

## Article

# Control of Problematic Weeds in Mediterranean Vineyards with the Bioherbicide Pelargonic Acid

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**Abstract:** Pelargonic acid (PA) is the only natural herbicide authorized for professional use in Spain. Incorporating PA into an integrated weed management strategy in vineyards may enable a more sustainable production method for grapes. In this work, PA of 55% concentration, formulated by a commercial company (PSEI), was evaluated and applied at 8, 10, 12, and 15 L/ha for weed control in Mediterranean vineyards during 2020 and 2021. A total of 22 different weed species, 16 dicotyledonous and 6 monocotyledonous, were identified in the experimental areas. Previously, greenhouse assays were performed against *Avena fatua* L. and *Chenopodium album* L. to determine the dose/response curves. PSEI proved to be a viable post-emergence herbicide with an efficacy of 40.79–80.90%, depending on the applied dose (higher doses were the most effective). Broader herbicidal activity (20% or more) was obtained against dicotyledonous weeds compared with monocotyledonous. The PA formulation was remarkable in achieving PSEI-similar effects as compared to the market reference but at lower concentrations (around 13% less PA) and doses (1–8 less L/ha). PA has proved to be a good candidate to control weeds in Mediterranean vineyards when used as a post-emergence broad-spectrum herbicide in the first stages of weed development.

**Keywords:** weeds; grapevine; natural herbicides; secondary metabolites; post-emergence; phytotoxicity

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## 1. Introduction

Vineyard cultivation in the Mediterranean area is characterized by a monoculture with a life cycle ranging from multiple years to several decades. Weed management is a major factor affecting establishment, production, and harvest in vineyards. Vine crops are very competitive with weeds once a canopy is established; however, wide intra- and interrow spacing and slow initial growth can leave them vulnerable to weed competition in the first few years after planting [1].

Global population increase, soil degradation and contamination, climate change, and the requirement to produce under increasingly severe biotic and abiotic stress conditions [2,3] make the use of sustainable strategies to improve crop efficiency necessary in modern agriculture. Soil quality is declining in many parts of the world, with implications for the productivity, resilience, and sustainability of agri-food systems [4,5]. Accumulation of contaminants in soil from intensive agriculture and pesticides with further emission and transformation to other media are important conditioners of soil quality and food

production [6,7]. It is estimated that <0.1% of pesticides applied to European crops reach the target pest, resulting in pollution depending on the capacity of soils to store, immobilize, and degrade contaminants [8]. Among all the pesticides, herbicides are the dominant group and are widely used in various sectors of agriculture [9]. Because of their characteristics of competitiveness in the agroecological systems by interfering in crop efficiency to obtain nutrients, moisture, light, or space to grow [10,11], weeds are one of the most critical factors affecting crop yield. Weeds are responsible for 10% of financial losses in agricultural production worldwide [12]. Since the end of the Second World War, with the discovery of auxin herbicides, weed control perspectives changed, and as new herbicides were discovered and available in the market, weed control was based on herbicides use [13]. Herbicides enabled weed control and increased crop yields but caused adverse effects due to their high persistence and off-target toxicity [14]. Some undesirable and unwanted effects of their usage are severe water and environmental pollution and hazards to human and animal health [15]. Glyphosate, the main herbicide used in Europe, is present in high concentrations in soils across the Mediterranean region [8]. Another problem is the development of herbicide-resistant weed biotypes. The repeated use of herbicides with a single mode of action has put a big selection pressure on weed species, leading to the rapid development of herbicide-resistant weed populations [14]. Worldwide legislation, such as the globally accepted International Code of Conduct on the Distribution and Use of Pesticides [16], the European Union Directive 2009/128/EC, or the US Food Quality Protection Act (FQPA), has adopted the principles of Integrated Pest Management (IPM), and alternative control methods to pesticides. The discovery of new active ingredients is still slow, herbicide resistance cases are steadily increasing, and effective active ingredients are expected from the markets [14,17].

Sustainable weed management comprises a suite of weed management options including integrated weed management (IWM) [18], which combines chemical, biochemical, biological, and mechanical methods and aims to optimize crop production and increase grower profit through the concerted use of preventive strategies, as well as scientific knowledge, management skills, monitoring procedures, and the efficient use of control practices [19]. This suggests that weed control should focus on interdisciplinary approaches to reduce chemical contaminants use [17].

Social environmental responsibility demands that final products such as grapes or wine be produced under more healthy control methods for the planet and consumers. The European Union's (EU) recent legislative frameworks set citizens' needs and demands as the major task for the organization of the agricultural sector in the member countries [20]. The use of natural products as new biotechnological management could be included in IWM strategies as one more available weed control method with good efficacies and reduction in crop residues. Bioherbicides are less persistent than synthetic herbicides [21,22]. Secondary metabolites such as phenolic compounds, short chain fatty acids, terpenoids, and alkaloids, among others, can be natural sources of new products with phytotoxic effects and new and multitarget-site activity and modes of action [23–26].

Pelargonic acid (PA) ( $\text{CH}_3(\text{CH}_2)_7\text{CO}_2\text{H}$ , n-nonanoic acid) is a saturated fatty acid with nine carbons in its structure [27]. Herbicidal fatty acids have a long history used as natural herbicides against broadleaf, grasses and mosses in professional agriculture, walkways, railways, parks, and urban and domestic areas [28]. The chain length of carboxylic acids determines the molecule's ability to penetrate through the cell membrane. This active ingredient can be derived from different natural sources, such as from vegetables and fruits including oranges, grape, apples, and potatoes, among others, but also from milk, cheese, and beef [28–31], and could be used as a non-selective, foliar-applied broad-spectrum herbicide [32,33]. The use of PA in soybean ecological management was proved with satisfactory results [34,35]. In 2015, a commercial reference (PA 68%) was authorized by the European Commission as a new plant protection product in Europe. It is derived from rapeseed oil using a natural extraction process [31]. This active ingredient is also

authorized for markets in USA and Canada. The high dosage and cost are some of the drawbacks of its practical application in current agriculture.

This work is a collaboration between the Polytechnic University of Valencia and the company Seipasa S.A., with the participation of the University of Vigo and the State University of Milan. Seipasa is a company that develops and commercializes biopesticides, with the purpose to manage agricultural ecosystems in a more sustainable way. The objectives of the research were (1) to find an optimal formulation of PA capable to be effective with reduced doses and less active ingredient than the existing products on the market and (2) evaluate the herbicidal potential of different PA formulations, one of them with a concentration of 55% active ingredient that was developed by Seipasa (PSEI), against important cosmopolite weeds in vineyards as an alternative to synthetic herbicide management. The experiments were carried out across two consecutive years, 2020 and 2021.

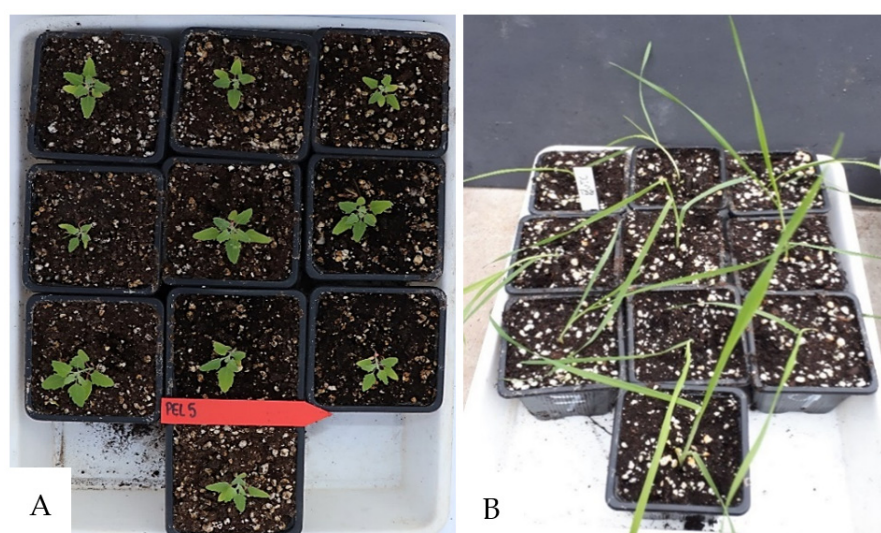
## 2. Materials and Methods

### 2.1. Post-Emergence Herbicidal Assays against Target Weed Species under Greenhouse Conditions Performed to Obtain Dose–Response Curves

#### 2.1.1. Weeds

For this experiment, seeds of *Avena fatua* L. and *Chenopodium album* L. purchased from HerbiSeed were used (year of collection 2017). Before the experiments, the seeds were tested to assure their germination viability in a growth chamber model EGH1501HR (Equitec, Madrid, Spain). Seeds were placed in Petri dishes (9 cm diameter) between two layers of filter paper (73 g/m<sup>2</sup>) wetted with 6 mL of distilled water. In the case of *A. fatua*, 5 seeds were placed in each Petri dish and 20 in the case of *C. album*. *A. fatua* seeds were incubated at 23.0 ± 0.1 °C for 8 h in light and 18.0 ± 0.1 °C for 16 h in dark, while *C. album* seeds were incubated at 30.0 ± 0.1 °C for 16 h in light and 20.0 ± 0.1 °C for 8 h in dark. These conditions for the germination tests were selected based on previous works [36,37].

Once tested for their viability, seeds were sown under greenhouse conditions (Table 1) in pots (8 × 8 × 7 cm) filled with 2 cm of perlite and 5 cm of a universal substrate (peat mixture; pH 5.5–6.5; EC 20 mS/m; organic matter > 80%). The pots were organized in trays (43 × 28 × 65 cm) (10 pots per tray for each treatment) and were bottom-watered throughout the experiment. When the plantlets reached the phenological stage of two to four leaves, corresponding to 12–14 Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale, the herbicide treatments were applied (Figure 1).



**Figure 1.** Weeds ready for being treated in the post-emergence herbicidal assays under greenhouse conditions, (A) *Chenopodium album*, (B) *Avena fatua*.

**Table 1.** Greenhouse conditions during the herbicidal tests.

Species	Start/End Date	Temperature (°C)			Relative Humidity (%)		
		Mean	Max.	Minim.	Mean	Max.	Minim.
<i>A. fatua</i>	10 December 2019– 10 January 2020	18.57	25.72	12.75	57.87	75.56	29.84
<i>C. album</i>	16 October 2020– 16 November 2020	18.90	25.52	12.30	45.88	50.26	40.40

### 2.1.2. Herbicidal Treatments Tested

Pelargonic acid was formulated as emulsifiable concentrate (EC) by Seipasa S.A., commercial name SEITHOR® (PSEI), to be evaluated for its post-emergence herbicidal activity in greenhouse and field conditions in the Mediterranean climatic zone (vineyards in Spain and Italy). The treatments assayed under greenhouse conditions were 5 different concentrations of PA formulation, ranging from 0% to 5.6% (Table 2). To each pot, PA formulation diluted with water was sprayed by using a glass sprayer model VFOC.712/10 (VidraFOC, Barcelona, Spain), distributing a total of 50 mL per tray (1 tray per treatment, 10 pots).

**Table 2.** Herbicidal treatments tested under greenhouse conditions.

	Treatments	Abbreviations
T1	Control treated with water	CW
T2	0.7% Pelargonic acid	PSEI 1
T3	1.4% Pelargonic acid	PSEI 2
T4	2.8% Pelargonic acid	PSEI 3
T5	4.5% Pelargonic acid	PSEI 4
T6	5.6% Pelargonic acid	PSEI 5

### 2.1.3. Evaluation of the Herbicidal Activity

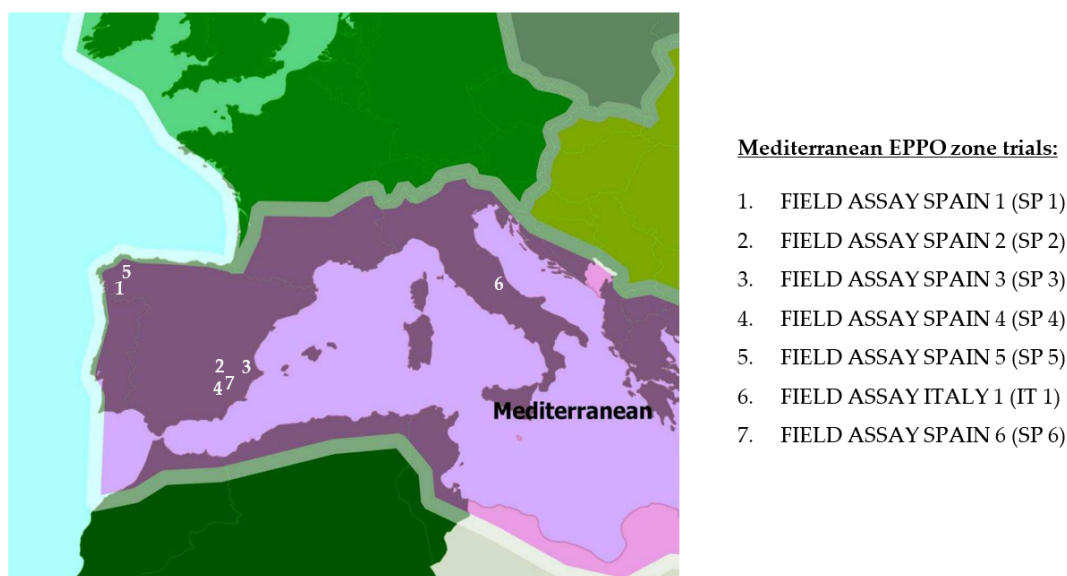
During the experiments, images from the plants were registered 1, 3, 7, 15, and 30 days after treatment to be processed with the software Digimizer v.4.6.1 (MedCalc Software, Ostend, Belgium). Previous studies reported that three days after treatment was the time when the highest susceptibility of treated plants was observed [31].

To obtain the dose–response curve for PA in both species, the efficacy of the treatment was assessed for each plant, which was defined as 0 for alive plants and 100 for dead plants.

## 2.2. Post-Emergence Herbicidal Assays against Important Weed Species in Mediterranean Vineyards

### 2.2.1. Location and Experimental Conditions

Seven trials were conducted in Spain and Italy: 6 in Spain (3 in Albacete province, 1 in Valencia province and 2 in the Galician community) and 1 in Italy (Abruzzo region), as indicated on the following map (Figure 2).



**Figure 2.** Zones of comparable climate in the European and Mediterranean Plant Protection Organization (EPPO) region as defined in EPPO Standard PP 1/241 Guidance on comparable climates for the purposes of efficacy evaluation trials on plant protection products. The borders are intentionally broad, indicating that there is an area of gradual change in climate between the zones proposed. Numbers in the map indicates the trial location.

The climatic conditions, averages of minimum and maximum temperatures, relative humidity (%), and precipitation were obtained from the official nearest weather station to each area for each trial (Table 3).

**Table 3.** Trial conditions in each field trial. All trials lasted 30 days.

Trial Code	Application Date		Temperature (°C)			Relative Humidity Mean (%)	Precipitation Mean (mm)
	1st	2nd	Min.	Max.	Mean		
SP 1	1 May 2020	15 May 2020	13.35	24.35	18.85	76.01	0.65
SP 2	2 May 2020	16 May 2020	10.75	26.75	16.67	75.70	0.19
SP 3	1 June 2020	16 June 2020	9.51	20.38	14.55	77.30	0.78
SP 4	3 May 2021	17 May 2021	11.41	25.02	18.30	65.80	1.71
SP 5	1 May 2021	15 May 2021	9.69	20.46	15.07	77.30	2.63
IT 1	5 May 2021	20 May 2021	13.25	25	21.34	64.80	4.00
SP 6	1 September 2021	15 September 2021	11.41	25.02	18.22	70.50	1.71

In this study, the field trials were conducted according to Good Experimental Practices standards (GEP). For this purpose, the European and Mediterranean Plant Protection Organization (EPPO) guidelines settled by the European Union for the herbicides efficacy assessment in Mediterranean vineyards were followed [38] (Table 4).

**Table 4.** EPPO Guidelines used for field trials design [38].

Guideline	Discipline	Description
PP 1/135(4)	General standard	Phytotoxicity assessment
PP 1/90(3)	Herbicides	Weeds in grapevine
PP 1/152(4)	General standard	Design and analysis of efficacy evaluation trials
PP 1/181(6)	General standard	Conduct and reporting of efficacy evaluation trials including GEP

The plot size (net) was at least 17 vines. The whole area between the rows was treated. Every plot was designed with 4 replicates with a complete randomized trial design according to EPPO guideline 1/152 (4) (Design and analysis of efficacy evaluation trials).

### 2.2.2. Weeds

During the experiments, the weeds growing in each plot were identified at the early seedling stage. At the end of the experiments, the number of plants from each species grown in untreated plots was compared with those present on treated plots.

### 2.2.3. Treatments

The treatments assayed in field experiments are reported in Table 5. A 55 % PA emulsifiable concentrate (EC) formulation developed by Seipasa S.A (PSEI, commercially named SEITHOR®) was applied at doses ranging from 8 L/ha to 15 L/ha (4400 to 8250 g of active substance (a.s.) per hectare). A positive control was also used, a commercial 68% PA-based herbicide EC (Beloukha®) was applied at label recommended concentrations, at 16 L/ha (10,880 g of a.s. per hectare). An untreated control without any herbicidal treatment (UTC) was used to compare the evolution of the weed populations compared with the rest of the treatments. The time between herbicidal dilution and application was less than 1 h.

**Table 5.** Treatments applied on field trials.

Treatments	Active Substance (a.s.)	Doses (L/ha)	g of a.s. per ha per Application	Abbreviations
T1	Untreated check	-	-	UTC
T2	55% Pelargonic acid SEITHOR®	8	4400	PSEI8
T3	55% Pelargonic acid SEITHOR®	10	5500	PSEI10
T4	55% Pelargonic acid SEITHOR®	12	6600	PSEI12
T5	55% Pelargonic acid SEITHOR®	15	8250	PSEI15
T6	68% Pelargonic acid reference Beloukha®	16	10,880	Ref. 16

### 2.2.4. Evaluation of the Herbicidal Activity

To evaluate the efficacy and selectivity of PSEI against different annual weeds in grapevine fields, two applications were carried out, one (A) as a broadcast application on the weeds at first stages of development (BBCH 10-12), and the second (B) after 7–14 days of the first application, being the weeds at BBCH 14-17. Selectivity and efficacy assessments were performed before treatment (A0-Preliminary), 1, 3, and 7 days after first application (DAA), and 1, 7, and 14 days after second application (DAAB). The untreated plot was used as a control to compare its results with the treated plots and for checking the growth and development of the crop under those conditions. The evaluations were performed randomly, according to EPPO n° 1/64 standard, which include how to perform trials against weeds in grapevine. The herbicidal activity was measured by calculating/estimating the following parameters:

- Weed coverage, which is the % of the surface covered by the weeds. This was assessed by visual estimation of the total surface covered by weeds in each treatment (whole area between the rows of 17 vines). This parameter was measured for each trial and pooled by year to compare the stability of the behavior of the formulations.
- Weed density, which is the number of weeds per square meter (plants/m<sup>2</sup>). The number of individuals from each weed species present in each plot was counted and

considered as representative when it was higher than 5 to ensure a minimum number of individuals representative per weed species.

- Weed control or efficacy, which is the percentage of mortality. It was assessed for each individual weed species by visual evaluation of the whole area of treated plots compared with the values found in the untreated plots (UTC). This percentage was measured for each species (both monocots and dicots) and for the total of each plot. The untreated control was settled to compare the weed populations compared with treated plots.
- Photographs of all the experiments were taken as data recording.

Additionally, grapevine phytotoxicity symptoms of damage or deformation were assessed at each evaluation date. Vigor (visual estimate in relation to the vegetative development of the crop) and growth stage of the crop were visually assessed in comparison with the untreated plots.

According to the SANCO/10055/2013 Rev. 4 (Guidance Document on the Renewal of Authorisations according to Article 43 of Regulation (EC) No 1107/2009) the following scale was proposed for the description of effectiveness against weeds (Table 6).

**Table 6.** Efficacy scale categories for weeds depending on the registered damage in field trials.

Category of Weed	Efficacy Scale
Highly Susceptible (HS)	95–100%
Susceptible (S)	85–94.9%
Moderately Susceptible (MS)	70–84.9%
Moderately Tolerant (MT)	50–69.9%
Tolerant (T)	0–49.9%

### 2.3. Statistical Analyses

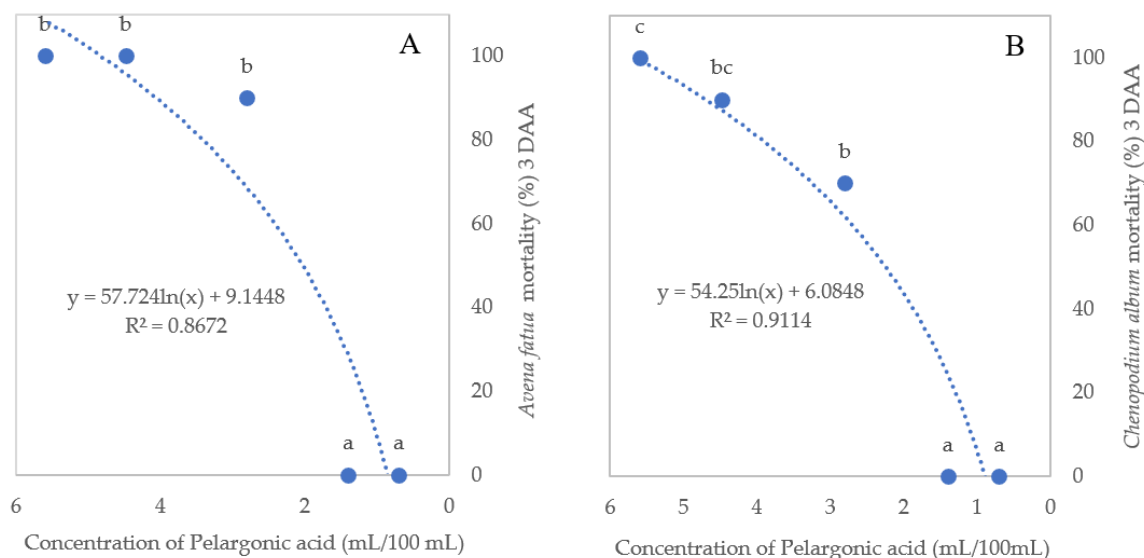
Data from greenhouse experiments were processed using the software Statgraphics® Centurion XVII (StatPoint Technologies Inc., Warrenton, VA, USA). A multifactor analysis of variance (ANOVA) was performed on efficacy including all species and treatments, followed by Fisher's multiple comparison test (LSD intervals, Least Significant Difference, at  $p \leq 0.05$ ) for the separation of the means.

The statistical design of field trials was a completely randomized blocks design including 4 replicates per treatment. The homogeneity of variance was tested for all treatments and assessment dates by means of the Bartlett's test. To study the effect of the applied treatments for each assessment data, means were compared using Student–Newman–Keuls ( $p = 0.05$ ). The statistical procedures were applied using ARM 2020, 2021 software (GDM Solutions, Brookings, SD, USA).

## 3. Results and Discussion

### 3.1. Greenhouse Experiment: Herbicidal Efficacy of PA Formulation on *A. fatua* and *C. album* Dose–Response Curves

In *A. fatua*, PSEI applied at the three highest concentrations (PSEI 3, 2.8% of PA; PSEI 4, 4.48% of PA, and PSEI 5, 5.60% of PA, Table 2) significantly affected plant viability, causing a mortality of higher than 90%. In fact, whereas the lowest doses did not induce plant mortality, the doses of 2.8 mL/100 mL, 4.48 mL/100 mL, and 5.60 mL/100 mL provoked mortality of 90, 100, and 100%, respectively (Figure 3A). Similar effects were observed in *C. album* (Figure 3B). In particular, the PSEI 3 treatment induced 70% plant mortality, while the highest concentrations assayed (4.48 mL/100 mL and 5.60 mL/100 mL) reached 90% and 100% mortality (Figure 3B).



**Figure 3.** Dose–response curve of PSEI against *Avena fatua* (A) and *Chenopodium album* (B) three days after treatment application (3DAA) under greenhouse conditions. Mortality was assessed as percentage of death plants per plot. Different letters mean statistical differences between concentrations.

Finally, the raw data of the dose-dependent curves built for both species were fitted through non-linear regression to calculate the lethal dose  $LD_{50}$ , which is considered the effective dose inducing the 50% of population mortality. The highest concentration (5.6) of PA formulation PSEI achieved 100% mortality in both species. Of the two species, *A. fatua* was the most susceptible, achieving a lethal dose ( $LD_{50}$ ) of 2.03 mL/100 mL (Figure 3A), whereas for *C. album*, it was 2.25 mL/100 mL (Figure 3B). The dose–response results allowed for identifying the key concentration to be used for the field experiments. In particular, we focused our attention on the concentration of 4.28 mL/100 mL, which induced mortality of higher than 90% in both species.

Field trials, due to variable climatic conditions and weed resistance, require higher dosage to achieve the same effect observed in laboratory tests [39]. Greenhouse experiments were performed previously to the field assays to assess the initial dose to be used in field assays based on the results obtained in *A. fatua* and *C. album* trials, which finally established the minimal effective dose of 8 L/ha PA (4400 g/ha), using a volume of treatment of around 200 L/ha (concentration of 4%).

### 3.2. Field Experiment: Herbicidal Efficacy of a New PA Formulation (PSEI) on Mediterranean Vineyards Weeds

All emerged species were identified during the field experiments, and it was noted that some species were common, because the experiments were carried out in the same climatic zone and at the same season (Table 7). There is a recent growing trend towards the replacement of traditional Mediterranean rainfed vineyards by irrigated vineyards that promotes the development of weeds [40,41]. In all the performed trials (7/7), it was possible to obtain a significant percentage of weed control (efficacy) in all treated plots compared to the untreated. The responses of all the identified weeds to the bioherbicide were categorized according to the achieved efficacy level (Table 6). The results per species were separated in two groups: monocotyledonous and dicotyledonous. Iterative experimentation under different climatic and crop scenarios are necessary to validate the efficacy of bioherbicides for their future implementation in agro-systems [20].

The most effective treatment was the Seipasa PA formulation at 15 L/ha (PSEI 15) with 8520 g/ha of a.s., achieving 71%, 91%, and 81% efficacy for monocots, dicots, and total weeds, respectively (Table 8). The results obtained with the new PA formulation were



similar to those obtained with the commercial reference, which demonstrated efficacies of 69% (monocots), 88% (dicots), and 81% (total weeds).

**Table 7.** Identified weed species in each field trial.

Trial Code	Identified Weed Species
SP 1	<i>Oxalis pes-caprae</i> L. (OXAPF); <i>Sonchus</i> sp. L. (SOONSS); <i>Cyperus rotundus</i> L. (CYPRO); <i>Bromus</i> sp. L. (BROSS)
SP 2	<i>Chrysanthemum fuscatum</i> L. (CHYFU); <i>Chenopodium album</i> L. (CHEAL); <i>Diploaxis erucoides</i> L. (DIPER)
SP 3	<i>Sonchus arvensis</i> L. (SONAR); <i>Diploaxis erucoides</i> L. (DIPER); <i>Trifolium</i> sp. L. (1TRFG); <i>Erodium</i> sp. L. (1EROG); <i>Plantago lanceolata</i> L. (PLALA); <i>Hordeum</i> sp. (1HORG)
SP 4	<i>Sonchus oleraceus</i> L. (SONOL); <i>Fumaria officinalis</i> L. (FUMOF); <i>Chrysanthemum fuscatum</i> L. (CHYFU)
SP 5	<i>Oxalis pes-caprae</i> L. (OXAPF); <i>Sonchus</i> sp. L. (SONSS); <i>Cyperus rotundus</i> L. (CYPRO); <i>Bromus</i> sp. L. (BROSS)
IT 1	<i>Taraxacum officinale</i> L. (TAROF); <i>Diploaxis erucoides</i> L. (DIPER); <i>Mercurialis annua</i> L. (MERAN); <i>Convolvulus arvensis</i> L. (CONAR); <i>Lolium perenne</i> L. (LOLPE); <i>Cynodon dactylon</i> L. (CYNDA)
SP 6	<i>Taraxacum officinale</i> L. (TAROF); <i>Diploaxis erucoides</i> L. (DIPER); <i>Convolvulus arvensis</i> L. (CONAR); <i>Sonchus oleraceus</i> L. (SONOL); <i>Bromus arvensis</i> L. (BROAV)

**Table 8.** Effects of PA formulation and commercial reference PA-based herbicide on monocots, dicots, and total weeds 14 days after second application. Data are expressed as percentage of weed control compared to untreated treatment  $\pm$  standard error (seven trials with four replicates,  $n = 28$ ).

Treatment (L/ha)	g of a.s. per ha per Application	% Control		
		Total Monocots	Total Dicots	Total (Monocots and Dicots)
PSEI 8	4400	33.67 $\pm$ 6.44 c *	47.92 $\pm$ 5.78 c	40.79 $\pm$ 4.68 c
PSEI 10	5500	53.87 $\pm$ 7.07 ab	65.15 $\pm$ 4.26 b	59.51 $\pm$ 3.65 b
PSEI 12	6600	58.76 $\pm$ 8.27 a	75.43 $\pm$ 3.49 b	67.09 $\pm$ 3.59 b
PSEI 15	8250	70.52 $\pm$ 9.52 a	91.27 $\pm$ 1.77 a	80.90 $\pm$ 3.95 a
Ref. 16	10,880	68.88 $\pm$ 9.80 a	87.68 $\pm$ 3.10 a	78.28 $\pm$ 3.73 a
Average		57.14	73.49	65.31

\* Different letters along the columns indicate significant differences ( $p < 0.05$ ).

Dicotyledonous species were more susceptible than monocots to the application of PA (to both PSEI and the reference) (Table 8). Regarding total control of all weed species (average of monocot and dicot), PSEI showed a clear dose effect, obtaining higher efficacy at higher doses. Comparing the reference (Ref. 16), at 16 L/ha (10,880 g of a.s. per ha), having a PA concentration of 68%, with PSEI, which has 55% of PA at a dose of 15 L/ha (8250 g of a.s. per ha), shows similar results, which suggests that PSI could be an improved bioherbicide formulation for weed control.

### 3.2.1. Percentage of Weed Control per Species

A total of 22 different species, 16 dicotyledonous and 6 monocotyledonous (Table 9), were identified in the treated plots of the seven vineyard trials.

Regarding dicotyledonous (Table 9, species named by EPP codes), the most susceptible species were CHYFU, CHEAL, and 1SONG, for which all treatments achieved more than 70% control. The most tolerant species was SONAR, for which no treatment exceeded 50% of control.

PSEI, at 8 L/ha (4400 g of a.s. per ha), obtained low-medium control results with minimum efficacies of 6.30% in PLALA, being considered as a tolerant weed (T) for this treatment. The highest efficacies, around 95–100% control, were achieved by PSEI at 12 L/ha (6600 g of a.s. per ha) against CHEAL, PSEI at 15 L/ha (8250 g of a.s. per ha) against DIPER, MERAN, and SONOL, and by the reference at 16 L/ha (10,880 g of a.s. per ha) against 1SONG, MERAN, and SONOL, being considered as a highly susceptible species (HS) to the treatments.

**Table 9.** Herbicide efficacy per species (dicotyledonous and monocotyledonous identified weeds).

Dicot Species *	Treatment (L/ha)				
	PSEI 8	PSEI 10	PSEI 12	PSEI 15	Ref. 16
OXAPF	45.7	77.6	78.2	85.0	83.8
SONSS	62.6	81.9	87.5	91.3	85.0
CHYFU	72.5	77.5	92.5		87.5
CHEAL	82.5	88.8	100		90.0
DIPER	65.9	77.4	85.0	99.4	90.7
SONAR	32.0	30.8	50.0		45.0
1TRFG	41.3	51.7	76.3		80.0
1EROG	20.0	61.0	76.3		94.5
PLALA	6.3	56.3	65.8		82.5
1SONG	77.5	83.8	88.8	91.3	96.3
FUMOF	53.8	62.5	62.5	81.3	93.8
1CHYG	61.3	73.8	73.8	87.5	93.8
TAROF	51.3	61.9	68.8	90.7	90.6
MERAN	25.0	36.9	50.0	100	100
CONAR	21.3	43.2	66.0	91.3	89.4
SONOL		77.5	85.0	95.0	100
<b>Monocot species *</b>					
CYPRO	34.4	51.9	78.2	88.8	71.3
BROSS	54.4	72.6	74.4	81.3	75.7
1HORG	38.3	65	60		100
LOLPE	25	47.5	56.3	80	83.8
CYNDA	16.3	23.8	21.3	35	33.8
BROAV		62.5	62.5	67.5	48.8

\* Species were coded according to EPPO standards: *Oxalis pes-caprae* L. (OXAPF); *Sonchus* sp. L. (SONSS); *Chrysanthemum fuscatum* L. (CHYFU); *Chenopodium album* L. (CHEAL); *Diploaxis erucoides* L. (DIPER); *Sonchus arvensis* L. (SONAR); *Trifolium* sp. L. (1TRFG); *Erodium* sp. L. (1EROG); *Plantago lanceolata* L. (PLALA); *Sonchus* spp (1SONG); *Fumaria officinalis* L. (FUMOF); *Chrysanthemum* sp. (1CHYG); *Taraxacum officinale* L. (TAROF); *Mercurialis annua* L. (MERAN); *Convolvulus arvensis* L. (CONAR); *Sonchus oleraceus* L. (SONOL); *Cyperus rotundus* L. (CYPRO); *Bromus* sp. L. (BROSS); *Hordeum* sp. (1HORG); *Lolium perenne* L. (LOLPE); *Cynodon dactylon* L. (CYNDA); *Bromus arvensis* L. (BROAV). Results were compared by color codes according to the obtained efficacy (Table 6). Assessments were made 14 days after second treatment application in vineyard fields located in Spain and Italy during 2020 and 2021.

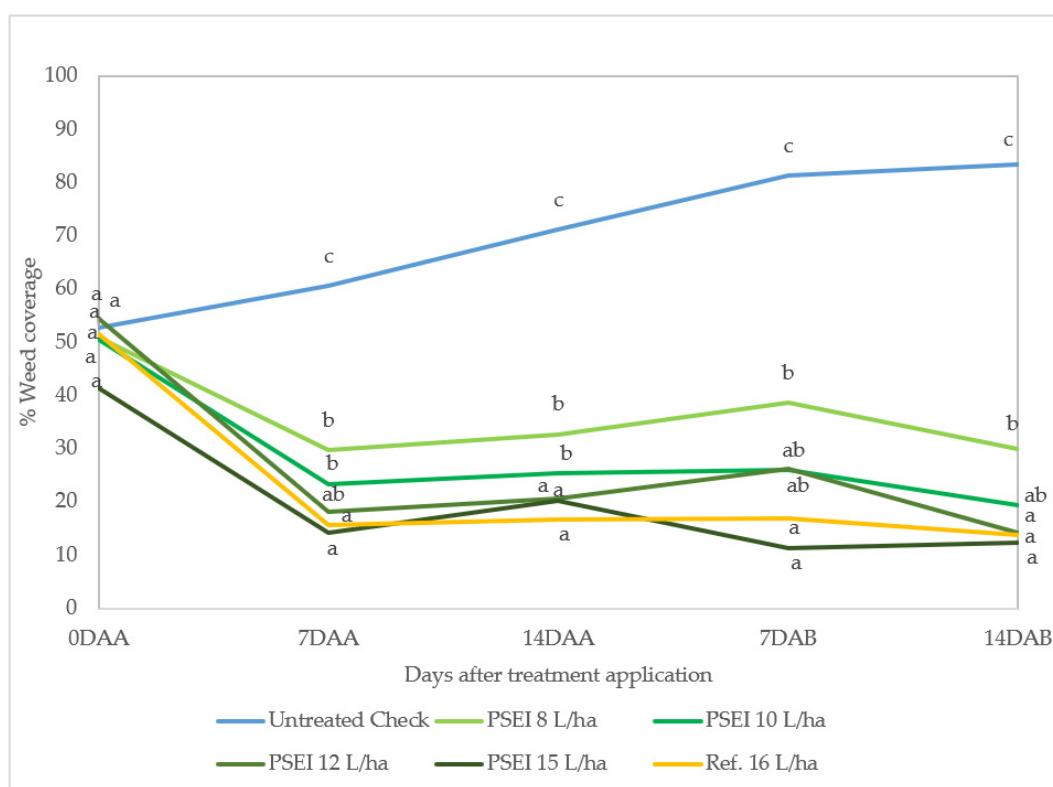
Regarding monocotyledonous species (Table 9), CYNDA, for which all treatments tested achieved less than 33.8% control, was considered tolerant (T) according to the results. In the control of 1HORG, the highest efficacy (100%) was achieved by the PA reference at 16 L/ha (10,880 g of a.s. per ha). PSEI at 15 L/ha (8250 g of a.s. per ha) obtained efficacies between 67.5 and 88.8% against CYPRO, BROSS, LOLPE, and BROAV.

A clear dose effect was observed from the overall data analysis for monocot species (Table 8). PSEI at 8 L/ha (4400 g of a.s. per ha) obtained low efficacies; thus, the minimum effective dose for PSEI could be set up to 10 L/ha (5500 g of a.s. per ha).

The results demonstrated the herbicidal potential of PA to control weeds in Mediterranean vineyards and confirmed that it could be a sustainable alternative to synthetic herbicides. These results also offer a new mode of action to control weeds that have developed resistant biotypes to many herbicides [24–26,42].

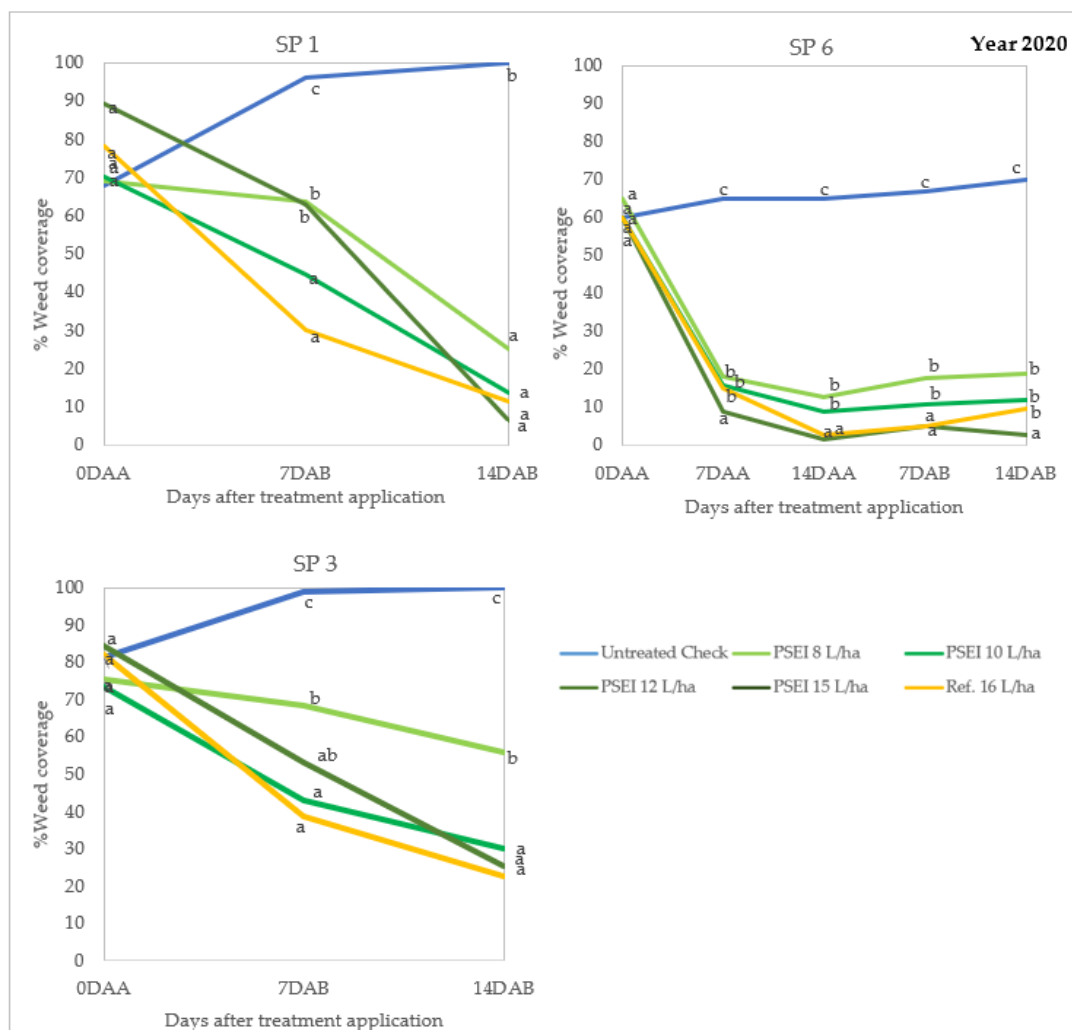
### 3.2.2. Weed Coverage Evolution

Data of weed coverage from the seven field efficacy trials were analyzed up to 14 days after the second treatment application (Figure 4). After the first application, all PA treatments achieved significant reductions in total weed coverage and in all cases differed significantly from the untreated control.



**Figure 4.** Global coverage percentage (monocots + dicots) of weed coverage in seven trials after two applications of PA formulation, PSEI, at different doses of 8, 10, 12, and 15 L/ha (4400, 5500, 6600, and 8250 g of a.s. per ha, respectively) compared to PA reference of PA at 16 L/ha (10,880 g of a.s. per ha). Assessment of weed coverage was made 7 and 14 days after the first application (A), and 7 and 14 days after the second application. A total of seven trials were established in different locations of Spain and Italy over 2020–2021 in vineyard fields. Different letters in the same assessment date point indicate significant differences ( $p < 0.05$ ) between treatments.

Fourteen days after the first application (14DAA), the total coverage of the plots started to increase, which suggested that a second application could be beneficial to maintain the weed control in the field. As shown in Figure 5, this second application kept the total weed coverage below 30% until 14 days after the second application (14DAB), a total of 30 days from the beginning of the trial.



**Figure 5.** Percentage of weed coverage evolution during 2020 vineyard trials in Spain: SP 1 in Galicia, SP 6 in Albacete, and SP 3 in Valencia region. Evaluations of % of weed coverage per plot were performed before treatment (0DAA), 7 and 14 days after the first treatment application (7DAA and 14 DAA), and 7 and 14 days after the second treatment application (7DAB and 14 DAB). Four doses of PSEI (8, 10, 12, and 15 L/ha or 4400, 5500, 6600, and 8250 g of a.s. per ha, respectively) were evaluated compared to untreated check control and PA reference at 16 L/ha (10,880 g of a.s. per ha). Different letters in the same assessment point indicate significant differences between treatments ( $p < 0.05$ ).

### Weed Coverage per Trial

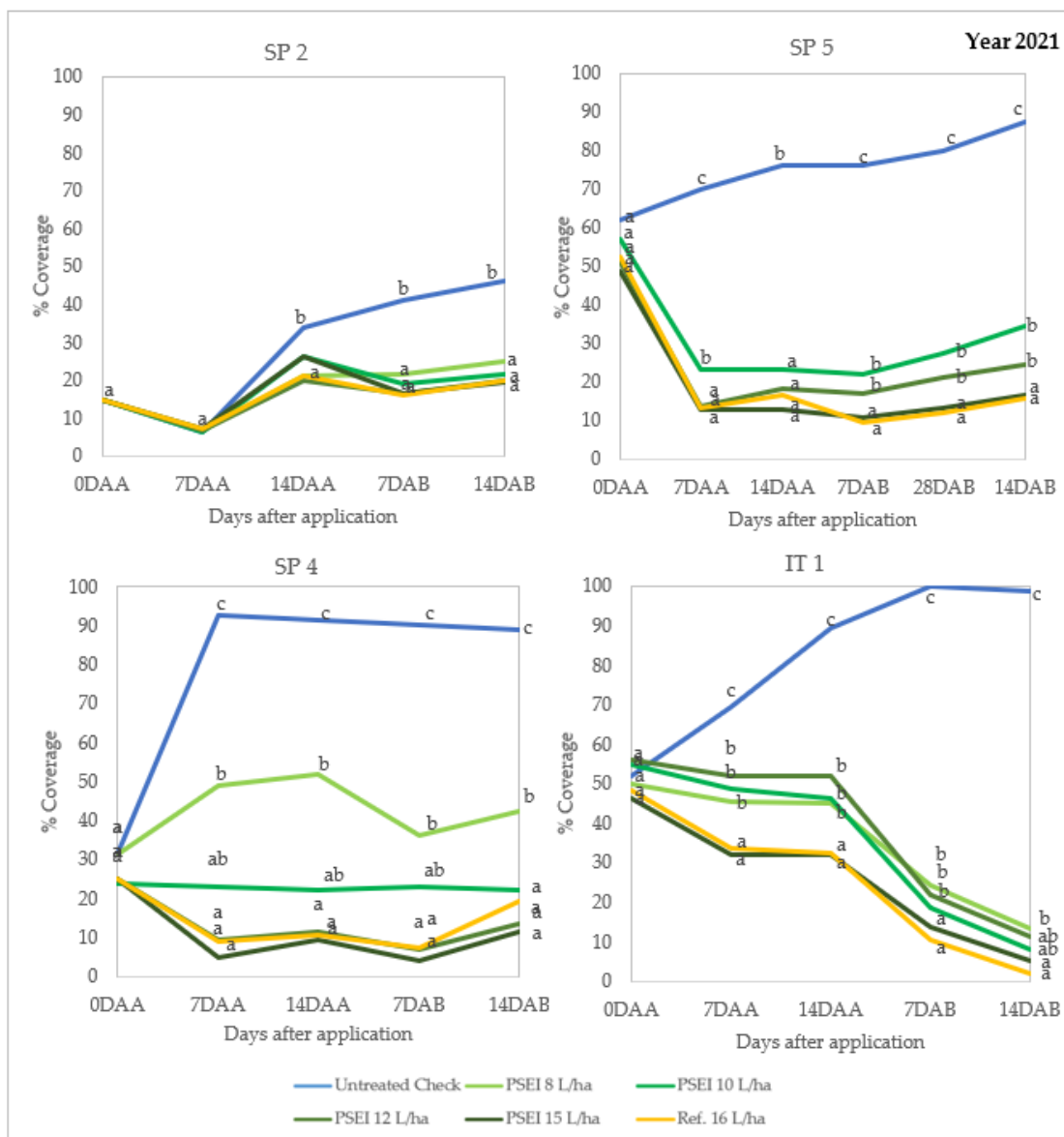
Regarding the evaluation of weed coverage percentage per trial, it could be observed that the treatments analyzed in the 2020 trials showed similar effects compared to those of 2021. In Table 10, results of weed coverage per year (without untreated control) fourteen days after the second application were compared, showing no statistical differences between years.

The trials conducted in the EPPO Mediterranean zone showed satisfactory reductions in plot weed coverage. All trials achieved significant weed reductions up to 1 month after two applications as shown in Figures 5 and 6.

**Table 10.** Results of final coverage in seven trials; 3 carried out in 2020 and 4 in 2021.

Year	Number of Trials	Number of Values	% of Final Coverage (14 DAB)
2021	4	19	17.28 ± 4.11 a
2020	3	12	19.49 ± 2.08 a

Values of all PA treatments were compared per year, fourteen days after the second application. Values are mean of % of weed coverage ± standard error. Different letters in the same column indicate significant differences ( $p < 0.05$ ).



**Figure 6.** Percentage of weed coverage evolution during 2021 in vineyard trials, 3 in Spain and 1 in Italy: SP 2 in Albacete, SP 5 in Galicia, SP 4 in Albacete, and IT 1 in Abruzzo. Evaluations of % of weed coverage per plot were performed before treatment (0DAA), 7 and 14 days after first treatment application (7DAA and 14 DAA), and 7 and 14 days after second treatment application (7DAB and 14 DAB). Four doses of PSEI (8, 10, 12, and 15 L/ha or 4400, 5500, 6600, and 8250 g of a.s. per ha respectively) were evaluated compared to untreated check control and PA reference at 16 L/ha (10,880 g of a.s. per ha). Different letters in the same assessment point indicate significant differences between treatments ( $p < 0.05$ ).

The immediate knockdown effect after the first application was variable depending on the trial. Trials with medium-high coverages at the beginning of the trial obtained significant initial reductions, whereas trials with lower initial coverages decreased it slightly (Figures 5 and 6). All PA treatments in all trials differed statistically from the untreated control. Plots treated with PA showed phytotoxicity symptoms a few hours after treatment, and this was concordant with previous research [31,43].

Weeds treated at BBCH 12–16 obtained more visible effects in the reduction of weed coverage per plot, while weeds treated at BBCH up to 18 presented moderate control and decreased their growth rate. In the open field treatments in commercial vineyards plots, where different species could be present in different phenologies, weeds were treated at the most homogeneous stage possible, between BBCH 12 to 18 depending on the climate conditions.

According to the results of weed coverage shown in the experimental plots (Figures 5 and 6), the dose of PSEI 8 was less effective. The PA reference at 16 L/ha (10,880 g of a.s. per ha) and PSEI at 12 and 15 L/ha (6600 and 8250 g of a.s. per ha, respectively) were the most efficient treatments for weed management in all the field trials. PSEI had a concentration of 55% PA, while the commercial reference had 68% of this active ingredient. Furthermore, PSEI was used at lower doses (between 4400 and 8250 g of a.s. per ha respectively) than the PA reference. The selection of an appropriate dose and its use in the field is necessary to avoid, as far as possible, the development of weed-resistant biotypes. In some cases, low or high doses may cause weed adaptation and reduced herbicide efficacy [44]. In natural weed populations, herbicide resistance is a phenomenon that occurs at a low frequency and that has evolved over millions of years [45]. The first cases of herbicide resistance were reported around 1970; since then, resistance of mono- and dicotyledonous weeds to herbicides has become an increasing problem worldwide, including 267 species and 21 modes of action (MoA) [45–47]. Global weed species are accumulating resistance mechanisms, displaying multiple resistance across many herbicides, and posing a great challenge to herbicide sustainability in world agriculture [47]. That is why building sustainable solutions to herbicide resistance evolution are necessary and worthy challenges.

Pelargonic-acid-based formulations available in the market need high doses to show a significant weed control; in, for example, amateur applications normally used in home gardens and ornamentals, the recommended doses are 166 L/ha (112,880 g of a.s. per ha) for the best-known natural herbicide in Switzerland [31]. A reduction of product doses in field application is important to facilitate and reduce the cost of bioherbicide treatments for growers. PSEI dosage reduction without decreasing efficacy is a key point in order to be able to make several applications per crop cycle. An appropriate formulation is essential to ensure a homogeneous mixture in water, as well as selection of nozzles to reach the weed surface effectively [48,49]. Probably due to the effect of PA on the cuticles, with the later alteration on membrane permeability and peroxidation of thylakoid membranes [21,30,31], leaves appeared desiccated, with chlorosis signs but without punctual damages on the leaves, resulting in a stop of growth and development of the whole plant. Knowledge of the mode of action is key to developing improved formulations with this active ingredient. Clear physico-chemical differences were observed between the formulations of PSEI and the PA reference: while PSEI shows homogeneity in the water emulsion before applying, the reference product was separated in different phases, needing continuous agitation to ensure a good foliar coverage. Two applications of the PA-based products significantly affected the final efficacy against weeds: while the first application acted as a desiccant on the weeds according to the effects described, the second application affected the regrowth or the parts where the product could not arrive due to overhead foliage or lack of spray efficiency. The application performance and efficacy of herbicides are highly related to their chemical structures, the stability of their formulations, their target, and the application environment [9,50]. The use of PA bioherbicides can be integrated with other tools for weed control, such as mechanical

methods to control species in advanced stage of development, applying the PA after the use of said mechanical control. The application time for PA formulations is one of the requirements to ensure an optimal weed control. Only weeds treated in first stages of development, as described in the trials in this work, could be controlled with high efficacy in field conditions with the active substance. Ensuring a good coverage and correct distribution of the product in the leaves are other important considerations for field applications.

Natural compounds with herbicidal activity have several advantages compared with synthetic herbicides. For example, they have new modes of action or different target sites in weeds that can prevent the development of weed resistance problems. They have better public acceptance and may be cheaper to register by companies for their use in agriculture, because of their low potential risk. Currently, they are limited by their expensive synthesis procedures, their toxicological issues, the difficulties in the supply process, and the shelf life of the molecules in field conditions [51].

As well as PA, other natural compounds, such as shikimic acid derivatives, simple phenols, benzoic acid derivatives, flavones, and tannins, have been studied as substances with herbicide potential. All of them are considered phenolic compounds, the largest group of allelochemicals, which, although being the most abundant, require high production costs and have low stability and selectivity. For these reasons, they have not been developed as commercial products as much as fatty acids like PA [52]. Some natural compounds with herbicidal potential could be evaluated in mixture or combination with PA to find possible synergistic effects that combine modes of action and reduce field dosage and costs for farmers. Kava root (*Piper methysticum* L.) [53], carvacrol, trans-cinnamaldehyde [35], some essential oils or terpenoids [30], and acetic acid could be potential active substances in combination with PA to control weeds and avoid resistance problems [54].

Pelargonic acid could be considered as an alternative to other herbicides that are more toxic for the environment and health. Pelargonic acid is considered a compound of low toxicity and low environmental impact but has no residual activity or translocation potential [24,55]. For example, it can substitute applications of glyphosate or other contact herbicides, such as paraquat or diquat, that have restrictions on their use in professional plantations and in non-agricultural uses (industrial, roads and highways, gardens, urban applications) in Europe [56–58]. The use of PA in other agricultural regions and climatic zones depends on weed tolerance, traditional practices, and regulatory issues. Further research is needed to establish the range of use of PA according to climatic conditions, crops, and requirements.

Despite the potential benefits of PA, sometimes it is mistakenly compared to and used like glyphosate and other post-emergence herbicides and is applied to well-established weeds (50 cm tall), which leads to a false expectation of efficacy and improper use of the product [59]. The correct use of this active substance should be established by providing information and training to farmers about integrated weed management, making field applications at the indicated times, and respecting label instructions in order to establish an appropriate niche market for the active substance [57–60].

#### 4. Conclusions

The different PA formulations tested achieved high herbicidal activity in the majority of weeds against which they were applied, causing high mortality and reduction of weed coverage in the studied plots, demonstrating that they could be good candidates for weed management in woody crops such as vineyards in Mediterranean conditions. Monocotyledon species were less susceptible to the treatments. The formulation of PA, developed by Seipasa (PSEI) and with less active ingredient than the PA reference available in the market, could achieve good efficacies according to their improved formulation. Further investigations should focus on determining the optimum phenological stage of weeds for herbicide application, the persistence of the treatment, and the interaction of climate

conditions with the potential efficacy, which is very important in the context of integrated weed management and sustainable agriculture in different climatic areas. Field conditions can influence the efficacy of PA products, requiring a higher dosage to obtain the same effect as in trials under controlled conditions (greenhouse or growth chamber).

Some phytotoxicity symptoms were observed on grapevines in the trials conducted during 2020 and 2021 in the Mediterranean area. The product affected the green areas of the weeds, such as leaves, with no adverse effect on the woody part of the vines. An efficient application is necessary to ensure that the product reaches the weeds and not the crop leaves. In other horticultural crops where herbaceous parts are predominant, the product should be evaluated to ensure the optimal application time without producing any phytotoxic effects on the crop.

A better understanding of the natural compounds and their mode of action could lead to a more efficient formulation for their optimal administration. The potential risk of each active substance should be evaluated to ensure the viability of sustainable and environmentally friendly formulations.

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