90} value exceeding 12 µL/mL, indicating an enhanced dispersibility at higher concentrations compared to other formulations. Therefore, S1 × Ac and S2 × Ac emerged as the most suitable formulations in terms of water dispersibility, followed by S1 × Ab and S2 × Ab which showed progressively lower but still a positive dispersibility performance.

3.3. Biostimulant Effects of Formulations

In *L. sativa*, the results showed notable differences between inhibitory and biostimulant responses among the evaluated samples (Table 4).

The strong phytotoxic effect of EtOH markedly inhibited both germination and root development, highlighting the high sensitivity of the seeds to this solvent. In particular, when SrEE was dissolved in ethanol (positive control) it relieved this toxicity, reducing the inhibition of germination and seedling growth, and thereby demonstrating its biostimulant potential. Overall, most formulations showed clear biostimulant activity, with an average stimulation of approximately +17%, driven mainly by increased root elongation rather than changes in germination, which remained stable after 48 h. Conversely, the carriers generally produced moderate inhibitory effects in *L. sativa* seedlings.

Table 4. Screening of treatments (T) and carriers (C) on the model seed species *Lactuca sativa* and *Lolium perenne*, as well as against the fungus *Aspergillus flavus*. Results are expressed as mean values (%) ± SE. Positive values (+) indicate growth-promoting effects and negative values (−) indicate inhibitory activity regarding negative control (H_2O).

Sample	Conc. (%) [1]	T/C	*Lactuca sativa*				*Lolium perenne*					*Aspergillus flavus*
			RG_{24H} (%)	RG_{48H} (%)	RRG (%)	GI (%)	RG_{72H} (%)	RG_{144H} (%)	RRG (%)	RSG (%)	GI (%)	$\%H_2O$ [2]
EtOH [3]	0.125/1.25	T	−3.22 ± 6.2	−0.26 ± 3.0	−13.81 ± 5.1	85.96	−11.26 ± 7.3	−10.81 ± 9.2	−4.04 ± 7.1	−17.61 ± 4.6	84.48	−41.74 ± 1.2
		C	−38.17 ± 5.1	+2.36 ± 2.6	−57.06 ± 7.8	43.95	−39.79 ± 10.8	−8.10 ± 5.4	−55.99 ± 7.4	−51.54 ± 5.0	48.66	n.a.
	0.25/2.50	T	−35.48 ± 4.3	−0.26 ± 5.2	−29.47 ± 10.4	70.34	−27.11 ± 14.9	−8.1 ± 10.3	−8.63 ± 10.2	−30.60 ± 6.1	80.38	−50.57 ± 1.7
		C	−35.48 ± 15.2	−0.26 ± 3.0	−43.82 ± 6.4	56.03	−14.43 ± 14.0	−5.40 ± 6.8	−30.02 ± 9.1	−32.72 ± 4.3	66.82	n.a.
	0.50/5.00	T	−91.93 ± 5.1	−2.88 ± 5.0	−58.92 ± 10.1	39.89	−20.77 ± 6.0	+5.40 ± 2.7	−3.87 ± 9.1	−26.54 ± 4.0	89.25	−67.85 ± 4.2
		C	−100.00 ± 0.0	−5.51 ± 7.4	−66.46 ± 7.3	31.69	−14.43 ± 14.0	−10.80 ± 6.8	−39.33 ± 7.0	−33.18 ± 3.3	62.06	n.a.
	1.00/10.00	T	−100.00 ± 0.0	−2.88 ± 5.0	−54.76 ± 8.0	43.93	−39.78 ± 18.2	−13.51 ± 10.8	−45.43 ± 7.0	−58.37 ± 5.0	43.03	−72.06 ± 1.5
		C	−100.00 ± 0.0	−21.26 ± 6.7	−74.57 ± 7.0	20.02	−46.12 ± 18.9	−18.91 ± 13.6	−46.07 ± 6.2	−43.72 ± 5.0	53.65	n.a.
S1 × Aa	0.125/1.25	T	+4.84 ± 2.6	+2.36 ± 2.6	+2.86 ± 3.7	105.29	−11.27 ± 10.3	−5.40 ± 8.1	+3.11 ± 6.6	−1.98 ± 4.0	103.21	−37.54 ± 2.3
		C	−5.91 ± 8.0	−2.88 ± 5.0	+6.01 ± 5.2	102.95	1.40 ± 8.9	−2.70 ± 0.0	−26.56 ± 7.2	−11.45 ± 5.4	78.86	−25.67 ± 1.5
	0.25/2.50	T	+2.15 ± 3.1	−0.26 ± 3.0	+7.80 ± 5.4	107.52	−46.12 ± 7.9	−16.21 ± 8.1	−0.10 ± 8.6	−15.40 ± 5.4	92.24	−41.47 ± 3.0
		C	−3.22 ± 6.2	−2.88 ± 5.0	−18.51 ± 3.1	79.13	−27.11 ± 6.0	−2.70 ± 7.6	−40.12 ± 8.7	−26.43 ± 6.0	68.48	−26.02 ± 1.5
	0.50/5.00	T	−3.22 ± 7.6	−2.88 ± 5.0	+17.75 ± 5.5	114.35	−33.45 ± 7.9	−13.51 ± 6.2	+8.76 ± 10.7	−8.51 ± 6.1	100.12	−48.76 ± 2.5
		C	−5.91 ± 6.7	−5.51 ± 4.2	+14.34 ± 6.9	108.04	−27.11 ± 11.9	−5.40 ± 10.2	−15.63 ± 7.2	−11.80 ± 4.3	88.55	−28.14 ± 0.8
	1.00/10.00	T	+2.15 ± 3.1	−0.26 ± 3.0	+24.91 ± 5.7	124.59	−33.45 ± 14.9	−13.51 ± 7.6	+3.26 ± 8.3	−26.33 ± 4.8	88.46	−53.06 ± 1.5
		C	+4.84 ± 2.6	+2.36 ± 2.6	−9.32 ± 3.7	92.81	−33.45 ± 14	−10.81 ± 9.2	−25.21 ± 5.3	−19.38 ± 6.0	73.61	−30.13 ± 1.5
S1 × Ab	0.125/1.25	T	−0.54 ± 2.6	−0.26 ± 3.0	−18.38 ± 6.3	81.4	−8.10 ± 15.8	−5.40 ± 8.1	+16.39 ± 6.7	+0.08 ± 3.7	105.39	−42.85 ± 2.6
		C	−8.60 ± 6.9	−0.26 ± 3.0	−7.31 ± 4.6	92.44	+1.40 ± 11.5	+5.40 ± 2.7	+16.83 ± 5.4	+1.07 ± 2.7	114.69	−29.11 ± 1.5
	0.25/2.50	T	−3.22 ± 4.3	−0.26 ± 3.0	−10.42 ± 6.4	89.35	−20.77 ± 7.9	+0.00 ± 2.7	+34.29 ± 9.1	−2.76 ± 4.8	115.76	−48.41 ± 2.2
		C	−3.22 ± 4.3	−0.26 ± 3.0	−5.28 ± 3.7	94.46	−20.77 ± 14.0	−5.40 ± 6.8	−13.72 ± 7.2	−10.29 ± 5.2	92.62	−30.42 ± 1.4
	0.50/5.00	T	−13.97 ± 8.7	−8.13 ± 5.0	+8.98 ± 6.5	100.12	−23.94 ± 11.5	−8.10 ± 6.9	+29.84 ± 9.6	−2.15 ± 4.7	110.85	−50.57 ± 2.1
		C	−8.60 ± 9.3	−0.26 ± 5.2	−0.58 ± 5.4	99.16	−11.26 ± 5.1	+5.40 ± 2.7	+7.27 ± 8.9	−2.96 ± 4.0	107.53	−39.21 ± 1.0
	1.00/10.00	T	−11.29 ± 9.1	−5.51 ± 7.4	+22.27 ± 7.2	115.53	−30.28 ± 16.7	−16.21 ± 6.8	+13.36 ± 9.1	−21.26 ± 5.2	83.52	−57.76 ± 1.3
		C	−5.91 ± 2.6	−2.88 ± 5.0	+10.10 ± 5.6	106.93	−20.77 ± 9.5	+2.70 ± 3.1	−6.81 ± 8.7	−10.05 ± 4.9	96.39	−52.25 ± 7.1

Table 4. *Cont.*

Sample	Conc. (%) [1]	T/C	*Lactuca sativa*				*Lolium perenne*					*Aspergillus flavus*
			RG_{24H} (%)	RG_{48H} (%)	RRG (%)	GI (%)	RG_{72H} (%)	RG_{144H} (%)	RRG (%)	RSG (%)	GI (%)	$\%H_2O$ [2]
S1 × Ac	0.125/1.25	T	2.15 ± 3.1	−0.26 ± 3.0	+15.74 ± 6.0	115.44	−1.76 ± 10.8	−2.70 ± 7.6	+4.03 ± 5.5	+5.54 ± 3.4	107.55	−49.38 ± 0.9
		C	−5.91 ± 6.7	−2.88 ± 2.6	+6.39 ± 5.5	103.33	−4.92 ± 12.1	−5.40 ± 5.1	−0.86 ± 6.7	+2.04 ± 4.2	95.3	−37.57 ± 4.3
	0.25/2.50	T	−0.53 ± 5.1	−0.26 ± 3.0	+26.17 ± 3.6	125.85	+1.40 ± 11.5	−2.70 ± 4.4	+3.63 ± 5.2	−9.13 ± 3.7	97.25	−53.28 ± 0.3
		C	+7.53 ± 0.0	+4.99 ± 0.0	−19.64 ± 2.8	84.36	−20.77 ± 9.5	−10.80 ± 5.1	−11.91 ± 6.6	−18.66 ± 4.5	82.48	−44.33 ± 2.4
	0.50/5.00	T	−3.23 ± 0.0	−0.26 ± 3.0	+29.62 ± 4.1	129.29	−46.12 ± 7.9	−16.21 ± 2.7	−20.07 ± 9.3	−31.21 ± 6.6	68.49	−60.35 ± 0.8
		C	−5.91 ± 5.1	−2.88 ± 5.0	−2.87 ± 4.8	94.32	−17.60 ± 8.1	−10.80 ± 5.1	−11.87 ± 8.3	−20.97 ± 6.1	81.38	−52.30 ± 2.4
	1.00/10.00	T	−3.22 ± 4.3	−5.51 ± 4.2	+50.60 ± 5.2	142.3	−42.96 ± 3.6	−21.62 ± 6.8	−25.38 ± 4.7	−43.32 ± 5.0	62.19	−66.54 ± 3.4
		C	−3.22 ± 4.3	−0.26 ± 3.0	−9.73 ± 1.9	90.03	−42.95 ± 15	−13.51 ± 7.6	−21.10 ± 6.2	−29.96 ± 5.2	76.43	−54.96 ± 1.2
S2 × Aa	0.125/1.25	T	−5.91 ± 6.7	−5.51 ± 4.2	+2.13 ± 3.0	96.51	−36.61 ± 8.9	−5.40 ± 5.1	+12.79 ± 7.5	−21.83 ± 4.5	98.0	−40.79 ± 1.5
		C	+7.53 ± 0.0	+4.99 ± 0.0	−24.32 ± 2.4	79.45	−14.43 ± 6.0	−5.40 ± 5.1	+4.62 ± 10.2	−2.97 ± 5.6	103.48	−14.44 ± 3.1
	0.25/2.50	T	+2.15 ± 3.1	−0.26 ± 3.0	+13.51 ± 3.2	113.21	−39.79 ± 6.0	−21.62 ± 5.1	+8.41 ± 9.8	−26.00 ± 6.3	86.4	−45.39 ± 2.2
		C	+7.53 ± 0.0	+4.99 ± 0.0	−29.38 ± 3.7	74.13	−20.77 ± 7.9	−8.10 ± 3.1	−3.88 ± 6.9	−19.19 ± 4.3	86.13	−25.20 ± 2.9
	0.50/5.00	T	−3.22 ± 4.3	−0.26 ± 3.0	+37.22 ± 3.5	136.86	−30.28 ± 8.1	−13.51 ± 6.2	+10.03 ± 13.2	−30.31 ± 6.6	80.4	−48.75 ± 2.4
		C	+4.84 ± 2.6	+2.36 ± 2.6	−14.56 ± 4.8	87.45	−36.62 ± 12.6	−13.51 ± 0.0	−6.90 ± 6.5	−10.98 ± 6.0	93.45	−21.95 ± 1.8
	1.00/10.00	T	+4.84 ± 2.6	+2.36 ± 2.6	+47.78 ± 4.4	151.28	−42.95 ± 15.0	−16.21 ± 5.1	−6.34 ± 9.7	−34.33 ± 6.1	75.47	−56.05 ± 1.2
		C	+2.15 ± 5.3	−0.26 ± 5.2	−28.46 ± 2.5	71.34	−65.14 ± 13.0	−24.32 ± 6.2	−25.07 ± 8.4	−37.78 ± 7.0	59.55	−26.62 ± 2.8
S2 × Ab	0.125/1.25	T	−0.53 ± 5.1	−0.26 ± 5.2	−8.61 ± 3.3	91.14	−8.09 ± 6.0	0.00 ± 2.7	+16.26 ± 8.2	+7.41 ± 4.9	117.73	−42.83 ± 5.2
		C	−5.91 ± 2.6	−0.26 ± 3.0	−17.21 ± 2.8	82.56	−27.11 ± 6.0	−5.40 ± 2.7	−2.23 ± 8.6	−1.63 ± 5.0	100.65	−29.35 ± 0.8
	0.25/2.50	T	−3.22 ± 4.3	−2.88 ± 5.0	+14.5 ± 3.8	111.2	−30.28 ± 13.1	−8.10 ± 3.1	−1.02 ± 7.3	−16.42 ± 6.0	88.87	−45.94 ± 3.7
		C	−3.22 ± 4.3	−5.51 ± 4.2	−19.76 ± 2.6	75.81	−27.11 ± 6.0	−10.80 ± 5.1	−10.99 ± 7.0	−8.77 ± 4.9	92.48	−34.34 ± 1.1
	0.50/5.00	T	−0.54 ± 2.6	+2.36 ± 2.6	+29.22 ± 4.1	132.27	−14.43 ± 6.0	−10.80 ± 5.1	+17.42 ± 7.1	−4.92 ± 5.5	103.45	−49.29 ± 2.3
		C	+7.53 ± 0.0	+4.99 ± 0.0	−30.64 ± 2.4	72.81	−27.11 ± 7.9	−10.80 ± 2.7	−30.45 ± 6.1	−24.04 ± 5.1	70.83	−37.58 ± 1.0
	1.00/10.00	T	−3.22 ± 7.6	−5.51 ± 7.4	+38.44 ± 5.5	130.82	−33.45 ± 10.8	−13.51 ± 4.4	−1.42 ± 8.0	−21.79 ± 5.5	86.06	−57.43 ± 1.4
		C	−0.53 ± 5.1	+4.99 ± 0.0	−26.57 ± 2.2	77.09	−61.97 ± 10.3	−24.32 ± 0.0	−38.07 ± 8.1	−36.80 ± 7.9	57.62	−51.11 ± 1.2

Table 4. *Cont.*

Sample	Conc. (%) [1]	T/C	*Lactuca sativa*				*Lolium perenne*					*Aspergillus flavus*
			RG_{24H} (%)	RG_{48H} (%)	RRG (%)	GI (%)	RG_{72H} (%)	RG_{144H} (%)	RRG (%)	RSG (%)	GI (%)	$\%H_2O$ [2]
S2 × Ac	0.125/1.25	T	−0.54 ± 2.6	+2.36 ± 2.6	+1.62 ± 6	104.03	−17.6 ± 13.1	0.0 ± 2.7	+6.71 ± 5.3	−4.04 ± 3.4	101.3	−44.54 ± 3.3
		C	+2.15 ± 3.1	+2.36 ± 2.6	−26.47 ± 3.6	75.26	−20.77 ± 14	−5.4 ± 5.1	−7.49 ± 7.4	−7.62 ± 4.1	94.87	−42.29 ± 1.6
	0.25/2.50	T	−5.91 ± 6.7	−0.26 ± 5.2	−0.35 ± 5.2	99.38	−17.60 ± 10.9	0.0 ± 2.7	+10.09 ± 7.7	−8.38 ± 5.3	100.86	−50.42 ± 2.2
		C	+2.15 ± 3.1	+4.99 ± 0.0	−30.38 ± 4	73.09	−36.62 ± 8.9	−10.8 ± 5.1	−5.32 ± 8.6	−16.43 ± 5.6	86.77	−45.49 ± 2.5
	0.50/5.00	T	+2.15 ± 3.1	+2.36 ± 2.6	+17.50 ± 4.6	120.28	−42.95 ± 10.9	−16.21 ± 9.2	−5.96 ± 7.9	−24.08 ± 6.0	80.51	−54.96 ± 3.5
		C	−8.60 ± 6.9	−10.76 ± 6.7	−0.75 ± 5.2	88.56	−52.46 ± 9.5	−18.91 ± 5.4	−26.15 ± 6.6	−22.68 ± 4.7	69.61	−50.56 ± 1.0
	1.00/10.00	T	+2.15 ± 3.1	−0.26 ± 3.0	+31.51 ± 4	131.17	−52.46 ± 7.9	−27.02 ± 9.2	−53.20 ± 7.6	−57.86 ± 6.0	39.78	−65.31 ± 3.9
		C	−19.35 ± 3.1	−5.51 ± 4.2	−25.01 ± 2.2	70.86	−49.29 ± 12.6	−27.02 ± 5.1	−30.97 ± 8.9	−37.62 ± 7.4	60.51	−59.17 ± 1.1
S3 × Aa	0.125/1.25	T	−5.91 ± 5.1	−2.88 ± 5.0	+25.69 ± 6.2	122.06	−30.28 ± 12.1	−2.70 ± 7.6	+14.13 ± 8.4	−0.50 ± 5.1	106.81	−40.96 ± 2.0
		C	+4.84 ± 2.6	+2.36 ± 2.6	−8.81 ± 3.6	93.34	−17.60 ± 10.9	+2.70 ± 3.1	−8.78 ± 7.8	−2.30 ± 3.9	99.43	−23.53 ± 4.0
	0.25/2.50	T	+7.53 ± 0.0	+4.99 ± 0.0	+9.55 ± 3.5	115.02	−30.28 ± 10.9	−10.81 ± 8.1	+24.29 ± 12.1	−7.10 ± 6.7	100.02	−48.03 ± 5.2
		C	−11.29 ± 6.7	−13.38 ± 6.6	+18.68 ± 6.7	102.8	−58.80 ± 10.8	−13.51 ± 7.6	−19.32 ± 8.8	−20.59 ± 5.7	77.93	−24.22 ± 4.4
	0.50/5.00	T	−3.22 ± 7.6	−0.26 ± 3.0	+27.37 ± 6.9	127.04	−55.63 ± 8.1	−32.43 ± 8.1	−4.95 ± 10.5	−25.29 ± 6.9	78.18	−49.56 ± 1.9
		C	+4.84 ± 2.6	+2.36 ± 2.6	−24.89 ± 3.8	76.88	−46.12 ± 9.5	−29.72 ± 12.8	−26.38 ± 6.7	−15.02 ± 6.2	73.04	−29.33 ± 4.6
	1.00/10.00	T	−5.91 ± 5.1	+2.36 ± 2.6	+8.83 ± 6.5	111.41	−42.95 ± 8.1	−21.62 ± 5.1	+9.17 ± 8.2	−8.75 ± 11.4	89.66	−53.68 ± 2.3
		C	−0.53 ± 8.0	−2.88 ± 7.8	−7.44 ± 3.9	76.88	−61.97 ± 11.5	−48.65 ± 6.8	−27.28 ± 8.8	−40.15 ± 6.6	52.33	−29.23 ± 1.7
S3 × Ab	0.125/1.25	T	−5.91 ± 6.7	−2.88 ± 5.0	+27.27 ± 6.9	123.6	−17.60 ± 8.1	−10.8 ± 5.1	+24.34 ± 11.4	−5.11 ± 5.3	106.73	−35.72 ± 2.1
		C	−3.22 ± 4.3	−2.88 ± 2.6	−0.10 ± 5.2	97.01	−4.93 ± 8.1	8.11 ± 0.0	+21.35 ± 10.3	−5.16 ± 4.9	113.79	−29.23 ± 1.1
	0.25/2.50	T	−3.22 ± 7.6	−0.26 ± 3.0	+41.38 ± 5	141.02	−39.79 ± 9.5	−10.80 ± 5.1	+22.16 ± 11.2	−11.25 ± 6.3	97.13	−43.79 ± 1.3
		C	−0.54 ± 2.6	+4.99 ± 0.0	−18.4 ± 4.6	85.66	−11.26 ± 7.3	−10.81 ± 9.2	+7.64 ± 11.1	−10.01 ± 7.1	93.61	−31.84 ± 1.2
	0.50/5.00	T	−5.91 ± 2.6	−0.26 ± 3.0	+23.37 ± 4.6	123.05	−36.62 ± 0.0	−27.02 ± 2.7	−14.38 ± 9.7	−29.45 ± 6.0	69.86	−51.82 ± 2.2
		C	−16.66 ± 5.1	−5.51 ± 4.2	−18.62 ± 4.3	76.89	−27.11 ± 9.5	−8.10 ± 5.4	+11.70 ± 8.6	−4.59 ± 5.8	95.38	−35.96 ± 0.2
	1.00/10.00	T	+4.84 ± 2.6	+2.36 ± 2.6	+30.92 ± 4.1	134.02	−46.12 ± 6	−32.43 ± 10.2	−35.03 ± 7.7	−28.88 ± 7.9	59.09	−57.93 ± 1.3
		C	+2.15 ± 5.3	−0.26 ± 5.2	−15.62 ± 4.5	84.15	−23.94 ± 5.1	−18.91 ± 6.9	−23.73 ± 7.2	−29.07 ± 5.8	67.78	−45.19 ± 3.6

Table 4. *Cont.*

Sample	Conc. (%) [1]	T/C	*Lactuca sativa*				*Lolium perenne*					*Aspergillus flavus*
			RG_{24H} (%)	RG_{48H} (%)	RRG (%)	GI (%)	RG_{72H} (%)	RG_{144H} (%)	RRG (%)	RSG (%)	GI (%)	$\%H_2O$ [2]
S3 × Ac	0.125/1.25	T	−5.91 ± 6.7	−2.88 ± 5.0	+25.15 ± 5.8	121.54	−23.94 ± 5.1	−5.40 ± 5.1	+15.52 ± 7.5	−8.60 ± 5.3	108.9	−37.11 ± 2.3
		C	−3.22 ± 4.3	−2.88 ± 2.6	−8.45 ± 6.6	88.9	−30.28 ± 3.6	−8.10 ± 3.1	−8.63 ± 6.5	−15.06 ± 6.0	83.51	−29.39 ± 5.5
	0.25/2.50	T	−3.22 ± 7.6	−0.26 ± 3.0	+5.26 ± 3.2	104.98	−20.77 ± 10.8	−10.8 ± 5.1	+1.67 ± 11.8	−15.53 ± 5.3	97.97	−43.13 ± 1.0
		C	−0.54 ± 2.6	+4.99 ± 0.0	−3.54 ± 4.7	101.26	−23.94 ± 8.9	−8.10 ± 5.4	+2.39 ± 7.7	−14.01 ± 5.1	89.24	−42.25 ± 3.6
	0.50/5.00	T	−5.91 ± 2.6	−0.26 ± 3.0	+13.05 ± 2.8	112.76	−8.10 ± 7.9	−5.40 ± 5.1	+6.56 ± 7.4	−10.28 ± 4.4	100.73	−54.11 ± 2.1
		C	−16.66 ± 5.1	−5.51 ± 4.2	−16.47 ± 2.5	78.92	−46.13 ± 3.1	−16.21 ± 5.1	−16.33 ± 7.5	−27.24 ± 5.4	76.15	−45.44 ± 2.0
	1.00/10.00	T	+4.84 ± 2.6	+2.36 ± 2.6	+5.62 ± 7.1	108.12	−36.62 ± 8.9	−35.13 ± 6.2	−64.76 ± 3.6	−57.41 ± 4.8	35.84	−71.33 ± 2.7
		C	+2.15 ± 5.3	−0.26 ± 5.2	−6.08 ± 3.3	93.67	−61.97 ± 17.9	−27.02 ± 6.8	−37.69 ± 7.4	−42.76 ± 6.1	55.05	−55.18 ± 3.1
MEAN	0.125/1.25	T	−2.03 ± 1.5	−1.13 ± 1.2	+8.16 ± 2.0	104.70 ± 4.9	+0.78 ± 1.2	+10.92 ± 2.4	−4.66 ± 1.5	+4.01 ± 2.7	104.01 ± 2.7	−41.27 ± 0.9
		C	−2.03 ± 1.7	−0.26 ± 0.9	−8.92 ± 1.6	85.92 ± 5.5	+1.57 ± 1.0	−6.77 ± 2.7	−9.46 ± 1.7	−6.67 ± 6.1	93.32 ± 6.1	−28.67 ± 1.4
	0.25/2.50	T	−0.83 ± 1.6	+0.03 ± 1.0	+11.93 ± 1.7	107.79 ± 6.1	−1.84 ± 1.3	+9.47 ± 3.0	−14.26 ± 1.8	−4.31 ± 3.0	95.69 ± 3.0	−46.68 ± 0.9
		C	−0.53 ± 1.5	+0.32 ± 1.4	−14.02 ± 1.6	82.67 ± 4.5	−1.31 ± 1.2	−12.52 ± 2.7	−17.71 ± 1.7	−16.34 ± 3.0	83.66 ± 3.1	−33.45 ± 1.3
	0.50/5.00	T	−4.12 ± 1.6	−0.84 ± 1.1	+22.67 ± 1.7	113.59 ± 8.9	−3.94 ± 1.5	+2.46 ± 3.1	−19.18 ± 1.9	−11.81 ± 4.7	88.18 ± 4.7	−51.89 ± 0.9
		C	−5.01 ± 2.1	−2.30 ± 1.4	−10.56 ± 1.7	81.47 ± 6.6	−2.36 ± 1.5	−15.40 ± 2.5	−17.35 ± 1.7	−18.20 ± 4.4	81.80 ± 4.5	−36.82 ± 1.5
	1.00/10.00	T	−0.53 ± 1.7	−0.84 ± 1.4	+29.02 ± 2	119.32 ± 9.4	−6.84 ± 1.5	−19.67 ± 2.9	−35.56 ± 2.2	−32.69 ± 7	67.31 ± 7.1	−58.92 ± 1.0
		C	−2.03 ± 1.8	−0.55 ± 1.4	−13.12 ± 1.3	79.68 ± 7.5	−6.57 ± 1.9	−27.88 ± 2.4	−31.97 ± 2.0	−34.7 ± 4.3	65.29 ± 4.3	−43.28 ± 2.0

Note. [1] Concentrations were analyzed in ranges between 1.25 and 10 µL/mL for allelopathic effects and from 12.5 to 100 µL/mL for fungal inhibition tests; [2] Inhibition estimated at 120 h from linear regressions; [3] Positive control (SrEE at 25 mg/mL in EtOH or EtOH); n.a.: not assayed.

Among the analyzed samples, S1 × Ac, S2 × Aa, and S3 × Ab showed the greatest stimulatory potential, promoting root growth. In particular, S1 × Ac proved to be the most balanced formulation, which induced an average stimulation, with its carrier causing low inhibition. Likewise, EtOH also caused strong phytotoxicity in *L. perenne* seedlings, with SrEE partially ameliorating this toxicity, highlighting the protective role against the stress caused by the solvent. In general, the responses of these seeds to the treatments and carriers presented similar values, but certain formulations exhibited greater phytotoxicity than that detected for the carrier at increasing concentrations. However, certain treatments promoted certain stimulatory effects (5–10%), suggesting a positive response under low-dose conditions.

The bivariate Spearman correlation analysis (Figure 2) together with the log-logistic models (Table 5) revealed well-defined dose–response relationships for both species, as reflected by strong positive/negative correlations and robust goodness-of-fit values (R^2 > 90%), helping to distinguish between growth-promoting and growth-inhibiting trends.

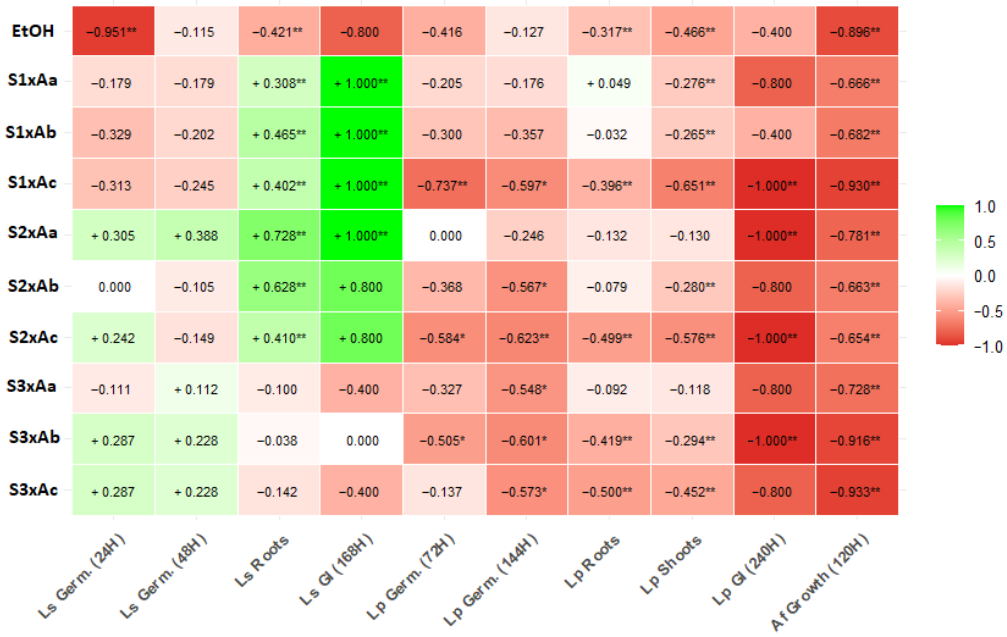

Figure 2. Bivariate Spearman correlations between formulations and biological responses, including biostimulation/phytotoxicity (*Lactuca sativa* (Ls) roots; *Lolium perenne* (Lp) roots and leaves) and inhibition activity against *Aspergillus flavus* (Af). Green indicates growth-promoting potential; red indicates inhibitory effects. *: $p < 0.01$; **: $p < 0.05$.

As suggested, when ethanol was used as a carrier, negative correlations were observed, especially in *L. sativa*, with particularly pronounced effects during early germination. Treatments with lower glycerol content (S1 and S2) produced the most favorable responses, associated with lower GI_{+25} values, indicating a high sensitivity even at lower doses. The effects of aqueous matrices varied depending on the solvent system: S1 positively combined with aqueous matrix C showing the strongest stimulatory response, while matrix A had the best performance in the presence of system S2. In *L. perenne*, inhibitory responses were more pronounced, as indicated by the negative correlations between treatments and observed outcomes. Accordingly, GI_{-25} values were determined based on the herbicidal effects of the treatments, representing a 25% reduction in the growth index. In particular, the combinations S1 × Ac, S2 × Aa, S2 × Ac, and S3 × Ab reduced the germination index (GI) of this model seed. Unlike *L. sativa*, inhibition in *L. perenne* affected not only root/shoot growth but also germination rates.

Table 5. Four-parameter log-logistic ($\log_{10}(x)$) model (R^2) for the different formulations tested on the model seed species *L. sativa* (GI_{+25}), *L perenne* (GI_{-25}), and the fungus *A. flavus* (EC_{50}).

Sample	Species	Four-Parameter Log-Logistic ($\log_{10}(x)$) Model Equation	R^2	Applications [1]	$GI_{\pm 25}$ [2]/EC_{50} [3] (µL/mL)
EtOH [4]	*L. sativa*	$y = 20.623 + (21.286)/(1 + (10^{85.955/x})^{0.41})$	0.994 ***		
	L. perenne	$y = 24.85 + (-151.723)/(1 + (10^{84.705/x})^{1.058})$	0.971 **		
	A. flavus	$y = 13.117 + (-85.843)/(1 + (10^{-41.194/x})^{1.493})$	1.000 ***	Antifungal	24.46
S1 × Aa	*L. sativa*	$y = -2.782 + (108.064)/(1 + (10^{161.114/x})^{1.258})$	1.000 ***	Biostimulant	>10.00
	L. perenne	$y = 6.499 + (87.109)/(1 + (10^{107.48/x})^{0.11})$	0.494		
	A. flavus	$y = 8.722 + (-63.892)/(1 + (10^{-36.822/x})^{1.582})$	1.000 ***	Antifungal	57.68
S1 × Ab	*L. sativa*	$y = -1.686 + (82.546)/(1 + (10^{419.388/x})^{3.635})$	0.999 ***	Biostimulant	>10.00
	L. perenne	$y = 11.84 + (-51.795)/(1 + (10^{110.757/x})^{1.189})$	0.803		
	A. flavus	$y = 2.467 + (-163.937)/(1 + (10^{-38.487/x})^{3.994})$	0.967 **	Antifungal	39.03
S1 × Ac	*L. sativa*	$y = -1.542 + (116.694)/(1 + (10^{198.726/x})^{1.68})$	0.952 **	Biostimulant	2.85
	L. perenne	$y = 5.122 + (55.637)/(1 + (10^{107.555/x})^{0.509})$	1.000 ***	Herbicide	3.97
	A. flavus	$y = 6.813 + (-79.472)/(1 + (10^{-48.22/x})^{1.702})$	1.000 ***	Antifungal	14.85
S2 × Aa	*L. sativa*	$y = -2.506 + (98.361)/(1 + (10^{168.955/x})^{0.634})$	1.000 ***	Biostimulant	3.45
	L. perenne	$y = 0.723 + (33.78)/(1 + (10^{106.907/x})^{1.458})$	1.000 ***	Herbicide	11.19
	A. flavus	$y = 2.946 + (-189.6)/(1 + (10^{-37.687/x})^{3.909})$	0.989 **	Antifungal	53.33
S2 × Ab	*L. sativa*	$y = -13.42 + (104.562)/(1 + (10^{131.556/x})^{0.398})$	0.999 ***		
	L. perenne	$y = 9.951 + (82.846)/(1 + (10^{118.27/x})^{0.143})$	0.728		
	A. flavus	$y = 4.378 + (-319.786)/(1 + (10^{-41.965/x})^{3.81})$	0.994 ***	Antifungal	51.72
S2 × Ac	*L. sativa*	$y = -12.233 + (113.917)/(1 + (10^{131.394/x})^{0.67})$	0.983 **		
	L. perenne	$y = 7.287 + (21.181)/(1 + (10^{101.333/x})^{0.793})$	1.000 ***	Herbicide	5.41
	A. flavus	$y = 3.221 + (-298.193)/(1 + (10^{-41.28/x})^{4.046})$	0.989 **	Antifungal	27.32
S3 × Aa	*L. sativa*	$y = 0.21 + (97.721)/(1 + (10^{139.065/x})^{0.486})$	0.099		
	L. perenne	$y = 21.188 + (62.73)/(1 + (10^{106.814/x})^{0.414})$	0.859		
	A. flavus	$y = 1.959 + (-61.976)/(1 + (10^{22.566/x})^{0.589})$	0.969 **	Antifungal	41.63
S3 × Ab	*L. sativa*	$y = -0.082 + (66.484)/(1 + (10^{202.156/x})^{1.628})$	0.108		
	L. perenne	$y = 4.219 + (50.576)/(1 + (10^{106.759/x})^{0.565})$	1.000 ***	Herbicide	
	A. flavus	$y = 4.015 + (-73.364)/(1 + (10^{-27.745/x})^{1.57})$	1.000 ***	Antifungal	42.19
S3 × Ac	*L. sativa*	$y = 5.84 + (102.778)/(1 + (10^{160.785/x})^{0.08})$	0.804		
	L. perenne	$y = 11.688 + (-14.785)/(1 + (10^{103.447/x})^{0.954})$	0.982 **		
	A. flavus	$y = 4.287 + (-262.008)/(1 + (10^{-33.757/x})^{2.906})$	1.000 ***	Antifungal	40.15
MEAN [5]	*L. sativa*	$y = -2.325 + (106.899)/(1 + (10^{133.026/x})^{0.97})$	1.000 ***		
	L. perenne	$y = 1.803 + (-356.229)/(1 + (10^{106.146/x})^{3.951})$	0.993 ***		
	A. flavus	$y = 2.565 + (-114.533)/(1 + (10^{-33.206/x})^{2.545})$	1.000 ***		

Note. [1] Agricultural applications were considered only when both criteria were met: a statistically significant bivariate Spearman correlation (positive or negative) (Table 4; $p < 0.05$) and an adequate fit of the log-logistic model ($R^2 > 0.90$); [2] Concentration required for a +25% (biostimulant) or −25% (herbicide) germination index in *L. sativa* or *L. perenne*; [3] Concentration required for a 50% inhibition in *A. flavus* (or −50% C); [4] Positive control (SrEE at 25 mg/mL in EtOH or EtOH); [5] Mean values do not consider the EtOH sample; ***: $R^2 > 99\%$. **: $R^2 > 95\%$.

3.4. Antifungal Capacities

The organism evaluated showed a high sensitivity to the *S. rosmarinus* extract (as the ethanol was allowed to evaporate completely), resulting in an average growth inhibition of approximately 60% (Table 4). The formulated products also showed strong effects, achieving inhibition close to 50%, as evidenced by the marked reduction in mycelial growth. Notably, the carriers also showed a significant inhibitory effect of around 35%, demonstrating the significant contribution of the carrier to the suppression of fungal growth. In general, most SrEE-based formulations showed moderate to strong antifungal activity, with inhibition values generally ranging from 40% to 60%, and in some exceptions reaching up to 70% at the major concentration tested. Among the treatments tested, S1 × Ac and

S2 × Ac consistently outperformed, as reflected by average inhibition values exceeding 50% for the assayed concentrations.

Spearman correlation analysis (Figure 2) along with log-logistic models for *A. flavus* (Table 5) revealed a consistent, statistically significant and concentration-dependent inhibitory effect for most formulations. In some treatments, fungal growth inhibition even showed coefficients of determination exceeding 95%, indicating excellent model fit and robust predictive capacity for the mathematical estimation of EC_{50} values. The SrEE showed a marked antifungal effect, characterized by a strong dose–response relationship ($R^2 > 95\%$) and a low EC_{50} value (24.5 µL/mL), confirming its high intrinsic antifungal potential. Similarly, most of the treatments showed significant inhibitory effects, often accompanied by comparable activity in their carrier matrices. In general, solvent systems S1 and S2 exhibited the lowest EC_{50} values, suggesting the contribution of PG to fungal growth inhibition. Conversely, formulations containing higher proportions of the soy lecithin:polysorbate-20 ratio similarly showed stronger antifungal effects, also reflecting low EC_{50} values and indicating a strong antifungal efficiency. Among all the treatments evaluated, S1 × Ac proved to be the most effective formulation, being the only one with an EC_{50} value below 15 µL/mL, followed by S2 × Ac (27.3 µL/mL).

4. Discussion

Previous works have supported the agrochemical potential of ethanolic extracts of distilled *S. rosmarinus* waste, in terms of their biopesticide, biostimulant, chelating, antimicrobial and antioxidant capabilities [8,9,12,21]. In particular, Ortiz de Elguea-Culebras et al. (2024) [12] evaluated the biostimulant potential of distilled plant extracts of *S. rosmarinus* collected from different Spanish populations and domesticated in crop fields. Among them, the population of Pina de Ebro (Spain) was particularly highlighted due to its adaptability to cultivation, essential oil production and balanced bioactivities (including antifungal, antioxidant, chelating and biostimulant). As a result, this study focuses on advancing that previous work and, in this way, developing the final formulation that incorporates that extract for the evaluation of agricultural applications and its subsequent commercialization, similar to that prepared by Chrysargyris et al. (2020) [22] using essential oil. One aspect to consider when developing a bioagrochemical is that plant extracts contain a large number of metabolites with different biological effects. Specifically, the *S. rosmarinus* selected in this study was previously characterized for its high content of carnosic acid, which has previously shown antimicrobial activity [23], but also phytotoxicity [24]. This dual behavior highlights the need to carefully optimize extraction methodologies (solvent selection, extraction time, temperature or equipment/system designs) to achieve a balanced and selective recovery of bioactive compounds while minimizing the co-extraction of undesirable components, as previously observed by Ferrando-Beneyto et al. (2024) [25].

In this regard, different natural ingredients were selected, focusing on their residual agroforestry origin, and were thoroughly evaluated under phytotoxicity tests to select the most suitable ones for the final formulation. According to the European Chemicals Agency (ECHA), ethanol (List no. assigned: 200-578-6), glycerol (200-289-5), propylene glycol (200-338-0), soy lecithin (232-307-2), polysorbate-20 (500-018-3), DMSO (200-664-3), acetic acid (200-580-7) and ascorbic acid (200-066-2) are all considered as safe and suitable for "plant protection products". Particularly, soy lecithin and ascorbic acid are also regarded as "active substances for plant protection products" by the UE in organic farming [26]. Overall, most ingredients showed moderate to low phytotoxicity at low to intermediate concentrations and, in some cases, even stimulated seedling growth, with responses clearly dependent on species and doses. These findings indicate that these by-products can act not only as inert carriers in formulations but even as growth-promoting additives,

acting alone or modulating the effects of active plant metabolites. Conversely, compounds that exhibited pronounced inhibitory effects within the analyzed range were considered allelopathic, justifying their exclusion from subsequent formulations.

Although ethanol exhibited acute phytotoxicity in both species tested and was therefore excluded from the final formulations, SrEE was initially dissolved in ethanol to ensure optimal solubilization (positive control). However, previous studies have reported that EtOH overcomes the dormancy of *Avena sativa* seeds, allowing germination at concentrations ranging from 50 to 200 mM or at higher concentrations at the beginning of emergence [27]. Glycerol and PG were initially considered due to their moderate phytotoxicity at lower concentrations; however, it was observed that formulations with higher concentrations of glycerol also showed greater phytotoxicity in *L. sativa* seedlings, making PG likely more suitable in the final formulations. Likewise, Kovács et al. (2015) [28] observed that glycerol released as a biodiesel by-product had a total inhibition on the germination of *L. perenne* seeds at a concentration of 1%, whereas Tisserat and Stuff (2011) [29] suggested opposite results with the increase in fresh and dry weight and shoot length in *Daucus carota*, *Zea mays* and *Mentha spicata* plants when sprayed with a solution containing 0.5 mL/L glycerol. Moreover, similar effects were previously observed by Pillard and Dufresne (1999) [30] for PG, who noted different effects in *L. sativa* and *L. perenne* seeds, where the emergence of *L. perenne* seeds was more affected than that of *L. sativa*. Finally, the co-solvent DMSO was considered in the final formulations due to its already expected biostimulant potential, as already demonstrated by Cruz-Campos et al. (2012) [31] on *Capsicum chinense*, at concentrations ranging from 4×10^{-8} M to 4×10^{-4} M, and by Tkachuk et al. (2024) [32] on the root elongation of *Lepidium sativum* with 0.05% DMSO, but also for its antifungal [33] and penetration capabilities.

Regarding adjuvants, soy lecithin was also selected for its expected biostimulant effects, as previously evaluated either directly [34] or as part of formulations [34–37], but also because this substance is approved as an antifungal treatment in organic farming [18]. The study carried out by Vannini et al. (2021) is particularly noteworthy [34], as they demonstrated the synergistic effect of a mixture of *Castanea sativa* wood distillate and soy lecithin when sprayed on *L. sativa* seedlings, highlighting the suitability of combining natural substances with soy lecithin. The non-ionic surfactant polysorbate-20 (or Tween-20) has also demonstrated previously higher values of fresh weight and plant height, suggesting that this substance favors the absorption and distribution of nutrients in the cultivation of *L. sativa* [38]. Similarly, Knypl (1977) [39] observed both stimulation and inhibition in the growth of the hypocotyl and epicotyl of *Amaranthus caudatus*, respectively, at a dosage of 2.5 µg per seedling. However, dissimilar effects were observed among the surfactants; while Tween-60/-65 exhibited pronounced stimulatory activity, Tween 40 showed no detectable effects. Acetic acid was discarded in the formulations for its herbicidal effects, as demonstrated alone or as the main component of wood vinegar [40], even being proposed as a natural and suitable alternative to glyphosate at concentrations above 20% [41]. Finally, despite not having found any evaluation directly focused on the stimulatory/inhibitory effects of ascorbic acid, some studies have claimed the potential antioxidant capabilities of this molecule by alleviating the toxicity effects of herbicides, such as Fusilade in wheat [42] or glyphosate in maize [43].

Nonetheless, the evaluation of individual components alone does not reliably predict the biological outcomes observed when these ingredients are combined, either among themselves or with a plant extract. Interactions between formulation constituents may give rise to synergistic, additive or antagonistic effects, ultimately modulating the overall biological responses. For this reason, the systematic evaluation of formulations with different ingredients at different concentrations is essential to ensure not only water solubility,

but also the safety and efficacy of the final product with enhanced beneficial effects and minimal adverse interactions. Overall, most formulations exhibited pronounced biostimulatory and antifungal activity in *L. sativa* seeds and against *A. flavus* growth, but induced strong phytotoxic effects in *L. perenne* roots. In addition, although the plant extract consistently reaffirmed its biostimulant and antifungal potential when incorporated into the formulations [12], these effects were positively or negatively influenced by the carrier. These findings emphasize the narrow concentration boundary and specific ingredient combinations required to achieve biocompatibility in the final formulation, while excluding inhibitory substances. Among the tested systems, formulation S1 × Ac emerged as particularly promising due to its balanced performance, combining suitable pH, good water dispersibility, strong biostimulant effects in *L. sativa* and antifungal effects against *A. flavus*, as well as moderate phytotoxicity in *L. perenne* at lower concentrations. These effects were largely attributed to the carrier, which lacked glycerol content, given its phytotoxic effects on *L. sativa*, and a higher soy lecithin:polysorbate-20 ratio, which improved water dispersibility and enhanced biostimulant and antifungal activities. Alternatively, Ortiz de Elguea-Culebras et al. (2023) [44] carried out an experimental trial in garlic, applying the S1 × Ac formulation either by immersing the clove or by spraying only the substrate, modifying the soy lecithin:polysorbate-20 ratio (1:2, 1:1 and 2:1). The results indicated that the 1:1 ratio (or 5.63% of each component in the final formulation) achieved the most balanced performance in terms of germination, growth, biomass, Fv/Fm, NBI and leaf color. Additionally, the inclusion of DMSO is expected to influence cellular permeability to active substances [16], while ascorbic acid should contribute to the antioxidant stability of polyphenols.

These findings indicate that the carrier substantially improves the bioavailability and biological performance of SrEE, while also enhancing the solubilization, diffusion and uniform distribution of the bioactive compounds within the matrix. This improved delivery facilitates more effective interactions with target organisms, maximizing efficacy in diverse application scenarios and supporting their potential as versatile tools for crop management and protection. Furthermore, the selected formulation consistently demonstrated significant effects in both seedlings and fungal growth, highlighting a promising dual functionality. Plant assays revealed clear species-dependent responses: while *L. sativa* generally exhibited growth stimulation, *L. perenne* was predominantly inhibited. This multifunctional behavior highlights the wide-ranging potential of the formulation for sustainable agricultural practices and reinforces the necessity of evaluating its effects across multiple crop species. Such testing is essential to accurately determine its intended functionality, whether as a biostimulant/antifungal or as an herbicide/antifungal agent, while accounting for species-specific responses, dose-dependent effects, and the broader ecological and agronomic implications of its application.

5. Conclusions

This study further supports the agrochemical potential of ethanolic extracts derived from distillation residues of *Salvia rosmarinus*. Building on previous work that identified a population from Pina de Ebro (Spain) as particularly promising due to its adaptability to cultivation, high essential oil content and balanced bioactivities (biostimulant, antifungal, chelating and antioxidant), this research advances toward the development of a suitable formulation, representing a crucial step for both agricultural application and commercialization. A major challenge in the development of plant-based bioagrochemicals lies in the wide chemical complexity of botanical extracts. In this case, the selected *S. rosmarinus* chemotype was rich in carnosic acid, a compound known for potent antifungal activity but also associated with phytotoxic effects. This dual behavior underscores the necessity of

optimizing extraction strategies and carefully selecting formulation components to balance solubility, efficacy and biocompatibility while minimizing toxic risks. Among the formulations tested, S1 × Ac emerged as the most balanced system, combining appropriate pH, good water dispersibility, strong biostimulant effects in *Lactuca sativa*, effective antifungal activity against *Aspergillus flavus*, and moderate phytotoxicity in *Lolium perenne* at low concentrations. This formulation included PG, DMSO, soy lecithin, polysorbate-20 and ascorbic acid, all obtained from residual agroforestry sources, highlighting the potential for integrating circular bioeconomy principles into biostimulant development.

Importantly, these findings demonstrated that the performance of multicomponent formulations could not be reliably predicted from single-component trials, since interactions between ingredients often lead to synergistic, additive or antagonistic effects. Collectively, these findings indicate that carefully optimized formulations substantially enhance the bioavailability and overall biological performance of vegetal extracts, functioning as effective delivery systems for plant-based active substances. This work not only reinforces the biological potential of distilled extracts of *S. rosmarinus* but also establishes a practical framework for the sustainable development of high-value products from agroforestry residues, opening new avenues for research and innovation in the emerging field of residue-based bioagrochemicals.

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Abbreviations

The following abbreviations are used in this manuscript:

CFU	Colony forming units
DMSO	Dimethyl sulfoxide
EC_{50}	Effective concentration that inhibits fungal growth by 25/50%
GI	Germination index
$GI_{\pm 25}$	Concentration required for a germination index of +25% or −25%
OD	Optical density
PG	Propylene glycol
RG	Relative germination

RRG	Relative root growth
RSG	Relative shoot growth
SrEE	Ethanolic extract of the distilled plant of *Salvia rosmarinus*
S_{90}	Concentration estimated to obtain a solubility of 90%

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