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ARTICLE

Lemon Catnip Hydrolate as a Dual-Function Bioresource: *In Vitro* Assessment of Phytotoxicity and Preservative Activity

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ABSTRACT: (1) Background: Plant hydrolates are widely generated as by-products of essential oil distillation, yet their potential biological properties remain insufficiently explored. This study evaluated whether that lemon catnip (*Nepeta cataria* var. *citriodora*) hydrolate exhibits allelopathic and antimicrobial effects in preliminary *in vitro* assays, contributing to the valorization of distillation residues within circular bioeconomy approaches. (2) Methods: Phytotoxic effects on germination and early seedling growth of crops (maize, soybean, and white clover) and weeds (common lambsquarters, amaranth, and wild carrot) were evaluated under *in vitro* conditions using hydrolate solutions (10%, 20%, 50%, and 100%). Germination kinetics were modelled using first-order, Elovich, double-constant, and Langmuir equations. Antimicrobial, antibiofilm, and antiadhesion activities were assessed against Gram-positive and Gram-negative bacteria and yeasts using agar diffusion and crystal violet screening assays. (3) Results: The hydrolate induced concentration-dependent inhibition of seed germination, with weed species showing greater sensitivity than crops. Complete suppression of weed germination occurred at ≥50% concentration, whereas maize retained partial tolerance. Germination performance indices declined significantly with increasing concentration. In antimicrobial screening assays, the hydrolate showed greater antimicrobial activity and reduced Gram-positive bacteria, reducing *Staphylococcus aureus* biofilm formation by 70% and adhesion by 75%. (4) Conclusions: Lemon catnip hydrolate demonstrated screening-level allelopathic, antimicrobial and biofilm-associated inhibitory effects under *in vitro* conditions. These findings highlight the potential of hydrolates derived from essential oil distillation as bioactive by-products and support further investigation using quantitative antimicrobial testing and applied models to assess their practical relevance.

KEYWORDS: Biofilm inhibition; circular economy; *Nepeta cataria* var. *citriodora*; natural preservatives; seed germination kinetics

1 Introduction

The rising worldwide demand for essential oils is promoting sustainable and circular economy solutions that utilize their by-products, such as hydrolates, post-distillation solid residues, and wastewater [1]. Among these by-products, hydrolates have found the widest practical application, as they do not require any additional processing before use [2]. They are often used as a replacement for the water phase in the cosmetic industry, for sanitation purposes in food processing, and in agriculture as biopesticides [3–5]. Moreover, they can also be applied in more complex technological processes, such as the production of edible coatings, or kombucha and kefir beverage fermentation [6–8]. Considering that hydrolates are aqueous dispersions containing a small proportion (usually less than 1%) of water-soluble and micro-dispersed volatile compounds from essential oils, their biological activity is generally lower than that of the corresponding essential oils [5,9]. Unlike essential oils, whose activity is often assessed in organic or emulsified systems, hydrolates function within a fully aqueous matrix, where compound behavior is governed by solubility, diffusion kinetics, adsorption onto biological surfaces, and partitioning into lipid membranes [10,11]. These physicochemical processes critically determine the effective concentration of bioactive compounds at the target site and, consequently, their bioavailability and biological response [12]. Therefore, the biological activity of hydrolates cannot be inferred solely from their chemical composition, as delivery-dependent factors such as limited membrane partitioning, rapid dilution, and diffusion-controlled transport may significantly modulate their interaction with plant tissues and microbial cells [13,14]. In this context, we hypothesize that the bioactivity of hydrolates is governed not only by their compositional profile but also by physicochemical constraints inherent to aqueous systems, including solubility, concentration gradients, and transport limitations, which may result in selective, concentration-dependent, and system-specific biological effects.

The concept of phytotoxic selectivity refers to the differential responses of plant species exposed to the same allelochemical and is strongly influenced by seed size, seed coat permeability, metabolic detoxification capacity, and membrane composition [15,16]. Smaller seeds generally exhibit greater susceptibility, partly due to higher surface-to-volume ratios that increase exposure per unit mass and facilitate more rapid uptake of phytotoxic compounds, resulting in stronger inhibitory effects on germination and early growth, as observed in studies comparing hydrolate effects across species with different seed sizes [17]. Terpenes, the main bioactive constituents of essential oils and hydrolates, have been shown to penetrate cell membranes, induce oxidative stress, alter mitochondrial function, and interfere with enzymatic processes essential for germination and early seedling development [18,19]. In antimicrobial systems, the designation of hydrolates as natural preservatives requires mechanistic interpretation [20]. Monoterpene alcohols exert antimicrobial effects primarily through adsorption to microbial cell surfaces, increased membrane permeability, leakage of intracellular constituents, and disruption of the proton motive force [11,21]. Biofilm inhibition further involves interference with quorum-sensing pathways and suppression of extracellular polymeric substance synthesis [22]. The effectiveness of such activity in aqueous matrices depends on concentration gradients, compound partitioning behavior, and interactions with microbial cell envelopes [23]. Recent advances in biologically mediated treatment and biosorption-based systems emphasize the importance of adsorption and surface interactions in determining contaminant fate and biological inhibition in water environments [24,25]. Although hydrolates are not classical biosorbents, their aqueous-phase activity may share conceptual similarities with such systems, particularly regarding compound–surface interactions, diffusion-controlled transport, and bioavailability constraints [26]. Incorporating these mechanistic perspectives allows a more rigorous interpretation of hydrolate bioactivity beyond descriptive phytotoxic or antimicrobial outcomes [9]. Despite growing interest in plant hydrolates, few studies critically assess their multifunctional behavior by

simultaneously evaluating *in vitro* phytotoxicity and antimicrobial activity within a unified experimental framework [17,27,28]. This gap limits the understanding and rational development of hydrolate-based agro-food applications.

Our previous investigation showed that the lemon catnip essential oil and hydrolate (*Nepeta cataria* var. *citriodora*), have very similar compositions, being predominantly composed of the terpene alcohol isomers nerol and geraniol, which give both the essential oil and the hydrolate a pleasant citrus scent [29]. Given that the essential oil of this plant species has an aroma very similar to that of lemon balm (*Melissa officinalis*), it can be used as an alternative source of lemon-scented essential oil to lemon balm, valued for its antinociceptive, antidepressant, and anxiolytic activities, as well as for its beneficial effects on gastrointestinal and heart-related symptoms accompanying anxiety disorders [30–32]. Moreover, research has shown that lemon catnip exhibits a wide range of bioactivities, including antioxidant, antihyperglycemic, anti-inflammatory, and antimicrobial potential, as well as insect-repellent properties [33,34]. Additionally, since geraniol represents a key terpene alcohol of significant commercial relevance to the flavor and fragrance industries and is a common constituent of their consumer products, lemon catnip essential oil can serve as a potential source of this compound [35].

Although the essential oil and hydrolate of lemon catnip share a broadly similar chemical profile, such compositional similarity does not necessarily translate into equivalent biological activity, as functional effects depend strongly on formulation, phase behavior, and compound availability in aqueous systems [9,27,36,37]. In particular, the hydrolate represents a dilute aqueous system in which the distribution, transport, and membrane accessibility of bioactive compounds differ substantially from those in the essential oil phase. Therefore, its biological effects must be evaluated independently rather than inferred from essential oil composition. Recent environmental research increasingly highlights the value of integrated biogeochemical control strategies capable of simultaneously addressing contaminant mitigation and resource efficiency within agroecosystems [38]. Such strategies move beyond single-function interventions, evaluating multifunctional systems that couple biological activity with reduced chemical inputs [39]. Nevertheless, these studies have not systematically assessed hydrolates as multifunctional agents combining phytotoxic and antimicrobial activities within a unified experimental design, limiting mechanistic understanding of their potential contribution to sustainable agro-food systems.

This study aimed to evaluate the bioactivity of lemon catnip hydrolate with a primary focus on its *in vitro* phytotoxic effects on selected cultivated plants and weeds. In particular, germination responses were analyzed using kinetic models to assess potential selectivity and concentration-dependent effects. As a secondary objective, the antimicrobial activity of the hydrolate was screened against a panel of Gram-negative and Gram-positive bacteria and yeasts to explore its broader biological potential within the same aqueous framework. Given the exploratory nature of this work, all experiments were conducted under controlled conditions as a preliminary screening to identify potential bioactivity patterns rather than to establish application-ready efficacy. This approach enables a comparative assessment of plant and microbial responses while providing a basis for more targeted, mechanism-oriented studies in future research.

2 Material and Methods

2.1 Plant Material and Hydrolate Production

Lemon catnip (*N. cataria* var. *citriodora*; BUNS vouch number 2-1401) was cultivated at the Institute of Field and Vegetable Crops in Novi Sad, in the Department of Alternative Crops located in Bački Petrovac. The plants were harvested at peak flowering, dried in a solar dryer to a constant weight, and then processed by steam distillation.

Essential oil extraction was performed by steam distillation using a semi-industrial stainless steel apparatus. Air-dried lemon catnip herba (approximately 30 kg per batch; three batches in total) was loaded into the distillation chamber, hermetically sealed (Inox Ltd., Bački Petrovac, Serbia), and subjected to steam generated by a separate high-pressure boiler, Vaporax (Ventilator Ltd., Zagreb, Croatia). Distillation was carried out in an open system at atmospheric pressure, with steam continuously passing through the plant material and conveying volatile compounds through a cooler and condenser to a Florentine vessel (Iskra Ltd., Poreč, Croatia). The essential oil floated to the surface of the aqueous layer, while some water-soluble components dissolved in the water, imparting a characteristic aroma and flavor resembling that of the essential oil. Based on previous studies on this species, a 4 h distillation time was identified as optimal for lemon catnip to maximize volatile oil recovery while maintaining the integrity of major volatile compounds [29,33]. After four hours of distillation, the essential oil was decanted, and the remaining hydrolate was filtered through *Macherey-Nagel MN 651/120 filter paper*, transferred to sterile plastic containers, and stored in a dark place at room temperature until further analysis, which was conducted within one month (chemical characterization, phytotoxicity, and antimicrobial bioassays). These storage conditions were selected to minimize oxidative and hydrolytic changes in the hydrolate, although no time-dependent stability analysis was performed, considering the short time elapsed between production and subsequent chemical and biological analyses. Hydrolates are generally stable for up to 12 months when stored in their original packaging at room temperature in a dry, dark place [40]. However, over an extended period of up to two years, their composition may change, possibly due to hydration processes during storage [2]. Physicochemical parameters, including pH and conductivity, were measured at 25°C using a multiparameter benchtop meter (HI2600) (Table 1).

Table 1: Physicochemical parameters of lemon catnip hydrolate and distilled water.

	Lemon Catnip Hydrolate	**Distilled Water**
pH	5.4 ± 0.2	6.0 ± 0.2
Conductivity	130 ± 21 µS/cm	0.5 ± 0.1 µS/cm

Prior to GC-MS and GC-FID analysis, the lemon catnip hydrolate was subjected to simultaneous steam distillation and extraction (SDE) using a Likens-Nickerson apparatus, one of the most widely employed techniques to isolate the volatile fractions present in the hydrolate [41]. Considering both its advantages and limitations as a sample preparation method, it is often the most effective approach for obtaining a representative extract with high overall recovery across a broad range of compounds [42].

Analysis of the volatile fraction was carried out using an Agilent 7890A GC system, equipped with a nonpolar HP-5MS fused-silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness), a flame ionization detector (FID), and an Agilent 5973 mass spectrometry detector (MSD). Compound identification was based on retention indices, calculated using a standard mixture of alkanes (C8–C24), and by comparing the obtained mass spectra with those in the Wiley and NIST reference libraries.

A total of 19 volatile compounds were detected in the extracted fraction of the lemon catnip hydrolate (Table 2), with nerol (48.5%), geraniol (31.4%), neral (6.8%), and geranial (6.2%) as the dominant compounds, accounting for 92.9% of the total volatile profile.

Table 2: Volatile profile of lemon catnip hydrolate (values are presented as mean ± SD of three independent replicates, expressed as percentages).

No.	Compound	Rt	RI_{exp}	RI_{lit}	% ± SD
1	6-methyl-5-Hepten-2-one	7.275	982	981	1.3 ± 0.92
2	*cis*-Linalool oxide (furanoid)	10.351	1069	1067	0.1 ± 0.00
3	Linalool	11.413	1097	1095	0.9 ± 0.85
4	*cis*-Rose oxide	11.885	1108	1106	0.1 ± 0.00
5	*trans*-Rose oxide	12.552	1127	1122	0.1 ± 0.03
6	Camphor	13.241	1144	1141	0.1 ± 0.11
7	β-Pinene oxide	13.502	1148	1154	0.2 ± 0.35
8	*trans*-Chrysanthemal	13.510	1144	1154	0.3 ± 0.29
9	iso-Isopulegol	13.644	1157	1155	0.1 ± 0.17
10	Nerol oxide	13.673	1153	1156	0.1 ± 0.06
11	Borneol	14.179	1165	1165	0.2 ± 0.40
12	Lavandulol	14.542	1168	1165	0.3 ± 0.46
13	Terpinen-4-ol	14.696	1177	1174	0.2 ± 0.35
14	α-Terpineol	15.298	1185	1186	0.3 ± 0.10
15	2,6,6-trimethyl-2-Cyclohexene-1-methanol	15.98	1200	1187	0.2 ± 0.40
16	Nerol	17.067	1223	1227	48.5 ± 5.44
17	Neral	17.560	1235	1235	6.8 ± 2.65
18	Geraniol	18.231	1251	1249	31.4 ± 1.77
19	Geranial	18.889	1265	1264	6.2 ± 2.89
	Total				97.4

Rt—retention time; RI_{exp}—retention index experimentally determined using an n-alkane mixture (C8-C24) on the nonpolar HP-5MS capillary column; RI_{lit}—retention indices from literature (Wiley and NIST) reference libraries.

2.2 In Vitro Germination

In vitro phytotoxicity bioassays were conducted using seeds of three cultivated species originating from commercially available seed lots provided by the Institute of Field and Vegetable Crops, Novi Sad, Serbia, namely maize (*Zea mays* L.; BUNS voucher number 2-0682), soybean (*Glycine max* (L.) Merr.; BUNS voucher number 2-0657), and white clover (*Trifolium repens* L.; BUNS voucher number 2-0656). Seeds of three weed species were collected from naturally established populations in the field and included common lambsquarters (*Chenopodium album* L.; BUNS voucher number 2-0655), redroot pigweed (*Amaranthus retroflexus* L.; BUNS voucher number 2-0680), and wild carrot (*Daucus carota* subsp. *carota* L.; BUNS voucher number 2-0654). For each species, 100 seeds per replicate were used, with four replicates per treatment, following standard protocols to ensure sufficient statistical power. Seeds were disinfected with 2% sodium hypochlorite for 2 min prior to sowing.

The selected concentration range of hydrolate (10%, 20%, 50%, and 100%) was chosen to capture both inhibitory and sub-inhibitory effects on seed germination and early seedling growth, enabling assessment of dose-dependent phytotoxic effects. Distilled water served as the primary control to account for baseline germination. Although additional buffered or isotonic controls could further refine the experimental design, the present study focuses on comparative phytotoxic screening under controlled conditions, and the small pH difference (5.4 vs. 6.0) is unlikely to be the primary factor influencing the observed effects. Seeds were placed in a sterile Petri dish (150 × 25 mm) on filter paper soaked with 10 mL of the hydrolate, its dilutions, or distilled water (control), with four replicate dishes per treatment. Petri dishes with seeds were maintained in a climate chamber at 22/20°C during a 12-h photoperiod and at 60 ± 2% humidity for 10 days. Petri dishes were arranged in a randomized complete block design within the climate chamber to minimize

positional effects. The seed germination rate was recorded daily, and shoot and root lengths were measured at the end of the experiment [28].

Germination indices and kinetic models were applied to provide a comprehensive quantitative description of germination dynamics and seedling growth across different hydrolate concentrations, enabling evaluation of temporal patterns and dose–response relationships. Several germination indices were calculated, including germination percentage (GP), coefficient of velocity of germination (CVG), germination index (GI), median germination time (t50), and germination rate index (GRI). In addition, these models were used to describe the relationship between seed growth and time, depending on hydrolate concentration [43]. When considering only changes in seed germination, empirical models such as the first-order kinetic, Elovich, double-constant, and Langmuir equations were applied [44,45]. In terms of error analysis, the accuracy of the developed models was evaluated through several key metrics, i.e.,: coefficient of determination (R^2), reduced chi-square (χ^2), mean bias error (MBE), root mean square error (RMSE), mean percentage error (MPE), the sum of squared errors (SSE), and average absolute relative deviation (AARD). The formulas used to calculate the kinetic models of seed germination are presented in Appendix A (Eqs. (A1)–(A10)).

2.3 In Vitro Antimicrobial Effects

Eight different microbial strains belonging to Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* serovar Typhimurium ATCC 13311), Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 19115), and yeasts (*Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763) were used in this study. Antimicrobial activity of lemon catnip hydrolate was evaluated using the agar disk-diffusion method. Briefly, standardised microbial suspensions (10^6 CFU/mL) were inoculated onto Mueller–Hinton agar (bacteria) or Sabouraud agar (yeasts). Sterile paper disks (6 mm) were impregnated with 15 µL of undiluted hydrolate and placed on inoculated plates. After incubation (24 h at 37°C for bacteria; 48 h at 28°C for yeasts), inhibition zones were measured in millimeters (mm). A negative control (sterile water) and positive control (standard antimicrobial agent) were incorporated to provide context for the observed activity. Biofilm biomass reduction and antiadhesion activities were assessed using the crystal violet staining method in 96-well microplates. Reduction in crystal violet-stained biofilm biomass was calculated as percentage reduction relative to untreated controls, while antiadhesion activity was determined as the percentage reduction in initial cell attachment. All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation.

2.4 Statistical Analysis

Prior to applying parametric analyses, the assumptions of normality and homoscedasticity were formally evaluated. Given the relatively small sample size, normality was primarily assessed using the Anderson–Darling test, which provides strong sensitivity to deviations in the tails of the distribution. The Shapiro–Wilk test was additionally applied as a confirmatory procedure. Homogeneity of variances was tested using Levene's test [46]. To address the issue of multiple comparisons, post hoc analyses were conducted using Tukey's HSD test with adjustment for family-wise error rate. Furthermore, the half-maximal effective concentration EC50, was determined for each species, the percentage of inhibition was calculated following the method of Williamson and Richardson [47]. All tests were performed in triplicate, and the statistical analysis of the data was conducted using Statistica 10 software.

3 Results

3.1 Seed Germination and Germination Kinetics Models

All parameters relevant to seed germination assessment (GP, CVG, GI, t_{50}, and GRI) in cultivated and weed species, following the application of different concentrations of lemon catnip hydrolate (10, 20, 50, and 100%), along with the control (distilled water), are presented in Table 3. As shown, the germination of all tested species was lower when the hydrolate was applied compared to the control, and increasing the hydrolate concentration further reduced germination. This effect was particularly pronounced in species with smaller seeds (white clover, common lambsquarters, amaranth, and wild carrot). In brief, the highest concentrations of hydrolate (50% and 100%) completely inhibited seed germination in all weed species, while, the highest CVG, GI, and GRI values, as well as the lowest t_{50} values were observed for white clover. Additionally, the concentration of hydrolate causing 50% inhibition of seed germination for each species (EC_{50}) was determined. Based on the results, it can be concluded that lemon catnip hydrolate exhibited the strongest inhibitory effect in weed species: amaranth (5.8%), followed by wild carrot (6.8%) and common lambsquarters (8.5%). In contrast, significantly higher concentrations were required to inhibit cultivated species: soybean (25.2%), white clover (32.7%), and maize (90.6%).

Table 3: Germination percentage and calculated germination indices of tested plant species treated with the control (distilled water) and different concentrations of lemon catnip hydrolate solution (10, 20, 50, and 100%).

Tested Plant	Concentration	GP (%)	CVG (%/Day)	GI	t_{50} (Days)	GRI (%/Day)	EC_{50} (%)
Maize	control	83 ± 4	24.20 ± 1.2	574 ± 29	3.17 ± 0.16	22.17 ± 1.10	90.6
	10%	75 ± 4	21.99 ± 1.1	484 ± 24	3.68 ± 0.18	18.94 ± 0.95	
	20%	77 ± 4	23.26 ± 1.2	516 ± 26	3.39 ± 0.17	19.88 ± 1.00	
	50%	59 ± 3	23.14 ± 1.1	394 ± 20	3.65 ± 0.18	14.95 ± 0.75	
	100%	36 ± 2	21.43 ± 1.1	198 ± 10	5.25 ± 0.27	7.61 ± 0.38	
Soybean	control	93 ± 5	24.54 ± 1.2	633 ± 32	2.81 ± 0.14	27.49 ± 1.38	25.2
	10%	53 ± 3	17.49 ± 0.9	280 ± 14	5.42 ± 0.27	11.80 ± 0.59	
	20%	47 ± 2	16.67 ± 0.8	235 ± 12	6.32 ± 0.31	9.89 ± 0.49	
	50%	24 ± 1	23.53 ± 1.2	141 ± 7	4.50 ± 0.22	5.86 ± 0.29	
	100%	8 ± 0	22.86 ± 1.1	53 ± 3	3.50 ± 0.17	2.15 ± 0.11	
White clover	control	93 ± 3	37.36 ± 1.9	778 ± 39	1.73 ± 0.09	40.12 ± 2.00	32.7
	10%	86 ± 1	36.13 ± 1.8	668 ± 33	2.22 ± 0.11	29.62 ± 1.47	
	20%	79 ± 0	26.69 ± 1.3	573 ± 29	2.67 ± 0.13	24.31 ± 1.21	
	50%	1 ± 0	33.33 ± 1.7	8 ± 0	2.50 ± 0.12	0.33 ± 0.02	
	100%	-	-	-	-	-	
Common lambsquarters	control	51 ± 3	24.40 ± 1.2	352 ± 17	3.50 ± 0.18	14.04 ± 0.70	8.5
	10%	21 ± 1	19.44 ± 1.0	123 ± 6	4.90 ± 0.25	4.46 ± 0.22	
	20%	8 ± 1	16.00 ± 0.8	38 ± 2	6.33 ± 0.31	1.4 ± 0.07	
	50%	-	-	-	-	-	
	100%	-	-	-	-	-	
Amaranth	control	83 ± 4	32.17 ± 1.6	655 ± 33	2.22 ± 0.11	30.98 ± 1.56	5.8
	10%	12 ± 1	16.67 ± 0.8	15 ± 1	8.50 ± 0.43	15.3 ± 0.76	
	20%	7 ± 0	16.67 ± 0.8	5 ± 0	7.25 ± 0.36	0.93 ± 0.05	
	50%	-	-	-	-	-	
	100%	-	-	-	-	-	

Table 3: *Cont.*

Tested Plant	Concentration	GP (%)	CVG (%/Day)	GI	t_{50} (Days)	GRI (%/Day)	EC_{50} (%)
Wild carrot	control	53 ± 3	21.37 ± 1.1	335 ± 17	4.18 ± 0.21	13.72 ± 0.68	6.8
	10%	14 ± 1	14.29 ± 0.7	56 ± 3	6.67 ± 0.34	2.22 ± 0.11	
	20%	11 ± 1	13.92 ± 0.7	42 ± 2	6.75 ± 0.34	1.63 ± 0.08	
	50%	-	-	-	-	-	
	100%	-	-	-	-	-	

Germination percentage; CVG—Coefficient of velocity of germination; GI—Germination index; t_{50}—Median germination time; GRI—Germination rate index; EC_{50}—represents the concentration of hydrolate causing 50% inhibition of seed germination.

Across all kinetic models, increasing concentrations of lemon catnip hydrolate consistently suppress seed germination (Appendix B.1). The First-order model shows a strong concentration-dependent decline in germination, with several species, such as white clover, common lambsquarters, and amaranth, reaching complete inhibition at higher concentrations. The Elovich model similarly indicates marked reductions in the germination rate, particularly in species with smaller seeds. The Double constants model also reflects a progressive loss of germination potential, with some species reduced to zero at elevated concentrations. In contrast, the Langmuir model produces unrealistic and extremely high Y_{max} values for several species, indicating overfitting and reduced reliability under stress conditions. The Langmuir model, although occasionally showing acceptable R^2 values, produced unstable Y_{max} estimates under high inhibition levels, indicating over-parameterization for those conditions. In summary, all models except the Langmuir model suggested the time and concentration-dependent inhibition of germination by lemon catnip hydrolate. Figs. 1–6 present the germination kinetics in germination maize, soybean, white clover, common lambsquarters, amaranth, and wild carrot, over ten days (1–10) depend on different concentrations of lemon catnip hydrolate solution.

3.1.1 Maize Germination Kinetics and Model Verification

Maize germination after 10 days was highest in the control treatment (83%) and decreased progressively with increasing hydrolate concentration with a pronounced inhibitory effect at 50% and in the undiluted treatment (Fig. 1). Germination speed indicators (CVG, GI, and GRI) followed the same trend, showing faster and more uniform germination in the control and at lower hydrolate concentrations, whereas higher concentrations significantly delayed germination, as reflected by increased t_{50} values. Kinetic modeling of maize germination demonstrated that the Elovich equation provided the best overall fit across all treatments, exhibiting consistently high coefficients of determination and the lowest error values. The Langmuir model also performed well, but only at higher hydrolate concentrations, while the double constants model showed moderate accuracy. In contrast, the first-order kinetic model displayed the weakest predictive performance, indicating limited suitability for describing maize germination dynamics under lemon catnip hydrolate exposure. Verification calculations for this model are provided in Appendix B.2.

3.1.2 Soybean Germination Kinetics and Model Verification

After 10 days, soybean germination was highest in the control treatment (93%) and decreased markedly with increasing concentrations of lemon catnip hydrolate. Even low hydrolate doses (10% and 20%) substantially reduced germination, while a pronounced inhibitory effect was observed at 50% concentration and in the undiluted hydrolate, where germination dropped to 24% and 8%, respectively (Fig. 2). In contrast, CVG and t50 did not show a consistent response pattern across treatments. Germination indices

reflecting both rate and uniformity (GI and GRI) declined sharply with increasing hydrolate concentration, indicating strong suppression of germination performance. Kinetic modeling revealed that the first-order model showed the weakest predictive performance, with high error values, particularly in the control treatment. The Elovich equation generally provided a good fit, although some overestimation was observed at intermediate hydrolate concentrations. The double constants model demonstrated strong predictive ability, especially at lower and intermediate hydrolate concentrations. The Elovich equation showed the most reliable performance across treatments, exhibiting the highest coefficients of determination and consistently low error values. Verification calculations for soybean germination kinetics are presented in Appendix B.3.

3.1.3 White Clover Germination Kinetics and Model Verification

White clover germination after 10 days was high in the control treatment (93%) and declined with increasing concentrations of lemon catnip hydrolate. Low hydrolate concentrations (10% and 20%) moderately reduced germination, whereas higher concentrations (50% and undiluted hydrolate) completely inhibited germination (Fig. 3). Germination speed and performance indicators (CVG, GI, and GRI) decreased progressively with increasing hydrolate concentration, while t50 values increased, indicating delayed germination even at lower doses. Kinetic modeling showed that the first-order kinetic model performed poorest, exhibiting low coefficients of determination and high error values. The Elovich equation provided improved fits, particularly at low hydrolate concentrations, whereas the double constants model showed moderate and less consistent performance. Overall, the Elovich equation demonstrated the most reliable predictive ability across treatments, with relatively high R^2 values and low errors. Verification calculations for white clover germination kinetics are presented in Appendix B.4.

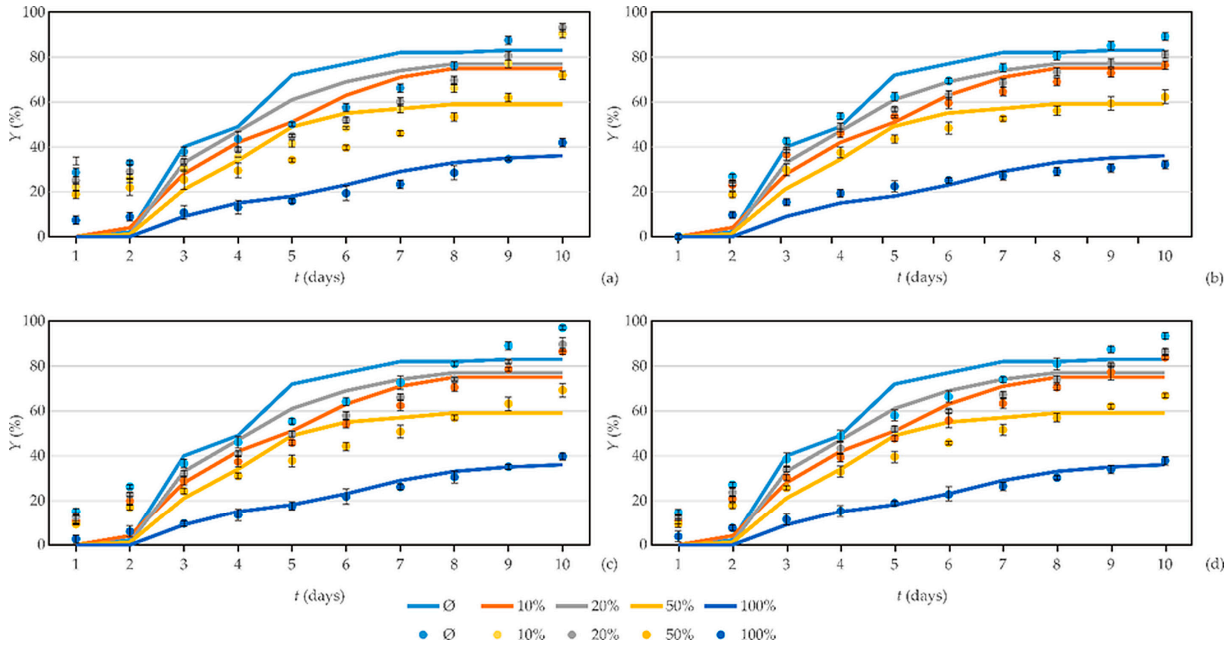

Figure 1: Germination kinetics in maize, over ten days depend on different concentrations of lemon catnip hydrolate solution (markers signify the experimental data; lines indicate predictive results): (**a**) first-order kinetic equation, (**b**) Elovich equation, (**c**) Langmuir equation, and (**d**) double-constant equation.

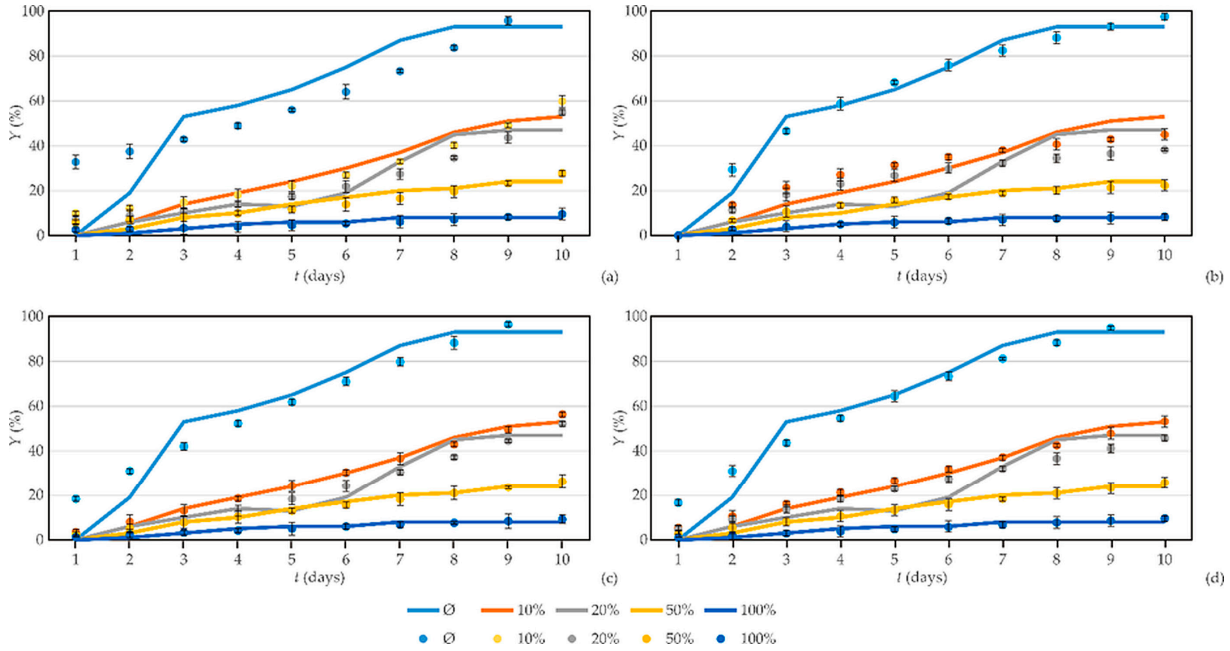

Figure 2: Germination kinetics in soybean, over ten days depend on different concentrations of lemon catnip hydrolate solution (markers signify the experimental data; lines indicate predictive results): (**a**) first-order kinetic equation, (**b**) Elovich equation, (**c**) Langmuir equation, and (**d**) double-constant equation.

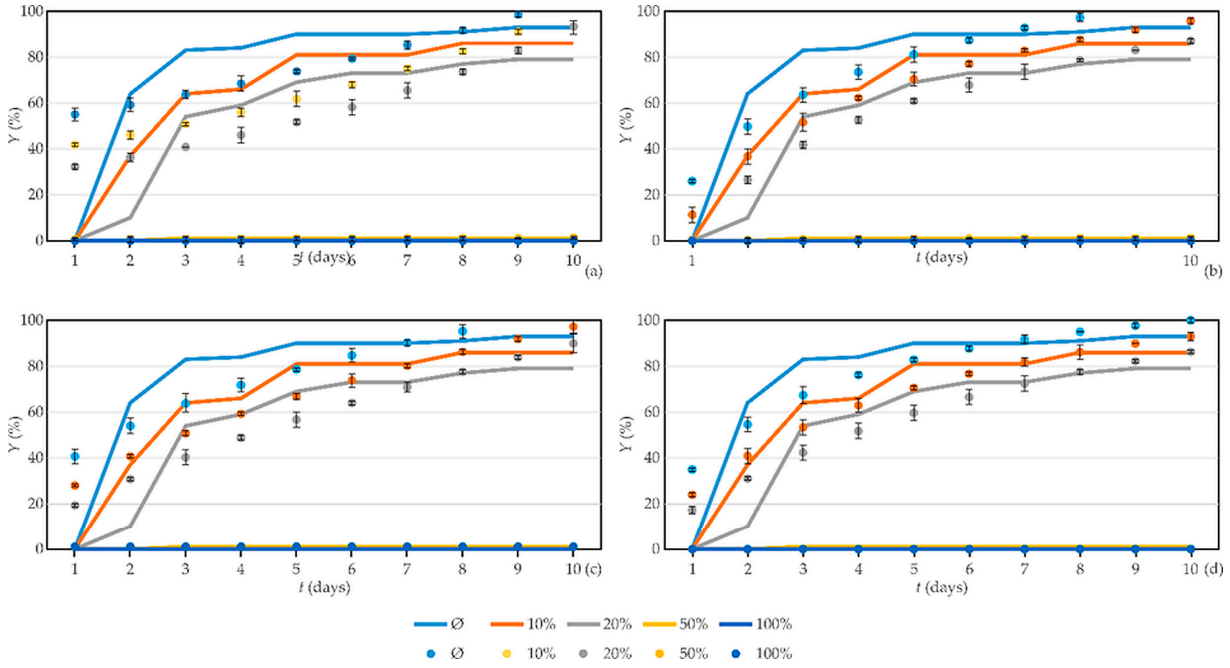

Figure 3: Germination kinetics in white clover, over ten days depend on different concentrations of lemon catnip hydrolate solution (markers signify the experimental data; lines indicate predictive results): (**a**) first-order kinetic equation, (**b**) Elovich equation, (**c**) Langmuir equation, and (**d**) double-constant equation.

3.1.4 Common Lambsquarters Germination Kinetics and Model Verification

Germination of common lambsquarters in the control treatment was moderate (51%) but decreased sharply with increasing concentrations of lemon catnip hydrolate. Even low hydrolate concentrations substantially inhibited germination, which dropped to 21% and 8% at 10% and 20% hydrolate solutions, respectively, while higher concentrations (50% and undiluted hydrolate) completely suppressed germination (Fig. 4). Germination performance indicators (CVG, GI, and GRI) declined progressively with increasing hydrolate concentration, whereas t50 values increased, indicating delayed germination at sublethal concentrations. Kinetic modeling revealed that the first-order kinetic model performed poorest, showing low coefficients of determination and high error values. The Elovich equation provided improved predictive accuracy, particularly at low hydrolate concentrations, while the double constants model exhibited moderate and variable performance. The Elovich kinetic equation showed the strongest predictive ability across treatments, with the highest R^2 values and relatively low error indices. Verification calculations for common lambsquarters germination kinetics are presented in Appendix B.5.

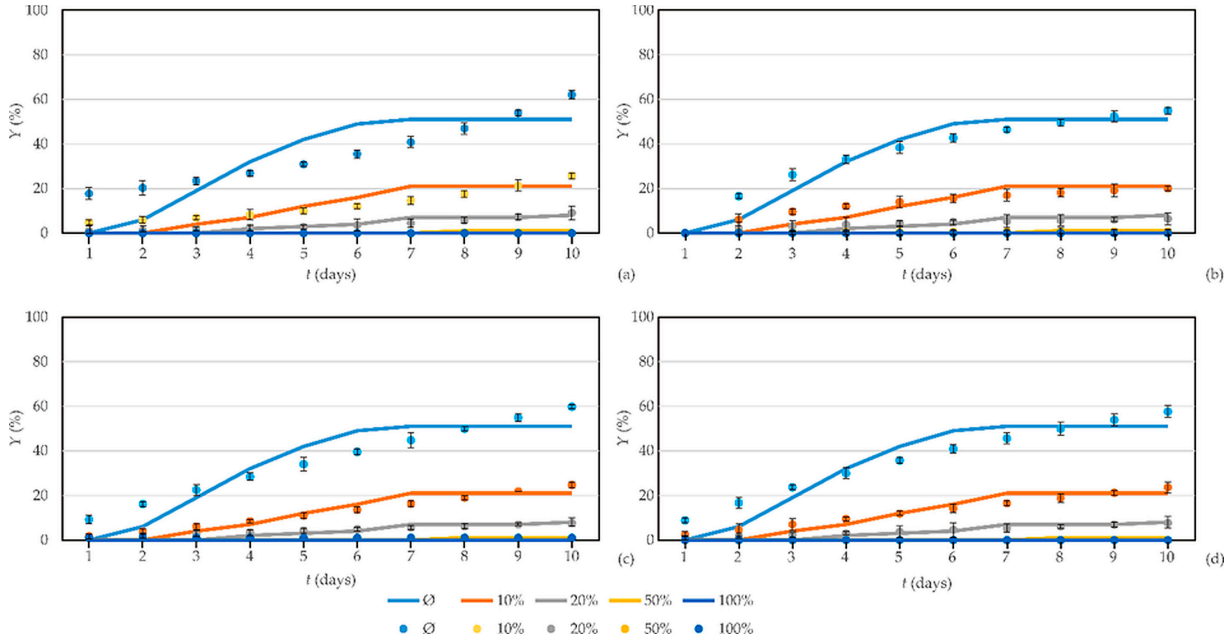

Figure 4: Germination kinetics in common lambsquarters, over ten days depend on different concentrations of lemon catnip hydrolate solution (markers signify the experimental data; lines indicate predictive results): (**a**) first-order kinetic equation, (**b**) Elovich equation, (**c**) Langmuir equation, and (**d**) double-constant equation.

3.1.5 Amaranth Germination Kinetics and Model Verification

Amaranth germination in the control treatment was high (83%) but was strongly inhibited by lemon catnip hydrolate even at low concentrations (Fig. 5). Germination dropped sharply to 12% and 7% at 10% and 20% hydrolate solutions, respectively, indicating pronounced sensitivity. Germination performance indicators (CVG, GI, and GRI) decreased markedly with hydrolate application, while t_{50} values increased substantially, reflecting delayed and irregular germination under hydrolate exposure. Kinetic modeling showed that the first-order kinetic model exhibited moderate predictive accuracy but relatively high error values, indicating limited reliability. The Elovich equation provided the best overall fit, with the highest coefficients of determination and lower errors across treatments. The double constants model showed

moderate performance with higher error values, whereas the Langmuir kinetic equation performed well, but only but only under higher hydrolate concentrations. Overall, the Elovich model was the most suitable for describing amaranth germination kinetics. Verification calculations for these germination models are presented in Appendix B.6.

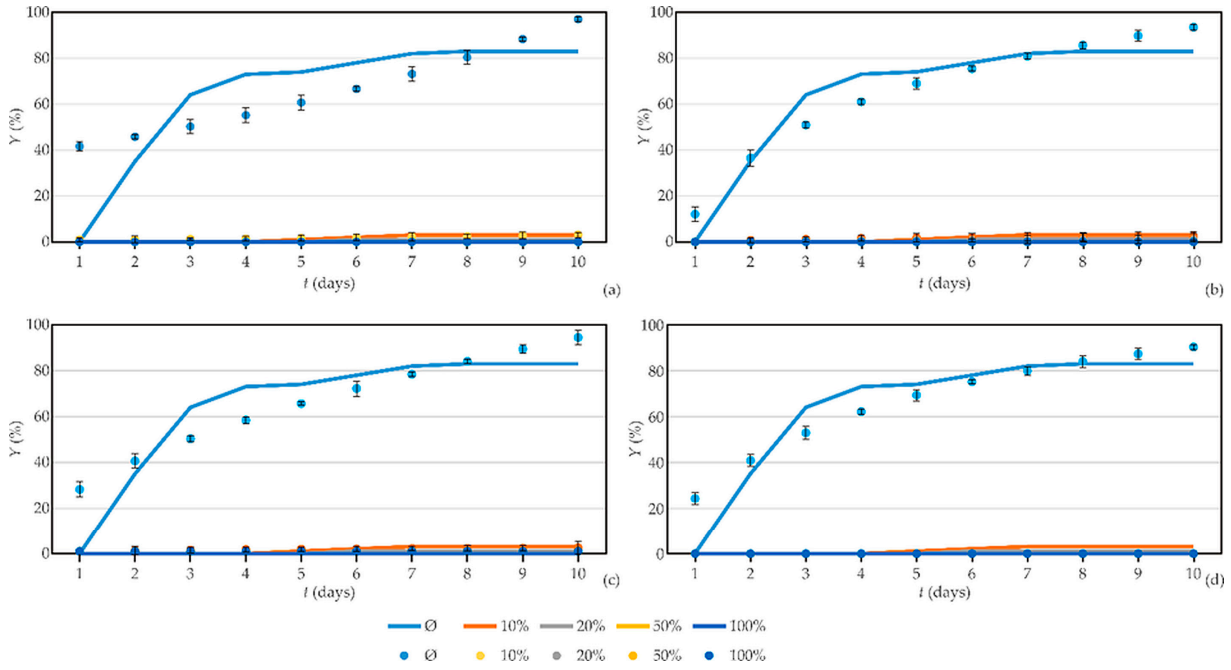

Figure 5: Germination kinetics in amaranth, over ten days depend on different concentrations of lemon catnip hydrolate solution (markers signify the experimental data; lines indicate predictive results): (**a**) first-order kinetic equation, (**b**) Elovich equation, (**c**) Langmuir equation, and (**d**) double-constant equation.

3.1.6 Wild Carrot Germination Kinetics and Model Verification

Wild carrot seed germination in the control treatment was moderate (53%) but decreased sharply with increasing concentrations of lemon catnip hydrolate (Fig. 6). Even low hydrolate concentrations strongly inhibited germination, which declined to 14% and 11% at 10% and 20% hydrolate solutions, respectively, while higher concentrations (50% and undiluted hydrolate) completely suppressed germination. Germination performance indicators (CVG, GI, and GRI) decreased progressively with increasing hydrolate concentration, whereas t_{50} values increased, indicating delayed germination at sublethal doses. Kinetic modeling showed that the Elovich equation provided the best fit under control conditions but its predictive accuracy declined at higher hydrolate concentrations. In contrast, the double constants and Langmuir kinetic models performed well, but only but only under higher hydrolate concentrations. The first-order kinetic model showed moderate accuracy under control conditions but improved at lower hydrolate concentrations, indicating concentration-dependent suitability. Verification calculations for wild carrot germination kinetics are presented in Appendix B.7.

Overall, lemon catnip hydrolate exerted a species-dependent phytotoxic effect, with the magnitude of inhibition varying among species. Crop species such as maize showed the highest tolerance, whereas soybean exhibited moderate sensitivity, and weed species, particularly amaranth and common lambsquarters, were highly susceptible even at low hydrolate concentrations. White clover and wild carrot also displayed strong inhibition, with complete suppression of germination at higher concentrations. Germination

performance indicators consistently declined with increasing hydrolate concentration, accompanied by delayed germination, as reflected by increased t_{50} values. Kinetic modeling demonstrated that the Elovich and Langmuir equations generally performed well, but only but only under higher hydrolate concentrations. These findings highlight the potential of lemon catnip hydrolate as a bio-based germination inhibitor, particularly against sensitive weed species, while underscoring the importance of species-specific responses and appropriate kinetic modeling for accurate interpretation of germination behavior.

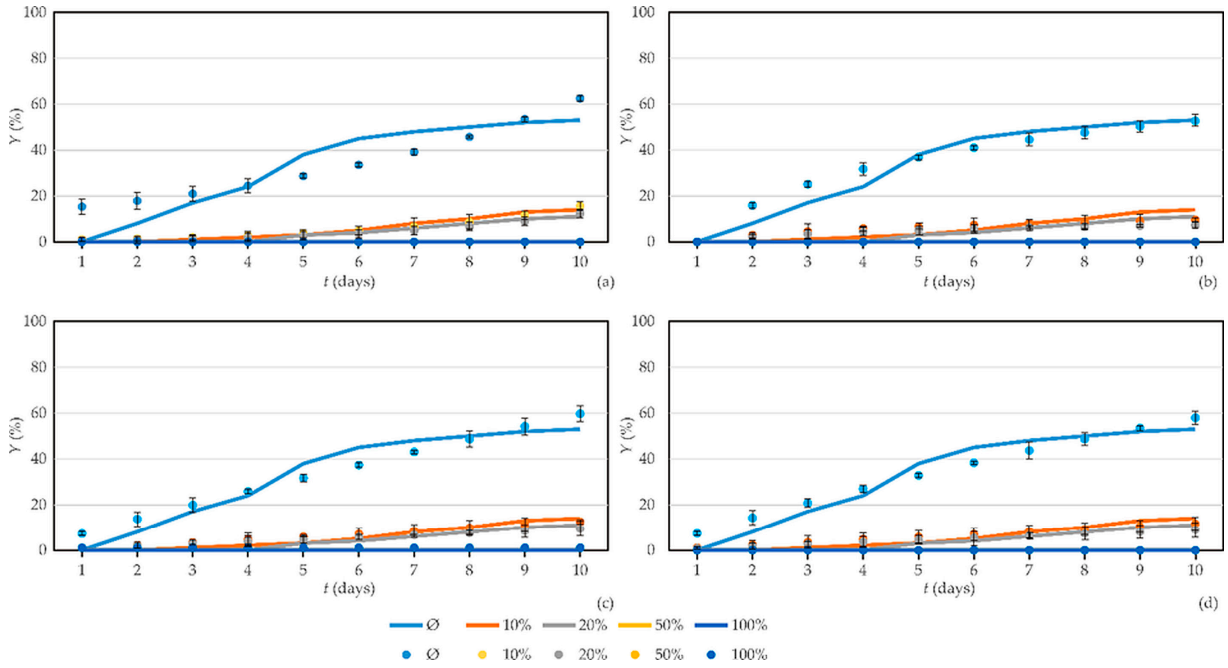

Figure 6: Germination kinetics in wild carrot, over ten days depend on different concentrations of lemon catnip hydrolate solution (markers signify the experimental data; lines indicate predictive results): (**a**) first-order kinetic equation, (**b**) Elovich equation, (**c**) Langmuir equation, and (**d**) double-constant equation.

3.2 Antimicrobial Assays

Lemon catnip hydrolate exhibited detectable but broad-spectrum antimicrobial activity, with a clear preference for Gram-positive bacteria (Table 4). The antimicrobial testing was done with undiluted hydrolate, since hydrolates are typically applied in undiluted form in sanitation or food-processing contexts; therefore, the present study prioritizes application-relevant conditions rather than pharmacological dose–response characterization. The largest agar-diffusion inhibition zones obtained by disk-diffusion assay were observed for *S. aureus* (14.3 mm) and *B. subtilis* (13.1 mm), whereas the Gram-negative *P. aeruginosa* was least affected (8.9 mm). *Listeria monocytogenes* (11.4 mm), *S.* Typhimurium (10.7 mm) and *E. coli* (12.5 mm) showed intermediate susceptibility. Among yeasts, *S. cerevisiae* (10.1 mm) was slightly more inhibited than *C. albicans* (9.8 mm). These patterns reflect the higher susceptibility of Gram-positive cells, likely because the outer lipopolysaccharide membranes of Gram-negative bacteria impede the hydrophobic monoterpenes like geraniol and nerol. Indeed, geraniol-rich hydrolates are known to act more effectively on Gram-positives, whereas Gram-negatives and yeast require higher concentrations for similar effects.

Reduction in crystal violet-stained biofilm biomass paralleled these trends. Lemon catnip hydrolate significantly reduced Gram-positive biofilm-associated biomass; however, given the absence of normalization to growth inhibition, this effect likely reflects a combination of antimicrobial activity and interference with

biofilm formation. Namely, *S. aureus* biofilm formation was reduced by 70%, and *B. subtilis* by 55%. In contrast, *Salmonella* and *E. coli* biofilms were only modestly affected (45–50% reduction) and *P. aeruginosa* biofilm was minimally inhibited (40%). Yeast biofilms were suppressed by roughly 45–50%. Overall, lemon catnip hydrolate prevented Gram-positive biofilm formation effectively, whereas its efficacy against Gram-negative and fungal biofilms was substantially lower. Anti-adhesion assays reinforced this pattern. Lemon catnip hydrolate reduced the attachment of *S. aureus* by 75% and *B. subtilis* by 65%, while *L. monocytogenes* and *S.* Typhimurium adhesion were also strongly inhibited (~60%). In contrast, *E. coli* adhesion was only moderately reduced (~35%) and *P. aeruginosa* adhesion barely changed (~15%). Yeast adhesion was modestly inhibited (≈50–60%). Thus, early-stage attachment of Gram-positive cells was effectively prevented by the hydrolate, whereas Gram-negative and yeast attachment was relatively resistant. These results again reflect structural differences: the terpenoid alcohols in the hydrolate can penetrate and disrupt the simpler cell envelopes of Gram-positives, but the additional lipopolysaccharide layer in Gram-negatives provides resistance. Similarly, geraniol targets fungal membranes by binding ergosterol and disrupting integrity, consistent with the observed antifungal/anti-yeast activity. In summary, lemon catnip hydrolate proved most effective against Gram-positive bacteria (especially *S. aureus* and *B. subtilis*), while Gram-negatives and yeasts were considerably less susceptible. The pronounced anti-adhesive activity against staphylococci and other Gram-positive bacteria suggests potential for lemon catnip hydrolate in natural sanitation or preservation applications. Importantly, because sub-inhibitory concentrations were not tested and biomass was not normalized to planktonic growth, the observed reductions likely reflect a combined effect of growth inhibition and interference with surface-associated biomass formation, rather than a specific antibiofilm mechanism.

Table 4: Antimicrobial, antibiofilm, and antiadhesion activity of lemon catnip undiluted hydrolate (100%).

Microorganism	Antimicrobial Activity (mm)	Antibiofilm Activity (%)	Anti-Adhesion Activity (%)
Escherichia coli	12.5 ± 1.8	50.0 ± 0.0	35.0 ± 5.0
Pseudomonas aeruginosa	8.9 ± 2.1	40.0 ± 0.0	15.0 ± 5.0
Salmonella Typhimurium	10.7 ± 1.5	45.0 ± 0.0	60.0 ± 0.0
Staphylococcus aureus	14.3 ± 2.0	70.0 ± 0.0	75.0 ± 0.0
Bacillus subtilis	13.1 ± 1.7	55.0 ± 5.0	65.0 ± 0.0
Listeria monocytogenes	11.4 ± 1.9	50.0 ± 5.0	60.0 ± 5.0
Candida albicans	9.8 ± 1.6	45.0 ± 0.0	50.0 ± 0.0
Saccharomyces cerevisiae	10.1 ± 1.8	50.0 ± 0.0	60.0 ± 5.0

In view of tested controls, the negative one (sterile distilled water) showed no inhibition zones and no measurable reduction in biofilm biomass formation or cell adhesion across all tested microorganisms, confirming that the observed effects were not due to experimental artifacts. The positive controls exhibited substantially higher antimicrobial activity compared to the hydrolate. In disk diffusion assays, inhibition zones for positive controls ranged between 18–30 mm for bacteria (gentamicin) and 21–25 mm for yeasts (amphotericin B). *S. aureus*, *L. monocytogenes* and *B. subtilis*, showed the highest susceptibility (25, 21, and 27 mm, respectively), while *E. coli*, *P. aeruginosa*, and *S. typhimurium* exhibited slightly lower, but still pronounced inhibition (18, 20, and 20 mm, respectively). Compared to these reference values, lemon catnip hydrolate demonstrated screening-level detectable antimicrobial and biofilm-associated inhibitory effects under *in vitro* conditions, supporting its classification as a mild but biologically active natural antimicrobial agent.

4 Discussion

4.1 Phytotoxic Effects In Vitro

Hydrolates are increasingly recognized for their diverse biological activities, encompassing phytotoxicity, herbicidal, biostimulant, and antimicrobial effects. They offer environmentally friendly alternatives to improve seed germination, enhance seedling growth, control weeds, and ensure food safety. Recent studies have demonstrated their potential across a range of crops, microgreens, and stress conditions, highlighting their relevance for sustainable agriculture and integrated crop management. However, some studies have reported variable or even negligible effects depending on plant species, hydrolate composition, and experimental conditions, emphasizing that bioactivity can be highly context-dependent (e.g., dose- and species-dependent effects observed in *Cistus ladanifer* hydrolate phytotoxicity tests) [48].

Hydrolates from the rhizomes, roots and aerial parts of *Angelica archangelica* and *A. sylvestris* demonstrated clear phytotoxic potential by reducing germination and inhibiting root and hypocotyl elongation in *Linum usitatissimum*, *Raphanus sativus*, *Cucumis sativus* and *Brassica oleracea*, with the strongest effects observed for *A. archangelica* and for hydrosols rich in aromatic and terpenoid compounds derived from younger plant material [49]. Thyme hydrolate similarly exhibited biological activity, showing low toxicity toward cultivated species such as soybean, sunflower and maize, but strong inhibitory effects on alfalfa, clover and several weed species (*Amaranthus retroflexus*, *Chenopodium album*, *Portulaca oleracea*, *Echinochloa crus-galli*, *Sorghum halepense* and *Solanum nigrum*), indicating its potential as a natural herbicide for integrated weed management [28]. It should be noted that the observed effects are influenced by the specific chemical composition of hydrolates. In the present study, the phytotoxic and antimicrobial profiles can be tentatively linked to the predominance of oxygenated monoterpenes, particularly geraniol and nerol, which are known to interact with biological membranes and may contribute to oxidative stress [50,51]. However, these interpretations remain inferential and should be considered hypotheses consistent with observed bioactivity, as their effects cannot be explained solely by specific functional groups (e.g., hydroxyl moieties) and are strongly modulated by molecular configuration, volatility, and solubility in aqueous systems such as hydrolates [52]. For example, although monoterpene alcohols are generally associated with membrane disruption, their effectiveness depends on stereochemistry and positional isomerism, as well as on their relative proportions within complex mixtures [53]. This is consistent with previous observations that structurally similar compounds (e.g., thymol and carvacrol) may exhibit markedly different phytotoxic or antimicrobial effects due to differences in hydroxyl group position and resulting interactions with lipid bilayers [54]. Importantly, the relatively moderate activity observed in this study, compared to essential oils, suggests that matrix effects and dilution in hydrolates significantly alter bioavailability and interaction dynamics, potentially attenuating direct structure-driven activity [20]. These physicochemical differences are directly reflected in functional outcomes observed in this study, including variation in seed germination, root elongation, and microbial inhibition [55]. Thus, differences in isomer distribution and relative abundance are not only compositional features but key determinants of biological response. In addition, laurel and rosemary hydrolates were tested as seed priming agents for wheat under salt stress, revealing that laurel hydrolate significantly improved germination dynamics and seedling vigour at moderate salinity, that rosemary hydrolate primarily promoted early seedling growth, and that their combined application produced synergistic enhancement of germination; however, none of the treatments were able to counteract severe salinity at 100 mM NaCl [56]. Biochemical analyses further showed that laurel hydrolate consistently increased phenolic content, supporting its suitability as a cost effective and environmentally friendly biostimulant for wheat cultivation in saline environments. Additionally, the

study evaluated six hydrolates (oregano, fennel, lavender, lemon catmint, peppermint, and hop) as natural sanitizers in alfalfa microgreen production, assessing their effects on seed germination, antimicrobial activity, and sensory qualities [27]. The results showed that peppermint hydrolate emerged as a promising natural sanitizer for alfalfa microgreens, supporting sustainable and organic urban agriculture while enhancing food safety.

Studies investigating catnip essential oil have demonstrated *in vitro* phytotoxic effects, including inhibition seed germination and early seedling growth in several noxious weeds, including *Hordeum spontaneum*, *Avena fatua*, and *Taraxacum officinale*, as well as in crops such as *Lepidium sativum*, *Ocimum basilicum*, and catnip itself. Notably, catnip exhibited the greatest susceptibility to its own essential oil [57]. Allelochemicals in catnip essential oil have been shown to inhibit ragweed shoot and root growth, induce shoot discoloration, suppress CAT activity, and stimulate POX activity in ragweed shoots [58]. Other *Nepeta* species also demonstrate strong phytotoxic effects. For instance, a water emulsion of *N. rtanjensis* essential oil exhibits phytotoxic effects on weeds such as *Stellaria media*, amaranth, and *Artemisia vulgaris* [59]. Catnip also negatively affects *Corchorus olitorius*, with densities of 100–500 plants per square meter resulting in reductions in multiple growth parameters [60]. Furthermore, water extracts of catnip inhibit the germination of *Alyssum hirsutum* and amaranth [61]. Although the precise mechanisms remain unclear, the phytotoxic effects of *Nepeta* essential oils have been linked to oxidative damage in plant tissues [62].

Dose–response relationships are likely critical, as different concentrations of hydrolates or essential oil constituents can result in contrasting effects, and future studies should evaluate threshold concentrations to better define bioactivity windows (nonlinear dose–response effects in phytotoxicity have been documented, underscoring the need for careful analysis of effective doses and thresholds) [63]. It should be noted that although distilled water was used as a control (pH 6.0), the higher acidity of lemon catnip hydrolate (pH 5.4) may alter osmotic potential, potentially contributing to germination inhibition independently of phytotoxic compounds. Elucidating such structure–activity relationships requires integrative approaches that combine chemical profiling with targeted biological assays and multivariate analysis, moving beyond correlative observations toward a mechanistic understanding [64]. From a broader phytochemical and toxicological perspective, interpretation of bioactivity based solely on compositional data remains limited. Factors such as compound stability, degradation pathways, and transformation during storage or application may significantly alter biological effects. Furthermore, the lack of constituent-level toxicological profiling complicates assessment of safety and selectivity, particularly in complex mixtures where interactions may modify both efficacy and toxicity. These analyses are increasingly recognized as essential in modern food, bioresource, and sustainable agriculture studies and will be prioritized in subsequent investigations.

4.2 Antimicrobial Potential

The antimicrobial potential of lemon catnip hydrolate demonstrated a screening-level *in vitro* selectivity toward Gram-positive bacteria, consistent with previous reports on the mode of action of monoterpene alcohols such as geraniol and nerol. The notably higher inhibition zones observed for *S. aureus* and *B. subtilis* indicate that the cell wall architecture of Gram-positive microorganisms, lacking an outer lipopolysaccharide layer, facilitates penetration of hydrophobic volatiles. In contrast, Gram-negative bacteria, such as *P. aeruginosa*, possess a more complex outer membrane that restricts diffusion of such molecules, resulting in lower susceptibility. This pattern parallels findings for other geraniol-rich hydrosols and essential oils, where Gram-positive strains consistently show higher sensitivity [65,66]. The detectable inhibition observed for *L. monocytogenes*, *S.* Typhimurium, and *E. coli* suggests that, although the hydrolate is less potent against Gram-negative species, partial activity occurs likely through disruption of the cytoplasmic membrane and

impairment of energy metabolism. Similar results have been reported for *Thymus vulgaris* and *Cymbopogon citratus* hydrosols, which act synergistically by destabilizing membranes and inducing protein leakage [9]. Moreover, the results indicate that synergistic or antagonistic interactions among constituents are likely more important than single-compound effects, particularly in hydrolates where minor components may disproportionately influence overall bioactivity. This is consistent with previous studies showing that the activity of whole essential oil matrices often exceeds that of individual constituents due to multi-target and interaction-driven effects [67,68]. Minor compounds can modulate the activity of dominant constituents and contribute to emergent bioactivity patterns [69], which may help explain the variability in phytotoxic responses across plant species and experimental conditions, as well as the selective antimicrobial activity observed against Gram-positive bacteria [70].

The yeast results further support a moderate antifungal potential, with *S. cerevisiae* and *C. albicans* showing comparable inhibition (9.8–10.1 mm). The observed inhibition patterns are consistent with previously reported membrane-associated effects of oxygenated monoterpenes [9,71]. However, as no membrane integrity or time-kill assays were performed in this study, these mechanisms remain inferential and require further investigation. The antibiofilm and antiadhesion assays strengthen this interpretation. The hydrolate suppressed *S. aureus* biofilm formation by 70% and *B. subtilis* by 55%. The weaker antibiofilm effects against *E. coli* and *P. aeruginosa* (40–50%) can be attributed to differences in quorum-sensing systems and biofilm matrix composition. Gram-negative bacteria often rely on acyl-homoserine lactone signalling molecules that are less affected by monoterpene alcohols than by phenolic compounds such as carvacrol or thymol [72]. The antiadhesive activity followed the same trend. Hydrolate treatment reduced initial attachment of *S. aureus* by 75% and *B. subtilis* by 65%, confirming strong interference at early colonization stages. Such effects are desirable in the prevention of biofilm initiation on surfaces relevant to food safety and healthcare. The significant inhibition of *Listeria* and *Salmonella* adhesion (~60%) further suggests the potential of lemon catnip hydrolate in sanitation formulations for food-contact environments, where *Listeria* persistence poses a major concern [73]. The limited impact on *P. aeruginosa* adhesion (15%) again reflects its robust biofilm physiology and resistance to monoterpenes. A key limitation of the present antimicrobial assessment is the absence of quantitative susceptibility testing. The use of disk diffusion and crystal violet staining provides only preliminary insight into biological activity and does not permit determination of dose–response relationships, or biofilm-specific inhibition. In particular, the crystal violet assay measures total surface-associated biomass and cannot distinguish between reduced cell viability and true inhibition of biofilm formation. Consequently, the observed antibiofilm and antiadhesion effects should be interpreted carefully as screening-level observations. Future studies should incorporate broth microdilution assays, time–kill kinetics, and normalization of biofilm biomass to viable cell counts, as well as molecular or microscopic approaches, to confirm mechanism-specific activity. The antimicrobial and antiadhesive profile observed for the lemon catnip hydrolate is consistent with the growing body of evidence supporting the use of plant hydrolates as natural sanitizing agents for food-contact surfaces and fresh produce. Recent studies have demonstrated that hydrolates effectively inhibit microbial adhesion and biofilm development *in vitro*, which are key prerequisites for maintaining hygienic conditions in food processing environments. In addition, their water-based composition, low odor, and ease of removal make them a practical and safer alternative to essential oils in industrial sanitation settings [9,74]. Similar mechanistic interpretations have been proposed in previous studies, initially inferred from bioactivity patterns and subsequently validated through membrane integrity assays, transcriptomic analyses, or molecular modelling approaches [75,76]. This highlights the importance of complementary experimental validation for confirming mechanism of action.

Practical applications of this concept are already being reported. For instance, *Thymbra capitata* hydrolate has been successfully used in washing solutions to control *L. monocytogenes* and spoilage microorganisms on leafy greens and shredded vegetables, with performance comparable to or exceeding traditional chlorine-based washes [77–79]. Moreover, hydrolates have been evaluated as processing aids *in vivo*, where thyme and lemon hydrolates reduced norovirus concentrations during oyster depuration [80], and as sanitizing agents for micro-sprout and seed production systems, effectively lowering microbial loads without compromising sensory quality [27]. The antimicrobial evaluation performed in this study provides screening-level evidence of hydrolate bioactivity. More rigorous quantitative approaches, including broth microdilution MIC determination, time-kill kinetics, and molecular analysis of biofilm-related genes, would strengthen mechanistic interpretation and should be addressed in future studies.

4.3 Potential of Lemon Catnip Hydrolate for Bioherbicidal and Antimicrobial Applications

As previously reported, lemon catnip hydrolate contains primarily oxygenated monoterpenes, with nerol, geraniol, and citral (geranial and neral) as dominant constituents, which are largely responsible for their aroma and biological activities [27,29,33]. Additionally, geraniol has been shown to exert phytotoxic effects, i.e., negatively impacts germination and seedling growth in wheat [81], inhibits radicle growth in maize seedlings [82], and reduces germination in garden cress and radish seeds [82]. In both *in vitro* and greenhouse studies, geraniol exhibits strong phytotoxic effects on a wide range of weeds and crops, including *Digitaria ciliaris*, *Setaria viridis*, *Poa annua*, *Echinochloa crusgalli*, *Aeschynomene indica*, *Abutilon theophrasti*, *Gossypium hirsutum*, maize, soybean, and *Ipomoea pandurata* [83]. The phytotoxic effects observed in this study can largely be attributed to this compound. Apart from phytotoxic effects, geraniol also possesses antimicrobial properties [35,65], the same as nerol [84]. From a safety and regulatory perspective, these compounds are documented as generally recognized as safe (GRAS) food flavoring substances, approved for direct addition to foods under U.S. FDA regulations (21 CFR Parts 182 and 172) and listed by both FEMA and JECFA [85,86]. Although individual constituents such as geraniol and nerol are classified as generally recognized as safe (GRAS) for use as flavoring agents, this designation does not directly extend to complex hydrolate systems or to their use as sanitizers or bioherbicides. The biological effects of multicomponent mixtures may differ substantially from those of isolated compounds due to interactions, matrix effects, and exposure conditions [87,88]. Therefore, regulatory acceptance and practical application require dedicated safety, efficacy, and exposure assessments at the product level rather than extrapolation from individual compounds. The obtained results provide a scientific and practical foundation for advancing lemon catnip hydrolate toward application as a natural, eco-friendly sanitizer in the food industry, particularly for surface decontamination and anti-biofilm hygiene programs targeting Gram-positive pathogens of concern [9,74,77,89].

A major limitation of the present study is the absence of comprehensive toxicological and ecological assessment, which is particularly relevant given the proposed agricultural and food-contact applications. While oxidative stress is often discussed as a mechanism underlying phytotoxic and antimicrobial effects, the broader biological impact of hydrolates *in vivo* remains unclear. Therefore, the observed bioactivity should not be interpreted as inherently safe or selective. Further *in vivo* studies, including chronic exposure and ecotoxicological evaluation, are essential before considering practical implementation.

5 Conclusions

Lemon catnip hydrolate demonstrated concentration-dependent *in vitro* phytotoxicity and antimicrobial activity under controlled *in vitro* conditions. Weed species exhibited higher sensitivity than most tested crops

(amaranth < wild carrot < common lambsquarters < soybean < white clover < maize), while Gram-positive bacteria were more susceptible than Gram-negative species. The combination of inhibition zone measurements, antibiofilm reduction, and antiadhesion activity indicates measurable multifunctional bioactivity within an aqueous matrix. However, these findings are restricted to laboratory-scale assays and do not account for environmental variables such as soil composition, microbial community interactions, compound persistence, dilution effects, or non-target organism sensitivity. Scalability, formulation stability, and ecological safety require further investigation before agricultural or industrial implementation can be considered. Future research should integrate greenhouse and field trials, environmental fate assessment, and systems-level evaluation frameworks to determine real-world performance and sustainability impact. The present findings are restricted to controlled *in vitro* assays and do not address toxicological, endocrine, or broader ecological effects, which would require dedicated risk assessment studies prior to practical implementation.

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Availability of Data and Materials: The authors confirm that the data supporting the findings of this study are available within the article [and/or] its Appendices A and B.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BUNS	Herbarium of the University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology
GC-MS	Gas Chromatography-Mass Spectometry
GC-FID	Gas Chromatography-Flame Ionization Detection
GP	Germination Percentage
CVG	Coefficient of Velocity of Germination
GI	Germination Index
t_{50}	Median Germination Time
GRI	Germination Rate Index
ATCC	American Type Culture Collection
R^2	Coefficient of Determination
χ^2	Reduced Chi-Square
MBE	Mean Bias Error

RMSE	Root Mean Square Error
MPE	Mean Percentage Error
SSE	Sum of Squared Errors
AARD	Average Absolute Relative Deviation

Appendix A

The formulas used to calculate the kinetic models of seed germination are presented below: first order kinetic equation (Eq. (A1)), Elovich equation (Eq. (A2)), double-constant equation (Eq. (A3)), Langmuir kinetic equation (Eq. (A4)), and error analysis (Eqs. (A5)–(A10)). In this study, the kinetic equations were applied as empirical models describing germination progress over time, following the approach previously reported by Zhou et al. [45] for seed germination kinetics.

Appendix A.1 First-Order Kinetic Equation

The first-order kinetic equation is widely used to model chemical reactions and is expressed as:

$$\ln Y = \ln Y_{\max} + B \cdot t \tag{A1}$$

where: Y presents represents seedlings parameters such as germination percentage over 10 days; $Y_{\max}$ is the maximum value of these parameters; B is a constant; and t is the germination time. A linear relationship can be obtained by plotting $\ln Y$ against t, and the model's goodness of fit can be assessed through linear regression parameters.

Appendix A.2 Elovich Equation

The Elovich equation is commonly used to describe adsorption kinetics in soils. Its simplified form is:

$$Y = A + B \cdot \ln t \tag{A2}$$

where: Y presents seed parameters, A and B are constants, and t is the adsorption time. A linear plot of Y versus t allows for evaluation of the model's fit based on the correlation coefficient.

Appendix A.3 Double-Constant Equation

The double-constant equation is an empirical model suitable for describing complex reaction kinetics. It is given as:

$$\ln Y = A + B \cdot \ln t \tag{A3}$$

where: Y presents seed parameters; A and B are constants; and t is the germination time. A straight line can be plotted between $\ln Y$ and $\ln t$, and the fitness of the model can be performed according to the correlation coefficient of the straight line.

Appendix A.4 Langmuir Kinetic Equation

The Langmuir kinetic equation is another approach for modeling seedling changes (germination percentage over 10 days) and is expressed as:

$$\frac{t}{Y} = \frac{t}{Y_{\max}} + \frac{1}{k} \tag{A4}$$

where: Y denotes seed parameters; $Y_{\max}$ is the maximum value of these parameters; k is the equilibrium constant; and t is the germination time. The model's fit can be assessed by plotting $\ln Y$ against t, and the model's goodness of fit can be assessed through linear regression parameters.

Appendix A.5 Error Analysis

In terms of error analysis, the accuracy of the developed models was evaluated through several key metrics, i.e.,: coefficient of determination (R^2), reduced chi-square (χ^2), mean bias error (MBE), root mean square error (RMSE), mean percentage error (MPE), the sum of squared errors (SSE), and average absolute relative deviation (AARD). These widely used parameters, were employed to assess the validity of the models, as follows:

$$\chi^2 = \frac{\sum_{i=1}^{N} (x_{\exp,i} - x_{pre,i})^2}{N - n} \tag{A5}$$

$$RMSE = \left[\frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i})^2 \right]^{1/2} \tag{A6}$$

$$MBE = \frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i}) \tag{A7}$$

$$MPE = \frac{100}{N} \cdot \sum_{i=1}^{N} \left(\frac{\left| x_{pre,i} - x_{\exp,i} \right|}{x_{\exp,i}} \right) \tag{A8}$$

$$SSE = \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i})^2 \tag{A9}$$

$$AARD = \frac{1}{N} \cdot \sum_{i=1}^{N} \left| \frac{x_{exr,i} - x_{pre,i}}{x_{exr,i}} \right| \tag{A10}$$

where $X_{exp,i}$ stands for the experimental values; $X_{pre,i}$ are the predicted values calculated by the model; and N and n are the number of observations and constants, respectively.

Appendix B

Appendix B.1

Table A1: First order, Elovitch, double constants equation, and Langmuir kinetic model coefficients, for germination for tested plant species treated with lemon catnip hydrolate solution.

Tested Plant	Model	Coefficient	Control	10%	20%	50%	100%
Maize	First order kinetic model	Y_{max}	24.930	19.071	21.652	16.286	6.051
		B	0.140	0.156	0.146	0.149	0.194
	Elovitch equation	A	0.000	0.000	0.000	0.000	0.000
		B	38.722	33.212	35.244	27.031	13.950
	Double constants equation	A	2.705	2.356	2.533	2.226	0.968
		B	0.812	0.914	0.853	0.874	1.179
	Langmuir kinetic equation	Y_{max}	239.965	341.408	256.666	213.485	4.2E+06
		k	15.296	11.112	13.040	9.725	3.784

Table A1: *Cont.*

Tested Plant	Model	Coefficient	Control	10%	20%	50%	100%
Soybean	First order kinetic model	Y_{max}	28.709	8.176	5.502	4.950	2.200
		B	0.134	0.199	0.230	0.172	0.147
	Elovitch equation	A	0.000	0.000	0.000	0.000	0.000
		B	42.385	19.530	16.593	9.704	3.600
	Double constants equation	A	2.909	1.240	0.484	0.928	0.249
		B	0.755	1.213	1.508	1.016	0.855
	Langmuir kinetic equation	Y_{max}	230.260	2.2E+05	2.2E+05	410.032	4.2E+06
		k	17.933	5.319	4.581	2.747	0.958
White clover	First order kinetic model	Y_{max}	51.136	37.921	28.634	0.457	0.000
		B	0.073	0.097	0.118	0.097	0.000
	Elovitch equation	A	26.007	11.388	0.433	0.073	0.000
		B	34.290	36.686	37.649	0.481	0.000
	Double constants equation	A	3.707	3.333	2.964	0.000	0.000
		B	0.409	0.540	0.667	0.000	0.000
	Langmuir kinetic equation	Y_{max}	126.052	136.553	155.061	141.553	4.2E+06
		k	48.311	29.195	19.432	0.000	0.000
Common lambsquarters	First order kinetic model	Y_{max}	15.428	3.930	0.887	0.000	0.000
		B	0.139	0.188	0.234	0.000	0.000
	Elovitch equation	A	0.000	0.000	0.000	0.000	0.000
		B	23.854	8.720	2.776	0.238	0.000
	Double constants equation	A	2.223	0.527	0.000	0.000	0.000
		B	0.811	1.165	0.883	0.000	0.000
	Langmuir kinetic equation	Y_{max}	149.557	1.9E+05	2.9E+06	0.000	0.000
		k	9.383	2.364	0.766	0.722	0.797
Amaranth	First order kinetic model	Y_{max}	37.964	0.899	0.000	0.000	0.000
		B	0.094	0.126	0.264	0.000	0.000
	Elovitch equation	A	12.112	0.000	0.000	0.000	0.000
		B	35.346	1.113	0.373	0.000	0.000
	Double constants equation	A	3.343	0.000	0.000	0.000	0.000
		B	0.524	0.414	0.000	0.000	0.000
	Langmuir kinetic equation	Y_{max}	129.432	0.000	0.000	0.000	0.000
		k	29.887	9.325	9.399	9.438	9.438
Wild carrot	First order kinetic model	Y_{max}	13.143	0.847	0.571	0.000	0.000
		B	0.156	0.292	0.307	0.000	0.000
	Elovitch equation	A	0.000	0.000	0.000	0.000	0.000
		B	22.891	4.153	3.168	0.000	0.000
	Double constants equation	A	1.985	0.000	0.000	0.000	0.000
		B	0.914	1.098	0.971	0.000	0.000
	Langmuir kinetic equation	Y_{max}	247.299	4.9E+06	1.1E+10	0.000	0.000
		k	7.579	1.166	0.896	0.797	0.797

Y_{max}; B; A; k—regression coefficients in: First order kinetic model, Elovitch equation, double constants equation, and Langmuir kinetic equation.

Appendix B.2

Table A2: Kinetcs model verification table for maize germination.

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
First order kinetic model	control	365.105	18.127	−1.141	167.302	3272.935	152.306	0.676
	10%	206.021	13.617	−1.097	67.200	1842.150	118.031	0.767
	20%	283.125	15.963	−1.109	292.370	2535.824	135.873	0.707
	50%	179.246	12.701	−0.915	223.988	1604.832	111.232	0.700
	100%	27.167	4.945	−0.591	11.066	241.006	42.121	0.864

Table A2: *Cont.*

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
Elovitch equation	control	98.426	9.412	−1.487	130.108	863.723	65.731	0.923
	10%	61.167	7.420	−1.765	54.737	519.353	53.303	0.951
	20%	77.578	8.356	−1.634	239.782	671.492	54.857	0.935
	50%	57.045	7.165	−1.428	186.700	493.014	52.793	0.924
	100%	25.496	4.790	−1.270	17.430	213.333	40.855	0.919
Double constants equation	control	178.044	12.659	−1.307	130.479	1585.306	105.747	0.848
	10%	80.599	8.517	−1.091	47.250	713.479	73.689	0.913
	20%	130.157	10.823	−1.223	226.113	1156.446	91.794	0.871
	50%	85.017	8.747	−1.000	170.235	755.147	75.905	0.863
	100%	8.361	2.743	−0.467	5.272	73.065	21.582	0.960
Langmuir kinetic equation	control	149.145	11.586	−1.914	132.289	1305.689	89.672	0.888
	10%	70.840	7.985	−1.594	48.587	612.146	66.411	0.935
	20%	111.085	9.999	−1.786	233.556	967.878	80.222	0.904
	50%	74.277	8.176	−1.466	177.517	647.008	68.713	0.896
	100%	10.679	3.100	−1.013	5.818	85.836	23.079	0.972

R^2—coefficient of determination; χ^2—reduced chi-square; MBE—mean bias error; RMSE—root mean square error; MPE—mean percentage error, SSE—the sum of squared errors, AARD—average absolute relative deviation.

Appendix B.3

Table A3: Kinetcs model verification table for soybean germination.

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
First order kinetic model	control	261.602	15.344	−0.885	20.668	2346.591	132.454	0.757
	10%	28.074	5.027	−0.637	17.173	248.604	41.405	0.925
	20%	32.724	5.427	−0.649	16.876	290.313	45.146	0.904
	50%	10.386	3.057	−0.301	21.436	92.564	24.846	0.866
	100%	2.300	1.439	−0.103	32.189	20.597	12.604	0.757
Elovitch equation	control	25.131	4.756	−0.420	8.979	224.412	35.843	0.977
	10%	46.413	6.463	−1.499	31.442	395.252	58.003	0.907
	20%	86.793	8.838	−1.663	46.681	753.486	77.712	0.787
	50%	5.277	2.179	−0.557	23.337	44.391	18.118	0.955
	100%	0.527	0.688	−0.137	21.608	4.551	5.006	0.955
Double constants equation	control	97.281	9.357	−0.942	13.307	866.667	80.974	0.914
	10%	4.404	1.991	−0.326	6.150	38.573	15.665	0.989
	20%	17.989	4.024	−0.108	15.387	161.780	33.779	0.945
	50%	2.472	1.492	−0.234	11.294	21.698	12.137	0.970
	100%	0.912	0.906	−0.104	21.416	8.097	7.892	0.908
Langmuir kinetic equation	control	71.413	8.017	−1.318	11.169	625.332	63.566	0.945
	10%	10.116	3.017	−1.249	13.500	75.439	25.685	0.993
	20%	37.695	5.825	−1.792	27.808	307.126	50.465	0.933
	50%	2.427	1.478	−0.338	11.953	20.701	11.991	0.976
	100%	1.037	0.966	0.029	19.138	9.327	8.457	0.887

R^2—coefficient of determination; χ^2—reduced chi-square; MBE—mean bias error; RMSE—root mean square error, MPE—mean percentage error, SSE—the sum of squared errors, AARD—average absolute relative deviation.

Appendix B.4

Table A4: Kinetcs model verification table for white clover germination.

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
First order kinetic model	control	474.885	20.674	−0.295	10.566	4273.089	146.013	0.425
	10%	325.163	17.107	−0.497	13.429	2923.996	135.311	0.591
	20%	320.048	16.972	−0.723	39.233	2875.207	145.979	0.623
	50%	0.109	0.313	−0.008	15.626	0.979	2.620	0.393
	100%	-	-	-	-	-	-	-
Elovitch equation	control	189.507	13.060	0.000	10.239	1705.560	110.725	0.769
	10%	62.353	7.491	0.000	6.583	561.180	61.387	0.921
	20%	71.043	7.996	0.000	23.599	639.390	63.321	0.915
	50%	0.053	0.219	0.000	12.701	0.480	1.750	0.700
	100%	-	-	-	-	-	-	-
Double constants equation	control	292.107	16.214	−0.566	9.647	2625.753	121.650	0.652
	10%	159.315	11.974	−0.728	8.853	1428.532	91.234	0.805
	20%	164.310	12.161	−0.952	30.404	1469.722	103.790	0.812
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Langmuir kinetic equation	control	195.030	13.249	−1.057	7.190	1744.090	94.411	0.788
	10%	100.971	9.533	−1.147	6.388	895.586	68.279	0.892
	20%	124.518	10.586	−1.429	28.157	1100.234	84.518	0.873
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-

R^2—coefficient of determination; χ^2—reduced chi-square; MBE—mean bias error; RMSE—root mean square error, MPE—mean percentage error, SSE—the sum of squared errors, AARD—average absolute relative deviation.

Appendix B.5

Table A5: Kinetcs model verification table for common lambsquarters germination.

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
First order kinetic model	control	124.370	10.580	−0.688	38.786	1114.600	94.308	0.699
	10%	17.573	3.977	−0.444	20.246	156.187	35.285	0.792
	20%	1.745	1.253	−0.185	9.909	15.363	10.276	0.848
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Elovitch equation	control	28.511	5.066	−0.830	26.040	249.711	39.995	0.940
	10%	14.061	3.557	−0.871	27.749	118.966	28.784	0.884
	20%	2.998	1.643	−0.392	23.900	25.440	14.599	0.810
	50%	0.159	0.379	−0.059	14.345	1.399	3.463	0.413
	100%	-	-	-	-	-	-	-
Double constants equation	control	55.544	7.070	−0.757	27.728	494.163	63.939	0.871
	10%	7.850	2.658	−0.386	14.998	69.162	23.623	0.910
	20%	2.008	1.344	−0.635	16.558	14.039	11.440	0.934
	50%	0.778	0.837	−0.700	0.000	2.100	7.000	-
	100%	-	-	-	-	-	-	-
Langmuir kinetic equation	control	45.103	6.371	−1.106	27.172	393.698	56.588	0.909
	10%	8.677	2.795	−0.702	17.027	73.169	24.091	0.923
	20%	1.551	1.182	−0.414	13.740	12.244	10.039	0.938
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-

R^2—coefficient of determination; χ^2—reduced chi-square; MBE—mean bias error; RMSE—root mean square error, MPE—mean percentage error, SSE—the sum of squared errors, AARD—average absolute relative deviation.

Appendix B.6

Table A6: Kinetcs model verification table for amaranth germination.

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
First order kinetic model	control	330.262	17.241	−0.464	14.620	2970.209	139.283	0.561
	10%	0.871	0.885	−0.420	13.096	6.077	7.487	0.842
	20%	-	-	-	-	-	-	-
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Elovitch equation	control	72.980	8.104	0.000	7.664	656.823	67.220	0.902
	10%	0.751	0.822	−0.181	16.318	6.435	6.847	0.736
	20%	0.128	0.339	−0.064	11.505	1.110	2.937	0.632
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Double constants equation	control	168.843	12.327	−0.711	10.324	1514.536	98.730	0.782
	10%	1.192	1.036	−0.441	17.697	8.783	9.048	0.820
	20%	-	-	-	-	-	-	-
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Langmuir kinetic equation	control	107.903	9.855	−1.146	7.659	958.007	74.238	0.878
	10%	-	-	-	-	-	-	-
	20%	-	-	-	-	-	-	-
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-

R^2—coefficient of determination; χ^2—reduced chi-square; MBE—mean bias error; RMSE—root mean square error, MPE—mean percentage error, SSE—the sum of squared errors, AARD—average absolute relative deviation.

Appendix B.7

Table A7: Kinetcs model verification table for wild carrot germination.

Tested Plant	Concentration	χ^2	RMSE	MBE	MPE	SSE	AARD	R^2
First order kinetic model	control	84.384	8.715	−0.707	24.795	754.456	74.763	0.791
	10%	1.535	1.175	−0.259	21.604	13.145	10.862	0.953
	20%	1.496	1.160	−0.258	7.688	12.796	10.620	0.930
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Elovitch equation	control	25.111	4.754	−1.076	20.622	214.426	36.740	0.954
	10%	10.133	3.020	−0.672	79.180	86.680	26.081	0.743
	20%	7.603	2.616	−0.586	19.636	64.996	22.165	0.712
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Double constants equation	control	30.003	5.196	−0.656	15.912	265.723	46.722	0.929
	10%	4.033	1.905	−1.009	53.416	26.111	17.029	0.957
	20%	4.422	1.995	−1.015	14.815	29.492	17.458	0.925
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-
Langmuir kinetic equation	control	26.071	4.844	−0.960	16.560	225.418	44.294	0.947
	10%	4.929	2.106	−0.814	56.216	37.729	19.166	0.947
	20%	4.094	1.919	−0.729	13.659	31.536	16.896	0.929
	50%	-	-	-	-	-	-	-
	100%	-	-	-	-	-	-	-

R^2—coefficient of determination; χ^2—reduced chi-square; MBE—mean bias error; RMSE—root mean square error, MPE—mean percentage error, SSE—the sum of squared errors, AARD—average absolute relative deviation.

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