SUSTAINABLE CONTROL OF *BOTRYTIS CINEREA* IN VITICULTURE USING BIOACTIVE PLANT EXTRACTS OBTAINED BY HYBRID ULTRASOUND-PERCOLATION TECHNOLOGY

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CONTROLUL SUSTENABIL AL FUNGULUI BOTRYTIS CINEREA ÎN VITICULTURĂ UTILIZÂND EXTRACTE BIOACTIVE DIN PLANTE OBȚINUTE PRIN TEHNOLOGIA HIBRIDĂ ULTRASUNETE-PERCOLARE

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ABSTRACT

Sustainable management of gray mold (Botrytis cinerea) in viticulture necessitates eco-friendly alternatives to synthetic fungicides to alleviate chemical residue risks and combat emerging pathogen resistance. This study evaluated the biocontrol potential of botanical extracts derived from Allium sativum, Lavandula angustifolia, Salvia officinalis, Cannabis sativa, and Melissa officinalis, obtained via an optimized hybrid ultrasound-assisted percolation process. The optimal extraction parameters were determined to be a particle size of 30 mm, a solid-to-liquid ratio of 1:30, a system pressure of 7 bar, and an ultrasound power of 120 W over a 120-minute extraction duration, achieving high polyphenolic yields while maintaining process energy efficiency. In vitro antifungal screening via the poisoned food technique revealed that A. sativum extract exhibited the most potent inhibition against B. cinerea (78.62% on day 2 and 39.84% on day 7 at a 50% v/v concentration), followed by L. angustifolia (69.62% on day 2). In vivo evaluations on Vitis vinifera L. corroborated a concentration-dependent reduction in disease incidence following post-inoculation application. However, a progressive temporal decline in antifungal efficacy was observed across all crude extracts, highlighting the critical need for microencapsulation strategies or condensed application intervals (3–5 days) under field conditions. Overall, hybrid ultrasound-assisted percolation represents a highly efficient green extraction platform for recovering bioactive compounds suitable for integration into sustainable vineyard crop protection regimes.

REZUMAT

Managementul sustenabil al putregaiului cenușiu (Botrytis cinerea) în viticultură necesită identificarea unor alternative ecologice la fungicidele de sinteză, cu scopul de a diminua riscurile legate de reziduurile chimice și de a combate fenomenul de rezistență al patogenilor. Acest studiu a evaluat potențialul de biocontrol al extractelor botanice obținute din Allium sativum, Lavandula angustifolia, Salvia officinalis, Cannabis sativa și Melissa officinalis, procesate printr-o tehnologie optimizată de percolare hibridă asistată de ultrasunete. Parametrii optimi de extracție au fost stabiliți la o dimensiune a particulelor de material vegetal de 30 mm, un raport solid-lichid de 1:30, o presiune de 7 bar și o putere a ultrasunetelor de 120 W pe o durată de 120 de minute, obținându-se randamente ridicate de compuși polifenolici cu menținerea eficienței energetice a procesului. Evaluarea in vitro a activității antifungice prin metoda Mediului Toxic (Poisoned Food Technique) a arătat că extractul de A. sativum a prezentat cea mai puternică inhibiție împotriva B. cinerea (78.62% în ziua 2 și 39.84% în ziua 7 la o concentrație de 50% v/v), urmat de cel de L. angustifolia (69.62% în ziua 2). Testele in vivo efectuate pe Vitis vinifera L. au confirmat o reducere dependentă de concentrație a incidenței bolii în urma aplicării post-inoculare. Cu toate acestea, s-a observat o scădere progresivă în timp a eficacității antifungice pentru toate extractele brute, ceea ce subliniază necesitatea dezvoltării unor formulări microincapsulate sau a scurtării intervalelor de aplicare (3–5 zile) în condiții de câmp. În ansamblu, percolarea hibridă asistată de ultrasunete se dovedește a fi o platformă ecologică de extracție extrem de eficientă pentru recuperarea compușilor bioactivi capabili să fie integrați în strategii durabile de protecție a culturilor de viță-de-vie.

INTRODUCTION

Grapevine (*Vitis vinifera L.*) is one of the most economically important horticultural crops worldwide, with global grape production exceeding 77 million tons annually (*Ohana-Levi and Netzer, 2025; Butiuc-Keul and Coste, 2023*). However, grape yield and quality can be significantly affected by numerous phytopathogenic agents, among which *Botrytis cinerea*, the causal agent of gray mold, is one of the most important fungal pathogens of grapevine. Infection can cause berry deterioration and decay, with negative effects on grape yield, quality, and commercial value. Disease development is particularly favored by high-humidity conditions, while the difficulties associated with its control justify the interest in identifying effective and sustainable prevention and management methods (*Rahman et al., 2024; Jiang et al., 2023; De Simone et al., 2020; Jacometti et al., 2010*).

Under certain controlled conditions, *Botrytis cinerea* can contribute positively to wine quality through the phenomenon known as “noble rot” (*Hegyi et al., 2022*; *Negri et al., 2017*). *Azzolini et al., (2013),* showed that the controlled inoculation of grapes intended for passito wine production with selected *B. cinerea* strains can modify the aromatic profile, promoting the accumulation of volatile and terpenic compounds associated with the complex aromas of sweet wines. In the study (*Kelly et al., 2022*), the use of a 10% proportion of infected grapes in appassimento-style red wines resulted in only minor chemical and aromatic changes, without significantly affecting consumer acceptance. The article (*Thakur et al., 2018*) mentions *Tokaji Aszú*, *Sauternes, Beerenauslese*, and *Trockenbeerenauslese* among the best-known wines produced through “noble rot”. Botrytization promotes sugar concentration and the accumulation of compounds such as glycerol and gluconic acid, while also contributing to the development of characteristic honey, caramel, and dried-fruit notes typical of premium botrytized wines.

The control of *B. cinerea* relies largely on the use of synthetic fungicides. However, repeated use of chemical products may favor the selection of resistant pathogen populations, while concerns regarding the reduction of the environmental impact of plant protection products have stimulated research aimed at identifying alternative control methods (*Hahn, 2014; Sofianos et al., 2023; Tăbăraşu et al., 2025a; Nenciu et al., 2023)*. In this context, extracts obtained from medicinal and aromatic plants are of interest due to the diversity of bioactive compounds they contain and may represent natural sources of substances with antifungal activity (*Dėnė and Valiuškaitė, 2021; Oprescu et al., 2023*).

Several studies have highlighted the potential of garlic (*Allium sativum L.*) extracts for the control of *Botrytis cinerea*. Aqueous and ethanolic extracts have demonstrated the ability to inhibit both mycelial growth and conidial germination, with the effect being concentration-dependent and potentially reaching complete inhibition (*Daniel et al., 2015*). Furthermore, volatile compounds derived from garlic have proved effective in limiting disease development, highlighting the contribution of sulfur-containing compounds to the antifungal activity of this species (*Campa-Siqueiros et al., 2017*). Antifungal activity has also been demonstrated for extracts obtained from garlic peels, for which inhibition of *B. cinerea* mycelial growth of up to 57.57% was reported, together with a reduction in disease development in *in vivo* tests on strawberries (*Carreón-Delgado et al., 2023*).

Antifungal activity against *B. cinerea* has also been reported for sage extracts. *Rabilu et al., (2021),* evaluated aqueous extracts obtained from *Salvia officinalis subsp. lavandulifolia* and *Salvia officinalis subsp. major*, reporting inhibition of the pathogen’s mycelial growth. Efficacy depended on the extract concentration and the harvesting period of the plant material, with the maximum reported inhibition reaching 59.34%.

The antifungal potential of lavender and lemon balm extracts was evaluated by (*Kasmi et al., 2017)*, who assessed aqueous extracts of *Lavandula officinalis* and *Melissa officinalis* against *B. cinerea*. Under *in vitro* conditions, both extracts affected pathogen development, with the effects being concentration-dependent and varying according to the developmental stage of the fungus. Lavender extract exhibited particularly pronounced activity against spore germination, resulting in 79.65% inhibition at a concentration of 1 mg/mL, whereas lemon balm extract showed lower antifungal activity. In *in vivo* tests, the effects of both extracts were considerably weaker, indicating that the efficacy observed *in vitro* is not necessarily maintained under more complex biological conditions.

Compared with other plant species, the antifungal potential of hemp extracts against *Botrytis cinerea* has been less extensively studied, and the available literature in this field remains limited. In this context, *Kursa et al., (2024)*, highlighted the antifungal potential of extracts obtained from *Cannabis sativa L.* inflorescences against several phytopathogenic fungi. In the case of *B. cinerea*, antifungal activity was concentration-dependent, with the most pronounced effect observed for the 20% extract, for which inhibition values ranging from 45.00 to 75.42% were reported.

The available literature thus highlights the potential of plant extracts to inhibit *B. cinerea*, while also showing considerable variability in their efficacy depending on the plant species, concentration used, and experimental conditions. Moreover, existing studies have predominantly focused on individual plant species or employed different experimental conditions, thereby limiting direct comparisons of their efficacy. In this context, evaluating several plant extracts under the same experimental conditions may contribute to identifying the species with the greatest antifungal potential.

Therefore, the present study aimed to comparatively evaluate the antifungal activity of lavender (*Lavandula angustifolia*), lemon balm (*Melissa officinalis*), sage (*Salvia officinalis*), garlic (*Allium sativum*), and hemp (*Cannabis sativa*) extracts against *Botrytis cinerea* isolated from grapes, in order to determine their ability to inhibit pathogen development and to identify extracts with potential for the development of alternative methods for gray mold control.

MATERIALS AND METHODS

Plant material and preparation

The plant material of *Lavandula angustifolia* Mill., *Melissa officinalis* L., *Salvia officinalis* L., and *Allium sativum* L. was obtained from experimental crops established at the National Institute of Research–Development for Machines and Installations Designed for Agriculture and Food Industry (INMA Bucharest, Romania). Lavender, lemon balm, and sage were harvested between May and June by cutting the aerial parts approximately 10–15 cm above the soil surface. Garlic plants were manually harvested by removing the entire plants from the soil, and the whole plant was used for extract preparation. For *Cannabis sativa* L., the monoecious industrial cultivar '*Marideea*', developed at the Agricultural Research and Development Station Secuieni (ARDS Secuieni, Romania), was used. The buds and inflorescences were used for extract preparation.

After harvesting, the plant materials were sorted and cleaned to remove impurities and damaged tissues and subsequently cut into fragments of 8, 10, 20, and 30 mm. The plant materials were dried in a UFE 500 Model 100–800 laboratory oven (Memmert, Germany) at 40 °C until a final moisture content of 8–10% was reached.

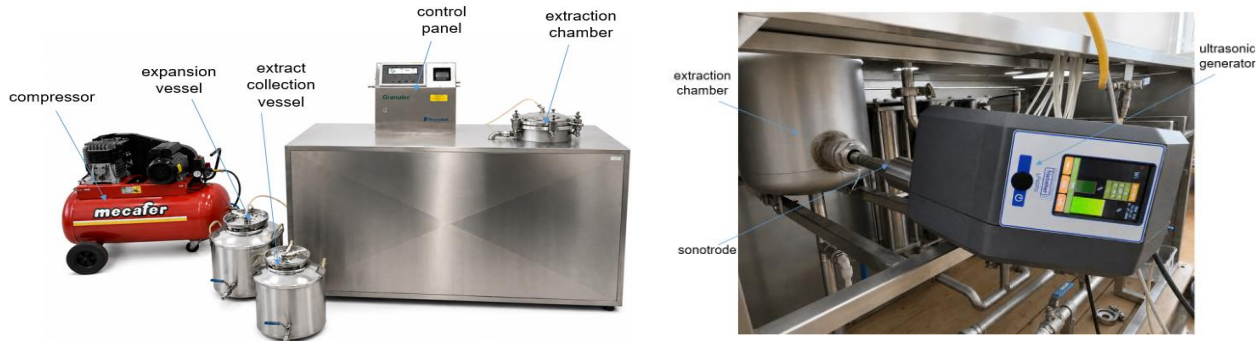
Cannabis sativa L. *Lavandula angustifolia* *Melissa officinalis* *Salvia officinalis* *Allium sativum* L.

Fig. 1 - Dried plant materials prepared for shredding and processing using the hybrid ultrasound-assisted percolation

Preparation of plant extract

The plant extracts were obtained separately from dried and ground material of *Lavandula angustifolia*, *Melissa officinalis*, *Salvia officinalis*, *Allium sativum*, and *Cannabis sativa* using the hybrid ultrasound-assisted pressurized percolation extraction method previously described by (*Tăbărașu et al. 2024b, Tăbărașu et al., 2025c*). For each extraction batch, 400 g of plant material and 12 L of water were used, corresponding to a solid-to-liquid ratio of 1:30 (g/mL). An additional 7 L of water was used as a buffer solvent to fill the pipelines and auxiliary components of the extraction system, thereby ensuring proper system operation.

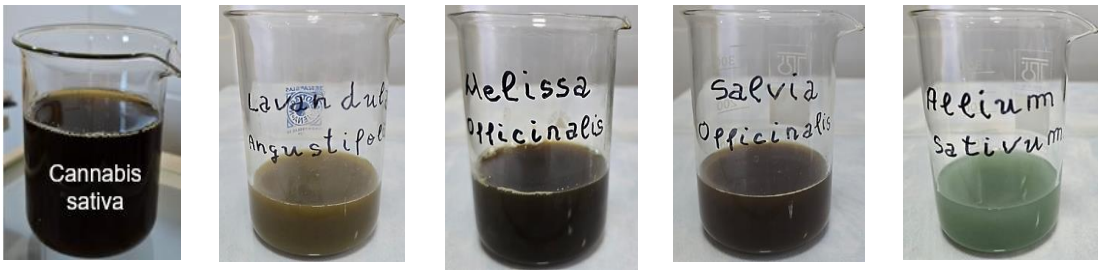
The extractions were performed using a hybrid extraction system consisting of a TIMATIC Duo percolator (Tecnolab SRL, Spello, Italy), adapted to operate with a single 12 L extraction chamber, and an UP400St ultrasonic generator (Hielscher Ultrasonics GmbH, Teltow, Germany) (Figure 2). The plant material was placed in permeable bags inside the extraction chamber, and water was used as the extraction solvent. The operating conditions were selected based on the results of previous studies (*Tăbărașu et al., 2024b; Tăbărașu et al., 2025c*), which identified the most efficient extraction parameters as an extraction time of 120 min, a pressure of 7 bar, and an ultrasonic power of 120 W. Pressurized percolation and ultrasonic treatment were applied simultaneously throughout the entire extraction process.



(a) (b)

Fig. 2 - Hybrid extraction equipment: (a) front view of the TIMATIC Duo extraction system; (b) rear view showing the Hielscher UP400St ultrasonic generator

At the end of the extraction process, the aqueous extracts obtained from each plant species were collected separately, filtered, and stored at 4 °C until further use in antifungal assays against *Botrytis cinerea*.



a) b) c) d) e)

Fig. 3 - Aqueous plant extracts obtained by ultrasound-assisted pressurized percolation

Based on previous studies, an extraction time of 120 min, a pressure of 7 bar, and an ultrasound power of 120 W were maintained as constant operating parameters. In the present study, the influence of plant material particle size was further evaluated using four fragmentation levels (8, 10, 20, and 30 mm), with 30 mm included as a reference condition based on its favorable performance in previous experiments.

Table 1

Experimental parameters used for the preparation of plant extracts

Parameter	Evaluated / selected values	Experimental approach
Plant material particle size	8, 10, 20, 30 mm	Variable
Extraction time	40, 60, 120 min	Variable
Extraction pressure	7 bar	Constant
Extraction power	120 W	Constant
Plant material	400 g	Constant
Solvent volume	12 L	Constant
Solvent	Water	Constant
Solid-to-liquid ratio	1:30 (w/v)	Constant

***Botrytis cinerea* isolation and culture conditions**

The *Botrytis cinerea* strain (ICDPP-BC-02) was obtained from the Microorganism Collection of the Bioproducts Laboratory for Plant Protection, Research and Development Institute for Plant Protection (ICDPP, Bucharest, Romania), and was originally isolated from grapes of the *Fetească albă* cultivar. Fungal identity was confirmed based on macroscopic and microscopic characteristics, together with phenotypic analysis of the metabolic profile using the Biolog Gen III MicroPlate™ system.

The pathogenicity of the isolate was confirmed prior to the antifungal assays by inoculating healthy grape berries and monitoring the development of characteristic gray mold symptoms. The fungal culture was maintained on Potato Dextrose Agar (PDA) at 4°C and periodically subcultured to preserve viability. For inoculum preparation, conidia were harvested from actively growing cultures and suspended in sterile distilled water containing 0.01% Tween 80.

In vitro antifungal activity of plant extracts

Antifungal effect of the plant extracts evaluated by the poisoned food method

Poisoned Food Technique is a standard *in vitro* microbiological method widely used to evaluate the antifungal activity of bioactive plant extracts or synthetic compounds against phytopathogenic fungi such as *Botrytis cinerea*. The method is based on the homogeneous incorporation of a defined concentration of the test extract into a sterile, melted agar growth medium, followed by solidification of the medium. Subsequently, a mycelial plug of *B. cinerea* is placed at the center of the Petri dish and incubated under controlled conditions. Antifungal activity is quantified by measuring the radial mycelial growth of the treated colonies and comparing it with that of the untreated control, with the percentage of mycelial growth inhibition calculated accordingly.

The plant extracts were tested at final concentrations of 10% and 20%. To prepare the experimental treatments, the appropriate amount of each extract was incorporated into 100 mL of Potato Dextrose Agar (PDA) previously cooled to approximately 45–50 °C. After addition of the extracts, the medium was thoroughly homogenized and aseptically poured into 90-mm-diameter Petri dishes, using 20 mL per plate. Five replicates were prepared for each extract and concentration, while PDA medium without plant extract served as the negative control. Approximately 1 h after medium solidification, each plate was aseptically inoculated at the center with a 5.5-mm-diameter mycelial disc, excised using a sterile cork-borer from the actively growing peripheral region of a 7-day-old *B. cinerea* colony. The inoculum discs were transferred using a sterile inoculating loop. The plates were subsequently sealed with Parafilm and incubated under a natural photoperiod at 22 ± 1 °C.

Antifungal activity was evaluated at 2, 5, and 7 days after inoculation by determining the mycelial growth of *B. cinerea*. The diameter of each colony was measured using a digital caliper along two perpendicular axes, and the mean of the two measurements was used for subsequent calculations. The initial diameter of the inoculum disc (5.5 mm) was subtracted from the measured colony diameter prior to calculating mycelial growth and the percentage of inhibition.

The percentage of mycelial growth inhibition (MGI) was calculated relative to the negative control using the following equation 1:

$$ICM(\%) = \frac{D_m - D_p}{D_m} * 100 \quad (1)$$

where:

D_m represents the mycelial growth diameter in the negative control, and D_p represents the mycelial growth diameter in the treatment containing the plant extract.

In vivo antifungal activity of the hydroalcoholic solutions on grape berries

The *in vivo* antifungal activity of the plant extracts against *Botrytis cinerea* was evaluated on grape berries of the Romanian wine grape cultivar *Fetească albă* (*Vitis vinifera* L. cv.). Healthy berries uniform in size and color, with no mechanical damage or visible symptoms of infection, were selected for the experiment.

Prior to inoculation, the berries were surface-disinfected by immersion in a 1% sodium hypochlorite solution for 2 min, followed by three consecutive rinses with sterile distilled water and drying under aseptic conditions. To verify the effectiveness of the disinfection procedure, a subset of surface-disinfected berries was maintained under the same experimental conditions without artificial inoculation with *B. cinerea*.

For controlled inoculation, each berry was superficially wounded to a depth of approximately 1 mm using a sterile needle. A 10 µL aliquot of a *B. cinerea* conidial suspension, adjusted to a concentration of 1×10^5 conidia/mL using a Thoma counting chamber, was applied to the wounded area. At 2 h after inoculation, aqueous extracts of *Lavandula angustifolia*, *Melissa officinalis*, *Salvia officinalis*, and *Allium sativum* were uniformly applied by spraying. Each extract was evaluated individually at concentrations of 5%, 10%, 15%, and 30%. The control treatment consisted of berries inoculated with *B. cinerea* and left untreated with plant extracts.

The experiment was conducted using a completely randomized design. Each treatment consisted of three biological replicates, with 30 grape berries per replicate, resulting in a total of 90 berries per treatment. After treatment application, the berries were maintained in closed plastic containers to ensure a high relative humidity of approximately 90–95% and incubated in the dark at 20 °C for 14 days. Gray mold development was evaluated 14 days after inoculation by determining disease incidence, expressed as the percentage of grape berries showing visible symptoms of *B. cinerea* infection relative to the total number of berries evaluated for each experimental treatment.

Disease incidence was calculated using the following equation 2:

$$DI(\%) = \frac{N_i}{N_t} * 100 \quad (2)$$

where DI represents disease incidence (%),N_i represents the number of grape berries showing visible gray mold symptoms, and N_t represents the total number of grape berries evaluated.

The treatment groups were structured as follows:

- Control: *Botrytis cinerea* inoculation, then after 4 hours sterile water;
- A1: *Botrytis cinerea* + 5% bio-solution in sterile water;
- A2: *Botrytis cinerea* + 10% bio-solution in sterile water;
- A3: *Botrytis cinerea* + 15% bio-solution in distilled water;
- A4: *Botrytis cinerea* + 30% bio-solution in distilled water.

Statistical analysis

Experimental data was analyzed using three-way Analysis of Variance (ANOVA). For the extraction experiment, extraction time, plant material particle size, and plant species were considered as fixed factors, and total polyphenol content was used as the response variable. For the in vitro antifungal assay, botanical extract type, treatment concentration, and incubation time were considered as fixed factors, and mycelial growth inhibition was used as the response variable. Main effects and all two-way and three-way interactions were evaluated. Prior to analysis, data normality and homogeneity of variance were verified. When significant effects were detected, mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) post-hoc test at a significance level of α = 0.05.

RESULTS

Three-way analysis of variance (ANOVA) was performed to evaluate the effects of extraction time, plant material particle size, and plant species on the total polyphenol content obtained by hybrid ultrasound-assisted percolation. The analysis revealed highly significant effects of extraction time ($F_{2,120}$ = 14152.33, p < 0.001), particle size ($F_{3,120}$ = 32.75, p < 0.001), and plant species ($F_{4,120}$ = 1289.40, p < 0.001). Significant interactions were also observed between extraction time and particle size ($F_{6,120}$ = 30.50, p < 0.001), extraction time and plant species ($F_{8,120}$ = 122.62, p < 0.001), and particle size and plant species ($F_{12,120}$ = 2.82, p = 0.0019). Furthermore, the three-way interaction between extraction time, particle size, and plant species was significant ($F_{24,120}$ = 3.87, p < 0.001), indicating that the effect of particle size on polyphenol recovery depended simultaneously on extraction duration and plant species.

Tukey's HSD post-hoc analysis (α = 0.05) showed significant differences among all three extraction times (p < 0.001). The mean total polyphenol content increased from 11.46 mg GAE/100 g after 40 min to 18.45 mg GAE/100 g after 60 min and 29.22 mg GAE/100 g after 120 min, demonstrating the strong influence of extraction duration on polyphenol recovery under the experimental conditions.

Table 2

Extraction method improvement for hybrid ultrasound-assisted percolation technology

Extraction time (min)	Particle size (mm)	Pressure (bar)	Ultrasound power (W)	TPC *Cannabis sativa* (mg GAE/100g)	TPC *Lavandula angustifolia* (mg GAE/100g)	TPC *Melissa officinalis* (mg GAE/100g)	TPC *Salvia officinalis* (mg GAE/100g)	TPC *Allium sativum* (mg GAE/100g)
40	8	7	120	9.67±0.665	10.16±0.290	10.29±0.619	14.57±0.661	10.32±0.481
40	10	7	120	10.13±0.670	10.68±0.310	10.67±0.583	15.03±0.620	9.76±0.488
40	20	7	120	10.92±0.597	11.22±0.321	11.34±0.550	16.11±0.601	8.83±0.479
40	30	7	120	11.29±0.601	11.85±0.387	11.97±0.545	17.18±0.599	7.18±0.471
60	8	7	120	17.73±0.663	18.96±0.670	19.32±0.619	27.33±0.661	13.01±0.480
60	10	7	120	17.10±0.594	17.85±0.559	18.98±0.603	26.84±0.645	12.72±0.472
60	20	7	120	16.78±0.669	17.03±0.576	18.30±0.608	26.11±0.653	12.19±0.483
60	30	7	120	15.83±0.663	17.93±0.598	17.10±0.619	25.90±0.661	11.89±0.480
120	8	7	120	27.15±0.559	33.11±0.631	29.65±0.596	34.79±0.760	27.82±0.465
120	10	7	120	26.43±0.551	32.28±0.631	28.84±0.611	34.18±0.754	27.06±0.454
120	20	7	120	25.71±0.563	31.24±0.562	28.06±0.599	33.25±0.767	26.58±0.470
120	30	7	120	24.03±0.559	30.46±0.549	26.47±0.596	31.92±0.760	25.34±0.465

*TPC – Total polyphenol content

Significant differences were also observed among the plant species evaluated. Across all experimental conditions, *Salvia officinalis* exhibited the highest mean total polyphenol content (25.27 mg GAE/100 g), followed by *Lavandula angustifolia* (20.23 mg GAE/100 g), *Melissa officinalis* (19.25 mg GAE/100 g), *Cannabis sativa* (17.73 mg GAE/100 g), and *Allium sativum* (16.06 mg GAE/100 g). Tukey's HSD test indicated significant differences among all pairwise species comparisons ($p < 0.001$).

Particle size also significantly influenced polyphenol recovery. The overall mean values were 20.26, 19.90, 19.58, and 19.09 mg GAE/100 g for particle sizes of 8, 10, 20, and 30 mm, respectively. Tukey's HSD test indicated significant differences among the particle-size levels ($p < 0.05$). However, because significant interactions involving particle size were detected, particularly the significant three-way interaction, these overall means should be interpreted cautiously, as the magnitude and direction of the particle-size effect depended on both extraction time and plant species.

At the shorter extraction time of 40 min, the influence of particle size varied among plant species, whereas at 120 min smaller particle sizes generally resulted in higher polyphenol recovery. At 120 min, the differences between 8 and 30 mm were approximately 3.12, 2.65, 3.18, 2.87, and 2.48 mg GAE/100 g for *Cannabis sativa, Lavandula angustifolia*, *Melissa officinalis, Salvia officinalis*, and *Allium sativum*, respectively. Although reducing particle size increased polyphenol recovery at the longest extraction time, the additional gain obtained by reducing the material from 30 to 8 mm remained relatively limited compared with the additional energy requirements associated with intensive size reduction.

Overall, the statistical results demonstrate that extraction time exerted a major influence on polyphenol recovery, while the effect of particle size varied according to both extraction duration and plant species. Under the experimental conditions, the combination of a 120 min extraction time, 7 bar pressure, 120 W ultrasound power, and a 30-mm particle size therefore represents a practical technological compromise between polyphenol recovery and process efficiency.

In Vitro Antifungal Efficacy of Selected Essential Oils Against Botrytis cinerea

To address the need for sustainable crop protection solutions, this study evaluated the *in vitro* antifungal efficacy of selected essential oils against *Botrytis cinerea*, systematically characterizing the interaction between oil selection, working concentration, and time-dependent bioactivity retention to support the development of novel botanical formulations.

Table 3

Mycelial growth and growth inhibition of *Botrytis cinerea* in response to hydroalcoholic extract treatments determined by the Poisoned Food technique at 2, 5 and 7 days after inoculation

Hydroalcoholic extract	Concentration (%)	Growth inhibition (%)		
		2 days	*5 days*	*7 days*
Lavandula angustifolia	10	39.84±4.72	25.26 ± 3.91	15.84 ± 3.46
	20	47.14±4.15	32.18 ± 3.67	22.52 ± 3.21
	50	69.62±2.23	44.37 ± 2.84	30.26 ± 2.71
Salvia officinalis	10	40.47±4.75	25.82 ± 4.12	15.46 ± 3.74
	20	48.66±3.36	32.71 ± 3.18	22.12 ± 2.86
	50	58.05±1.53	42.46 ± 1.76	28.27 ± 1.82
Melisa officinalis	10	38.79±3.81	24.74 ± 3.52	14.63 ± 3.17
	20	43.06±0.58	28.47 ± 0.81	18.16 ± 0.94
	50	51.15±1.64	35.38 ± 1.73	23.27 ± 1.69
Allium sativum	10	37.12±0.87	29.85 ± 1.14	20.17 ± 1.26
	20	53.14±4.15	39.72 ± 3.68	28.18 ± 3.51
	50	78.62±2.23	60.36 ± 2.41	39.84 ± 2.63
Cannabis sativa	10	36.22±0.51	23.58 ± 0.73	14.41 ± 0.86
	20	46.24±1.9	31.52 ± 1.83	20.68 ± 1.79
	50	51.31±2.17	34.14 ± 2.08	22.26 ± 2.02
Control (water)	-	0	0	0

Hydroalcoholic extract	Concentration (%)	Growth inhibition (%)		
		2 days	*5 days*	*7 days*
Control (alcohol 6%)	-	3.84 ± 0.72	2.71 ± 0.68	1.96 ± 0.61
Control (chemical)	-	92.10 ± 1.84	94.76 ± 1.42	96.18 ± 1.17

Three-way analysis of variance (ANOVA) showed that mycelial growth inhibition of *Botrytis cinerea* was significantly affected by extract type, concentration, and incubation time ($p < 0.001$ for all three main effects). Incubation time had the largest effect ($F_{2,180} = 1808.64$, $p < 0.001$), followed by extract concentration ($F_{2,180} = 795.32$, $p < 0.001$) and extract type ($F_{4,180} = 145.49$, $p < 0.001$). Significant interactions were also detected between extract type and concentration ($F_{8,180} = 38.68$, $p < 0.001$), extract type and incubation time ($F_{8,180} = 2.59$, $p = 0.010$), and concentration and incubation time ($F_{4,180} = 24.87$, $p < 0.001$). Importantly, the three-way interaction among extract type, concentration, and incubation time was also significant ($F_{16,180} = 3.23$, $p < 0.001$), indicating that the effect of extract concentration on fungal growth inhibition varied according to both botanical species and incubation period.

Tukey's HSD post-hoc test ($\alpha = 0.05$) confirmed significant differences among several extract–concentration combinations at each evaluation time. At 2 days after inoculation, *Allium sativum* extract at 50% produced the highest mycelial growth inhibition (78.62 ± 2.23%), significantly exceeding all other botanical treatments. *Lavandula angustifolia* at 50% showed the second-highest inhibition (69.62 ± 2.23%) and was significantly more effective than the remaining botanical treatments, whereas Salvia officinalis at 50% produced an intermediate inhibition of 58.05 ± 1.53%. The 50% treatments of M*elissa officinalis and Cannabis sativa* showed statistically comparable inhibition values (51.15 ± 1.64% and 51.31 ± 2.17%, respectively).

A similar statistical pattern was observed after 5 days of incubation. The 50% A. sativum treatment remained significantly more effective than the other botanical treatments, with an inhibition of 60.36 ± 2.41%. *L. angustifolia* (44.37 ± 2.84%) and S. officinalis (42.46 ± 1.76%) formed an intermediate efficacy group and did not differ significantly from each other, while *M. officinalis* (35.38 ± 1.73%) and *C. sativa* (34.14 ± 2.08%) showed statistically comparable lower inhibition levels.

After 7 days, A. sativum at 50% continued to exhibit the highest inhibition (39.84 ± 2.63%) and remained significantly superior to all other botanical treatments. *L. angustifolia* (30.26 ± 2.71%) and S. officinalis (28.27 ± 1.82%) showed statistically similar intermediate activity, whereas *M. officinalis* (23.27 ± 1.69%) and *C. sativa* (22.26 ± 2.02%) exhibited the lowest inhibition among the 50% botanical treatments and did not differ significantly from each other.

Tukey's HSD analysis further indicated that the three incubation periods differed significantly within each extract–concentration combination ($p < 0.05$), confirming a progressive reduction in antifungal efficacy between days 2, 5, and 7. Overall, the statistical analysis demonstrates that the antifungal activity of the tested botanical extracts was concentration-dependent but progressively decreased with incubation time, with A. sativum showing the strongest and most persistent inhibitory effect, followed by *L. angustifolia* and *S. officinalis*. The significant three-way interaction indicates that the magnitude of these concentration- and time-dependent effects was specific to the botanical extract evaluated.

A general trend observed across all botanical extracts is the regressive dynamic of inhibition over time. The maximum efficacy recorded on day 2 was followed by a progressive decline in fungistatic effect on days 5 and 7. This phenomenon can be attributed to the gradual volatilization of bioactive compounds or the metabolic capability of the fungus to degrade plant secondary metabolites during advanced stages of mycelial growth. The analysis of experimental controls confirms that the suppressive effect is exclusively attributable to plant biosynthesizes; the 6% alcohol control variant recorded negligible inhibition (ranging between 3.84% and 1.96%), contrasting sharply with the synthetic chemical control, which exhibited persistent and near-total pathogen suppression, increasing from 92.10% on day 2 to 96.18% on day 7.

From an applied perspective, the findings suggest that while botanical extracts—particularly garlic and lavender—represent promising eco-friendly alternatives for mitigating *Botrytis cinerea* infestation, their limited persistence necessitates technological optimization.

To achieve plant protection comparable to synthetic chemical fungicides, higher application concentrations, microencapsulation of bioactive compounds, or repeated applications at short intervals of 3–5 days are recommended.

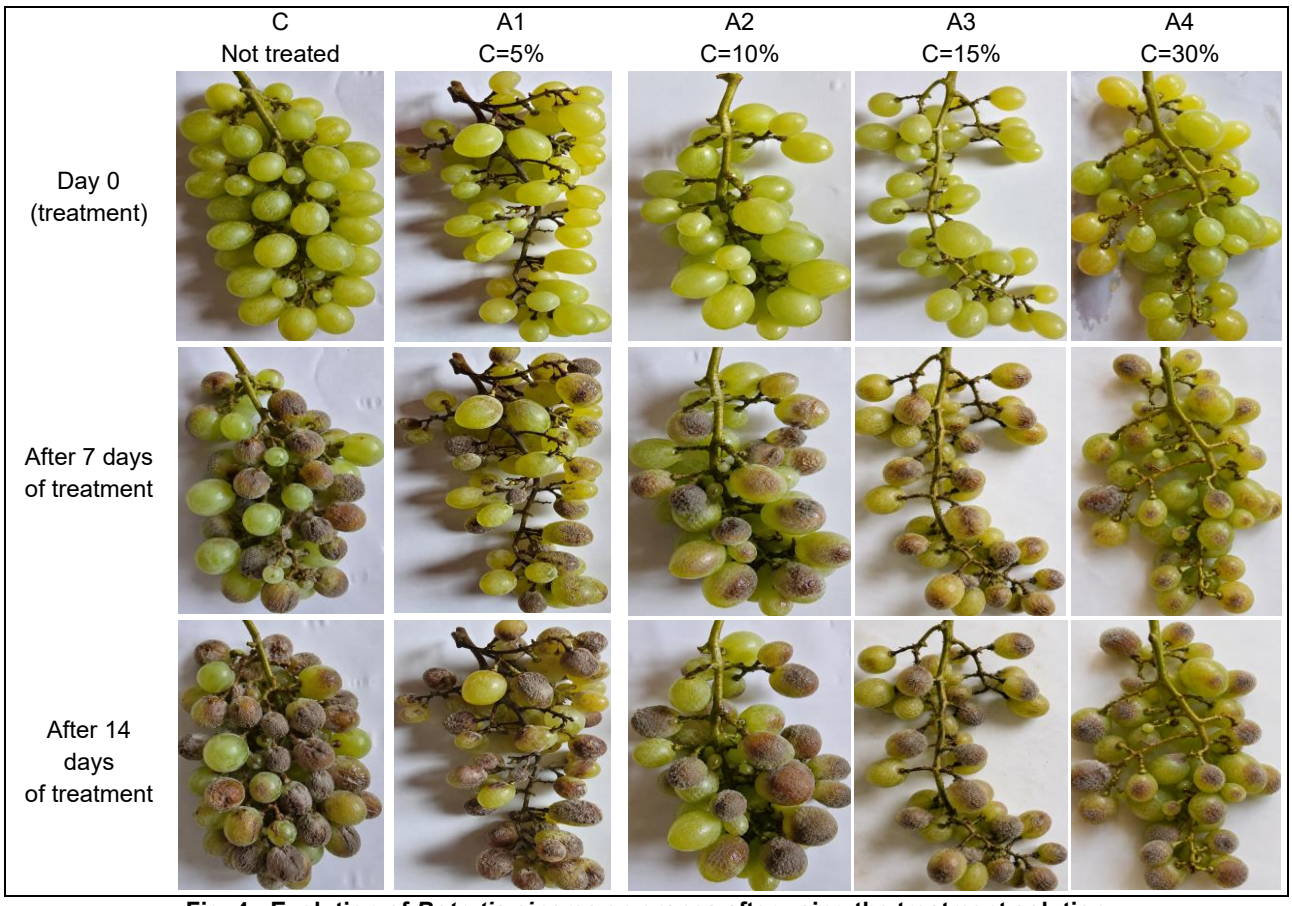


Fig. 4 - Evolution of *Botrytis cinerea* on grapes after using the treatment solution at different concentrations and time periods

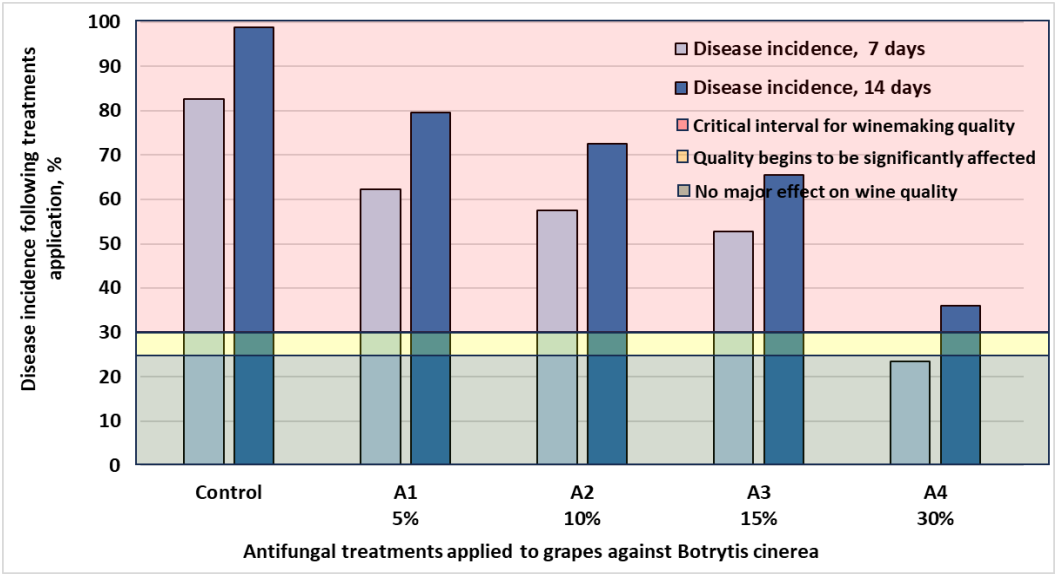


Fig. 5 - Impact of hydroalcoholic plant extracts on *Botrytis cinerea* incidence in grape clusters

The figure 5 demonstrates a clear concentration-dependent antifungal effect, with the incidence of *Botrytis cinerea* progressively decreasing as the concentration of the hydroalcoholic extracts increased. In the control, disease incidence increased from approximately 83% at 7 days to nearly 100% at 14 days, whereas treatments at 5–15% progressively limited infection development, although they were insufficient to maintain disease incidence below the thresholds associated with preserving grape quality. The 30% concentration showed the highest efficacy, reducing disease incidence to approximately 24% after 7 days and 36% after 14 days. The increase in incidence between the two assessment times indicates that the treatment did not completely suppress the infection but rather delayed and limited its progression, with the strongest effect observed at the highest concentration tested.

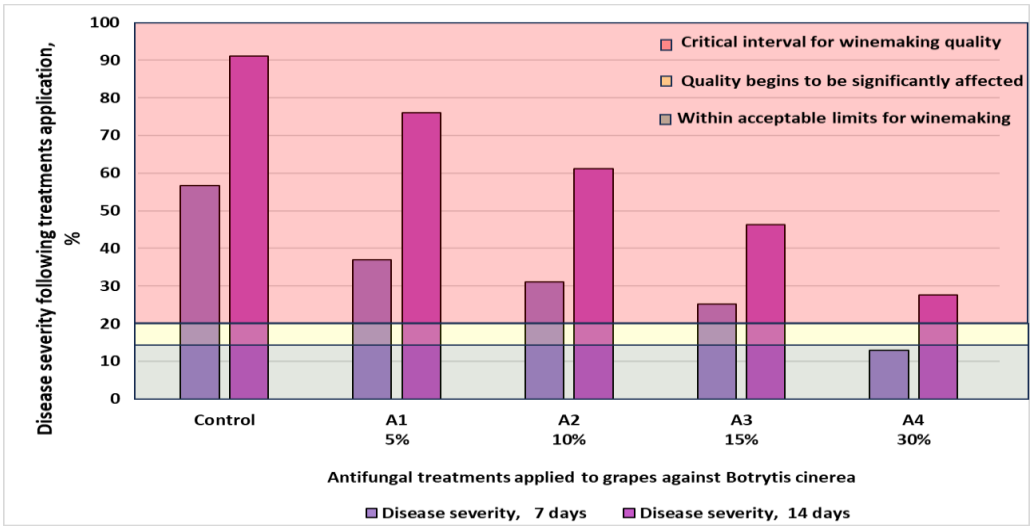


Fig. 6 - Impact of hydroalcoholic plant extracts on *Botrytis cinerea* disease severity in grape clusters

The figure 6 shows a progressive, concentration-dependent reduction in the severity of *Botrytis cinerea* infection. In the control, disease severity increased from approximately 57% after 7 days to 91% after 14 days, confirming the rapid progression of infection. Treatment with hydroalcoholic extracts limited disease development, with severity progressively decreasing as extract concentration increased, reaching approximately 13% at 7 days and 28% at 14 days for A4 (30%). The 30% concentration showed the highest efficacy, being the only treatment that maintained disease severity below 20% after 7 days and substantially limited its progression at 14 days. These results indicate that, although the extracts did not completely prevent disease progression over time, they exerted a clear concentration-dependent protective effect, with the greatest reduction in disease severity observed at the highest concentration tested.

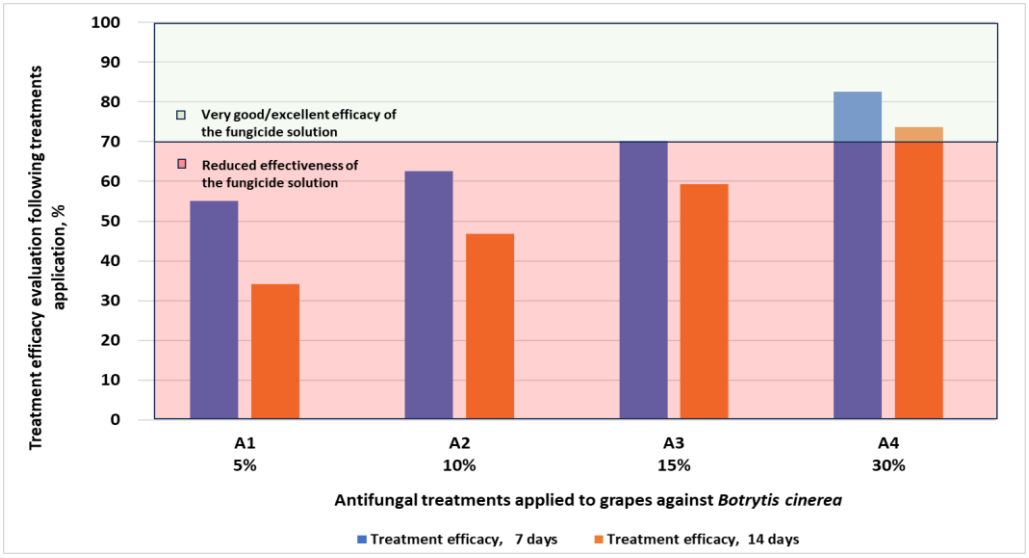


Fig. 7 - Treatment efficiency evaluation on antifungal treatment applied against *Botrytis cinerea* fungus

The figure 7 demonstrates a clear concentration-dependent increase in treatment efficacy against *Botrytis cinerea*. At 5% (A1), efficacy reached approximately 55% after 7 days but decreased to 34% after 14 days, indicating limited persistence of the antifungal effect. Increasing the concentration progressively improved treatment performance, with A2 (10%) and A3 (15%) reaching approximately 62% and 70% efficacy at 7 days, respectively. The highest efficacy was obtained with A4 (30%), reaching approximately 83% after 7 days and remaining at about 74% after 14 days, thus exceeding the 70% threshold associated with very good/excellent efficacy. Although efficacy decreased between 7 and 14 days for all treatments, the results indicate that the 30% concentration provided the strongest and most persistent antifungal effect, confirming the concentration-dependent response of *B. cinerea* to the hydroalcoholic extract treatment.

CONCLUSIONS

The findings of this study demonstrate that hybrid ultrasound-assisted percolation is a highly efficient green technology for recovering bioactive compounds from medicinal and aromatic plants, delivering rich polyphenolic profiles while preserving their structural integrity. Across the in vitro assays, the botanical extracts exhibited clear concentration-dependent antifungal efficacy against *Botrytis cinerea*. Among the single-plant treatments, garlic (*Allium sativum*) and hemp (*Cannabis sativa*) hydroalcoholic extracts achieved the most prominent inhibition of mycelial growth, effectively suppressing fungal development at elevated concentrations. Furthermore, the multi-extract formulation showed enhanced antifungal activity compared to individual extracts, indicating a synergistic action among diverse phytochemicals—such as organosulfur compounds, terpenes, and polyphenols—in disrupting fungal cell membranes and physiological pathways.

When evaluating these results against alternative botanical biopesticides, essential oils derived from aromatic species (e.g., *Lavandula angustifolia* or *Salvia officinalis*) often exhibit superior localized antifungal potency due to their high concentration of volatile lipophilic monoterpenes and sesquiterpenes (*Nenciu et al., 2026*). These volatile fractions penetrate fungal cell walls and disrupt membrane permeability more rapidly than hydroalcoholic extracts. However, pure essential oils present significant operational challenges, including high volatility, rapid photodegradation under field conditions, low extraction yields, and elevated production costs. Hydroalcoholic and aqueous extracts, by contrast, offer a broader spectrum of polar and non-polar phytocompounds, higher extraction yields, better environmental stability, and simpler, cost-effective scalability for bio-fungicide formulations.

Despite these promising biological properties, several practical limitations must be addressed before large-scale adoption. Hydroalcoholic extracts display lower physical persistence on plant tissue post-application, requiring frequent re-application during periods of high disease pressure. Additionally, batch-to-batch variation in secondary metabolite profiles—driven by environmental factors, harvest timing, and raw plant material quality—can result in inconsistent field efficacy.

Future research should focus on optimizing stability through eco-friendly nano-encapsulation or micro-emulsion delivery systems to protect volatile and sensitive polyphenolic compounds from solar degradation. Furthermore, extensive in vivo field trials on grapevine (*Vitis vinifera*) and horticultural crops are required to evaluate phytotoxicity, systemic host defense induction, optimal application timing, and long-term compatibility within integrated pest management (IPM) frameworks.

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