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Management of Chrysanthemum Verticillium Wilt through VIF Soil Mulching Combined with Fumigation at Label and Reduced Rates

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Abstract: Pre-plant soil fumigation for managing soilborne pathogens is practiced worldwide in several intensive ornamental and vegetable production areas. However, global regulations are currently implemented to minimize use of these chemicals. According to these policies, the efficacies of dazomet (DZ, Basamid Granulat®) applied alone to soil and under virtually impermeable film (VIF) at reduced (247.5 kg ha^{-1} a.i.) and label (495 kg ha^{-1} a.i.) rates were assessed in managing natural infections of Verticillium wilt and in reducing yield losses on greenhouse chrysanthemum plantations in two trials carried out in the 2012 and 2013 seasons. The performances of this fumigant at a lower rate combined with VIF application were also compared with standardized metam-sodium (MS, Divapan®) fumigation applied at 510 L ha^{-1} a.i. under VIF and application of dazomet at label rate alone to bare soil. Temperatures were monitored at 20-cm depth in plots covered (VIF) throughout the entire fumigation period. Although all fumigation treatments significantly reduced the infection level by *V. dahliae* on chrysanthemum, the performances differed among them. DZ and MS applied at label rates under VIF showed the most effectiveness in controlling Verticillium wilt of chrysanthemum. However, DZ applied to soil at a low rate under VIF mulching showed good performances, which were always better than application of DZ alone at label rate to bare soil. Moreover, when combining a reduced rate of DZ with VIF mulching, chrysanthemum yield losses were reduced in a similar manner to the label rate application under VIF. Based on these findings, DZ application at a reduced rate could be suggested on a large scale to prevent Verticillium attacks on chrysanthemum cultivated on a protected crop. Future studies should be performed to verify the ability of VIF application in inducing high temperatures in soil and above all in enhancing performances of soil fumigation at low rates performed with other natural and chemical sterilants.

Keywords: soilborne pathogens; *Verticillium dahliae*; virtually impermeable film; reduced rate; chrysanthemum production; sustainable management

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Citation: Castello, I.; D'Emilio, A.; Baglieri, A.; Polizzi, G.; Vitale, A. Management of Chrysanthemum Verticillium Wilt through VIF Soil Mulching Combined with Fumigation at Label and Reduced Rates. *Agriculture* **2022**, *12*, 141. https://doi.org/10.3390/agriculture12020141

Academic Editors: Giuseppe Lima and Catello Pane

Received: 3 November 2021
Accepted: 19 January 2022
Published: 21 January 2022

1. Introduction

The cultivation of chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) for cut flower production is a highly profitable industry in Europe. The Netherlands and, to a lesser extent, Italy are large producer countries. Italy intercepts the largest production area (approximately 1200 ha), although with a lower average yield [1]. In detail, Italian production of chrysanthemum cut flowers is mainly concentrated in Ragusa province (South Italy) where it reaches about 8% of Europeans thanks also to high-tech greenhouses, allowing average yields for these areas that are comparable with The Netherlands.

Unfortunately, chrysanthemum production is heavily threatened by Verticillium wilt, a disease caused by *Verticillium dahliae* Kleb., which is considered one of the most important soilborne fungi affecting many woody, vegetable and ornamental crops, including chrysanthemum [2–9].

Verticillium dahliae colonizes the vascular tissues of infected plants which at first show yellowing and wilting of lower foliage followed by plant collapse and death. The fungus differentiates in the necrotic tissues of resting thick-walled structures of plant debris, i.e., black microsclerotia, which may persist in the soil in absence of hosts for up to more than 10 years [3,10,11].

Once *V. dahliae* is well-established in a greenhouse, its control is very difficult and management strategies should be mainly addressed to reduce fungal resting structures in soil. Soil solarization, reported to be effective for sustainable managing of many soil-borne pathogens [12–16], is a very time-consuming technique whose performances are variable and depend on many factors [12,16]; for these reasons, it is not yet widespread enough for this crop. Otherwise, steam application and fumigation (mainly with metam sodium) of soil are employed in the Mediterranean basin and worldwide. However, the soil steaming is justified exclusively in intensive crop systems and it cannot be applied under open field conditions. Even soil fumigation has many limits since it is subjected to severe restrictions of EC and global regulations (e.g., Directive 2009 128/EC and the more recent Green New Deal-GND [17] that imposes progressive reduction of the amount and application number of fumigants. Metam sodium (sodium N-methyl dithiocarbamate) and dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5 thiadiazine-2thione) are chemical molecules that in soil undergo a process of chemical degradation which produces methyl isothiocyanate (i.e., M-ITC), a volatile substance which is responsible for the disinfestation effect against various soilborne pests [18–23]. Alternatively, M-ITCs from natural sources have been employed for eco-sustainable soil disinfection [20,24]. Nevertheless, the efficacy of fumigants can be compromised by many factors, such as incorrect application methods, unfavorable environmental conditions and high levels of dispersal into the atmosphere [18]. For example, these fumigants must be applied when soil temperatures and moisture levels allow a complete efficacy against several plant pathogens including *V. dahliae* [25]. For these reasons scientists have recently focused on developing good agricultural practices (GAPs).

In detail, wide research activity has been performed in the last two decades and is actually focused on development of low permeability materials (e.g., Virtually Impermeable Film-VIF). Use of colorless VIF materials, that differs from traditional high-density polyethylene (HDPE) film in that it has additional gas impermeable layers, such as nylon or polyaminides, between the PE layers [26], can enhance fumigant distribution in soil, in comparison with conventional PE films or bare soil [27,28]. Furthermore, VIF films perform better than PE films in increasing soil temperature up to a 30 cm depth [29]. Moreover, these innovative films are able to reduce fumigant loss and application rate and, at the same time, to increase fumigant retention over time and fungicidal performances [30–33].

Since *V. dahliae* represents the most limiting factor for chrysanthemum production in the Mediterranean basin, the objectives of this paper were to compare the efficacy of metam sodium and dazomet—for the latter also at a reduced rate—combined with VIF application to soil (i) for management of chrysanthemum Verticillium wilt in greenhouses and (ii) for reducing yield losses; (iii) the performances of VIF in increasing thermal regimes were also evaluated.

2. Materials and Methods

2.1. Description of Experimental Location and Operative Conditions

Two in field trials were conducted from June to October 2012 and 2013 in south eastern Sicily (36°08′ N, 14°32′ E) at an altitude of about 345 m above sea level (a.s.l.), in a multi-span tunnel greenhouse with a steel structure, in two adjacent spans each covering a 28.5 × 8.0 m soil surface (Figure 1).

Figure 1. Location and experimental scheme adopted for the first trial (source: https://www.google.it/intl/it/earth/ accessed on July 2018).

This experimental site was naturally infested by the fungal pathogen and had a history of widespread Verticillium wilt attacks observed over time on chrysanthemum (*Chrysanthemum* × *morifolium*,) cultivar Anastasia plantations under a greenhouse without any crop rotation. Anastasia cultivar is characterized by its large quilled spider double-type inflorescences with white ray florets, numerous ray florets, few inconspicuous disc florets and dark green foliage; it has strong flowering stems with a low number of lateral branches, a short response time and good postproduction longevity [34]. During the season before setting up experimental trials, approximately 15–20% of chrysanthemum plants showed characteristic wilting of the lower leaves in all bare plots. Symptomatic leaf area progressively increases, wilts and die. As the infection progresses up the stem, affecting all foliage, the number of marketable flowers was seriously reduced.

The soil samples were air dried, sieved at 2 mm and characterized for pH (7.6), texture (48% sand, 39% clay, and 13% silt), organic carbon (6.8 g kg^{-1}) and cation exchange capacity [16.1 cmol (+) kg^{-1}], according to Violante [35]. The soil texture was classified as loam and *Typic xerofluvents* soil, according to the USDA Soil Taxonomy [36].

2.2. Experimental Scheme, Treatments and Operative Conditions

For both trials soil was leveled and irrigated up to field capacity (about 40 to 50 L m^{-2}) before starting experimentation. Throughout the entire treatment period, no additional irrigation was supplied and the greenhouse was maintained uncovered. The commercial fumigant contained active compounds, which were Basamid Granulat® (dazomet 99%-DZ, water dispersible granules, Kanesho Soil Treatment, Bruxelles, Belgium) and Divapan 51® (metam sodium 51%-MS, suspension concentrate, Eastman Italia S.r.l., Milano, Italy) and, where it was due, the film used for mulching soil was colourless VIF (Ecobrom, Agriplast S.r.l., Vittoria, Ragusa, Italy). Inside the greenhouse (28.5 × 8.0 m), five different treatments were arranged in a randomized complete block design (RCBD) with four replications (plots of 4 × 2.50 m) for each treatment. Treatments for both trials included: (i) untreated and bare control; (ii) soil amendments of Basamid Granulat at 500 (495 a.i. DZ) kg ha^{-1} label rate followed by VIF mulching; (iii) soil amendments of Basamid Granulat at 250 (247.5 a.i. DZ) kg ha^{-1} reduced rate followed by VIF mulching; (iv) soil amendments of Basamid Granulat at

500 (495 a.i. DZ) kg ha^{-1} (label) rate without mulching; (v) application by injector poles of Divapan 51 at 1000 (510 kg a.i. MS) L ha^{-1} full rate followed by soil mulching with VIF (Figure 1). In all VIF mulched plots, the film sheets were laid on the soil surface, and their edges were buried 30 cm deep. The soil was slightly raised along the edges of each plot, and the film sheets were manually tensioned. The fumigation period ranged for both crop seasons (2012 and 2013) from 21 June to 8 July. After the fumigation period, the VIF tarping was removed from all mulched plots and left uncovered for three weeks before allowing re-entry into the greenhouse. The chrysanthemum with Anastasia cultivar transplanting was carried out on 28 July.

During both trials, soil temperature was measured at 20 cm depth in the middle of one plot for each treatment. Two platinum thermoresistance probes (PT100, model 108, Campbell Scientific Ltd., Shepshed, Lougborough, UK) were used for each depth. After the placement of probes, the film sheets were laid on the soil surface and their edges were buried up to 30 cm depth. Furthermore, air temperature and relative humidity were measured with sensors composed of a PT100 and a capacitive element (model RH1, Environmental Measurements, Ltd., North Shields, UK). Solar radiation flux was measured with pyranometers (model EQ08, Middleton Solar, North Geelong, Australia) in the wavelength range from 300 to 3000 nm. The sensors inside the greenhouse were placed in the middle area at a height of 1.8 m above the soil, whereas the sensors outside the greenhouse were placed above the ridge at a height of 4.5 m. All measured variables were sampled every 30 s with a datalogger (model CR10X, Campbell Scientific, Ltd., Loughborough, UK) and averaged to provide half-hourly values.

2.3. Verticillium Disease and Chrysanthemum Yield Assessment

The evaluation of treatment effectiveness was referred to disease incidence (DI) and severity of Verticillium wilt symptoms (SS) and it was always performed in the last decade of October at the end of the cropping season. The DI parameter was calculated for each plot on the basis of the percentage of chrysanthemum plants affected by Verticillium wilt on the total number of examined plants, whereas SS was always referred to an empirical 5-class scale taking into account the length of infected stems with withered foliages, where 0 = healthy stem, up to 4 = highest disease level based on stem length with infected foliage (Figure 2). This scale was set up with slight modifications according to a previous scale and used for the evaluation of Verticillium wilt SS [2]. Severity symptoms (SS) was calculated with the following formula:

$$\mathrm{SS} = \frac{\sum_{\mathrm{j}=0}^{n}(\mathrm{C}j \times n)}{\mathrm{N}}$$

where SS is the average index of disease severity, C*j* each class considered, *n* the number of infected plants in each class, j numerical values of classes from 0 to 4 and N the total number of plants examined. To this aim, about 70–80 plants were randomly selected from the middle area of each plot, excluding border rows, and evaluated. Following disease assessment, ten small samples were recovered from host tissue of five infected stems randomly selected within each plot to identify and confirm the causative agent and, consequently, the pressure level of natural infections. To this aim, the samples were sterilized with 0.8% NaOCl solution for about 1 min and rinsed with sterile distilled water before they were transferred onto PDA (Oxoid, Basingstoke, UK). Then, the plates were incubated at 25 °C for 10 days in the dark. On the basis of the recovery of *V. dahliae*, colonies from the symptomatic plants average DI and SS values were definitively adjusted.

Average yield from each plot of all treatments were also determined at the end of the cropping season by counting the number of marketable flower stalks of chrysanthemum in the total for each plot.

Figure 2. Disease scale consisting of 5 classes [0 = healthy stem (herein not reported), 1 = initial symptoms on basal leaves, 2 = low symptom severity, 3 = medium symptom severity, 4 = high symptom severity] used to evaluate mean severity index of Verticillium wilt of *Chrysanthemum* × *morifolium* cultivar Anastasia in field trials carried out in 2012 and 2013.

2.4. VIF Effects on Survival Verticillium dahliae Inoculum

Similarly to previous trials, the performances of VIF applied alone to soil in reducing viability of *V. dahliae* microsclerotia, obtained from previous chrysanthemum plantation (2011), was evaluated in both 2012 and 2013 crop seasons. To this aim, a monoconidial isolate from infected chrysanthemum tissue was used to produce inoculum. The mature microsclerotia were produced on sterilized paper placed on technical agar (Oxoid, Basingstoke, UK) plates. Subsequently, the plates were inoculated with pathogen and incubated for 4 weeks at 25 $\pm$ 1 °C. Ten paper pieces (0.5 $\times$ 0.5 cm in size) colonized by *V. dahliae* microsclerotia were removed from Petri dishes and placed into nylon mesh bags. Ten nylon bags were buried in uncovered and VIF mulched plots (three replicates for each treatment) at 20 cm depth in the adjacent greenhouse that was left uncovered for the entire treatment period. Soil temperatures were recorded in each plot, as previously described. Nylon mesh bags were sampled 17 days after inoculum placement and *V. dahliae* colonies re-isolated from the infected samples. Single paper pieces were washed with sterile water and placed onto (PDA) and maintained for 10 days at 25 ± 1 °C. The percentage of leaf pieces from which pathogen colonies developed was used to determine the survival of *V. dahliae* under two thermal regimes detected under VIF mulched and bare plots.

2.5. Data Analysis

Data about performance evaluation of soil treatments from the repeated experiment were analyzed by using the Statistica package software (version 10; Statsoft Inc., Tulsa, OK, USA). The arithmetic means of disease incidence (DI), symptom severity (SS) and yield were calculated, averaging the values determined for the single replicates of each treatment. Percentage data concerning DI were previously transformed into the arcsine ($\sin^{-1}$ square root x) and then subjected to analysis of variance (ANOVA), whereas SS and yield values were not transformed. Initial analyses of DI and yield were performed by calculating F and P values associated to evaluate whether the effects of single factor and treatment × trial interactions are significant. In the posthoc analyses, the mean values of DI and yield values were subsequently separated by the Fisher's least significance difference test (α = 0.05). Untransformed arithmetic means of DI, SS and yield are presented in the following table and figures.

Because an ordinal scale (0-to-4 class) was adopted for SS data evaluation (Figure 2), different nonparametric approaches were used. Kendall's coefficient of concordance (W) was calculated to assess whether the rankings of the SS scores among soil treatments are similar within each trial (treatment × trial interactions). Since in the efficacy experiment W was higher than 0.75, the SS scores were at first analyzed by using Friedman's nonparametric rank test, and subsequently followed by all possible pairwise performed with the Wilcoxon signed-rank at $p < 0.05$. On the other hand, when the only treatment effects were examined, the Kruskal-Wallis nonparametric one-way test was preliminarily applied, calculating χ^2 and p value associated.

3. Results

3.1. Monitoring Environmental Conditions and Soil Temperatures

Table 1 shows the statistical values of air temperature and solar irradiation calculated from the measured values. The weather was characterized by clear skies for almost the whole period of the two trials, except for a couple of partially cloudy days in 2013. For this reason, the mean values of solar irradiance were similar during the two trials, but with a lower minimum value and a higher standard deviation in the second trial than in the first. Air temperature during the 2013 trial was generally lower than in the 2012 trail. Specifically, in 2013 the minimum, the mean and the maximum values of air temperature were 5.5 °C, 3.8 °C and 1.0 °C less than in 2012, respectively. The relative humidity during the two trials was similar, with a mean value just over 50% and a maximum value of 100% (saturation) during night-time and early hours of the morning.

Table 1. Statistical values of air temperature (T), air relative humidity (RH) and solar irradiation (Irr) calculated from values measured outside the greenhouse during the two trials (from 21 June to 8 July 2012–21 June to 8 July 2013).

	2012			**2013**		
	T (°C)	**RH (%)**	**Irr (MJm^{-2})**	**T (°C)**	**RH (%)**	**Irr (MJm^{-2})**
Min	17.9	17.0	23.4	12.4	23.0	16.0
Mean	26.8	51.8	28.2	23.0	59.7	26.9
Max	37.7	100	30.5	36.7	100	30.1

Table 2 reports the main statistical values calculated for the soil temperatures measured in bare soil and VIF mulched soil at 20 cm depth during the two trials.

Table 2. Minimum (Min), mean of daily minimum (Mmin), mean, mean of daily maximum (Mmax) and maximum (Max) values, calculated for the soil temperature measured at 20 cm depth during the duration of two trials (from 21 June to 8 July 2012–21 June to 8 July 2013).

	2012		2013	
	Bare Plots (°C)	**VIF (°C)**	**Bare Plots (°C)**	**VIF (°C)**
Min	30.2	32.9	27.8	30.6
Mmin	31.0	33.8	29.8	32.7
Mean	32.4	35.9	31.1	34.5
Mmax	33.6	38.2	32.4	36.9
Max	34.9	39.3	34.5	39.4

The analysis of the table shows that the temperatures measured in the bare soil were always lower than the corresponding values measured in the VIF mulched soil, with differences in the mean values of 3.5 °C during 2012 and 3.4 °C during 2013. This difference decreases to values slightly below 3 °C when means of daily minimum were considered and increases up to about 4.5 °C, when means of daily maximum were considered.

As expected on the basis of the climatic conditions previously exposed, the comparison between the two years shows slightly higher temperatures during the 2012 trial compared to the 2013 trial. Specifically, these differences on the mean values are 1.3 °C and 1.4 °C for bare and mulched soil, respectively.

3.2. Evaluation of Treatment Performances, Disease and Yield Assessment and Pathogen Viability

In greenhouse experiments about soil treatment performances, there was always a significant effect of treatments on all parameters, i.e., average incidence and severity of Verticillium wilt and mean yield (p value < 0.002) (Table 3). Besides, Kendall's coefficient of concordance was 0.86 (>0.75) for SS data, thus indicating very high concordance between the two trials (Table 3). Therefore, the two trials were combined.

Table 3. Analysis of variance for disease incidence, severity (nonparametric approach) and n. of marketable chrysanthemum stalks among 5 soil treatments in two trials.

Factor(s)	**Parameter**								
	Disease Incidence ^{y}			**Disease Severity** ^{y}			**Yield (No. of Marketable Stalks)** ^{z}		
	df	*F*	*P value*	χ^2	*W*	*P value*	*df*	*F*	*P value*
Treatment	4	25.1985	*<0.0001*	22.00	. . .	*0.002*	4	11.260	*<0.0001*
Treatm. × Trial	4	0.4533	0.769183 ^{ns}	. . .	0.8577	...	4	0.034	0.997692 ^{ns}

^{y} F test of fixed effects, df = degrees of freedom and P value associated to F; ns = not significant data. ^{z} the χ^2 value for Kruskal-Wallis one-way analysis of variance test; W = Kendall's coefficient of concordance between repeated trials in the experiment.

Data on treatment effectiveness were definitively adjusted based on the recovery of fungal colonies from examined infected samples collected from each plot of soil treatment. In this regard, fungal colonies yielded on PDA were white-creamy colored with an abundance of mycelia and later developed into black color due to the formation of microsclerotia embedded in the artificial media. Microscopic examination showed conidiophores and phialides both appeared in a verticillate arrangement, whereas conidia were hyaline, smooth-walled and ellipsoidal to oval with average size 5.4 × 2.5 µm (n = 100). Microsclerotia appeared in elongate to irregularly spherical shape and were variable in size. Morphological characteristics of fungal colonies were comparable to *Verticillium dahliae* Kleb. described by Hawksworth and Talboys [37].

All fumigation treatments were always effective in reducing disease incidence and severity of natural infections of Verticillium wilt on chrysanthemum when compared to control plots. Comprehensively, both MS and DZ applied under VIF mulching at label rates revealed the best effectiveness among all tested treatments (significant data) in reducing the natural infections of *V. dahliae* on chrysanthemum. In addition, DZ applied at a reduced rate under VIF soil mulching showed better performances in reducing infections than application of DZ alone at label rate to bare soil (only for DI) and was even comparable (not significant data for only SS values) to those of DZ at label rate under VIF (Table 4).

Table 4. Compared performances of fumigation treatments in reducing natural infections (DI = disease incidence; SS = severity symptoms) caused by *Verticillium dahliae* on *Chrysanthemum* × *morifolium* cv "Anastasia" during 2012 and 2013 crop seasons.

Treatment	DI (%) ^{x,y}	SS (0-to-4 Scale) ^{x,z}	Sum of Ranks ^{z}
Untreated (bare)	22.04 ± 4.28 ^{a}	1.64 ± 0.24 ^{a}	267.50
Dazomet (495 kg ha^{-1}) plus VIF	4.34 ± 0.73 ^{d}	1.12 ± 0.04 ^{c,d}	93.00
Dazomet (247.5 kg ha^{-1}) plus VIF	7.80 ± 0.58 ^{c}	1.22 ± 0.06 ^{b,c}	151.50
Dazomet (495 kg ha^{-1})	11.93 ± 0.97 ^{b}	1.37 ± 0.12 ^{b}	218.00
Metam-Na (510 kg ha^{-1}) plus VIF	3.55 ± 0.42 ^{d}	1.10 ± 0.04 ^{d}	90.00

^{x} Combined data from 2 trials. Values followed by standard error of the mean (SEM) and means derived from 50-to-100 young plants. ^{y} Disease incidence values followed by same letters within the column are not significantly different according to Fisher's least significance difference test ($\alpha = 0.05$). ^{z} Differences among average SS (0-to-4 scale) data between treatments (different letters within the column) were analyzed with Friedman two-way analysis of variance by mean rank scores ($p < 0.001$) followed by all pairwise multiple comparison with Wilcoxon signed-rank test ($p \leq 0.05$).

As regards performances on yield, all treatments increased the number of marketable flower stalks from 13.8% up to 59.8% if compared to yield recorded on control plots. However, significant increases of yield were detected only in all plots simultaneously fumigated and mulched with VIF (Figure 3). In detail, DZ, when also applied at a reduced rate (247 kg ha^{-1}) under VIF, did not provide mean yield significantly different from those detected in the remaining plots fumigated with MS and DZ at label rate under VIF (Figure 3). Otherwise, DZ applied at label (495 kg ha^{-1}) rate in plots left uncovered for the entire period of treatment did not induce a significant increase of marketable stems (Figure 3).

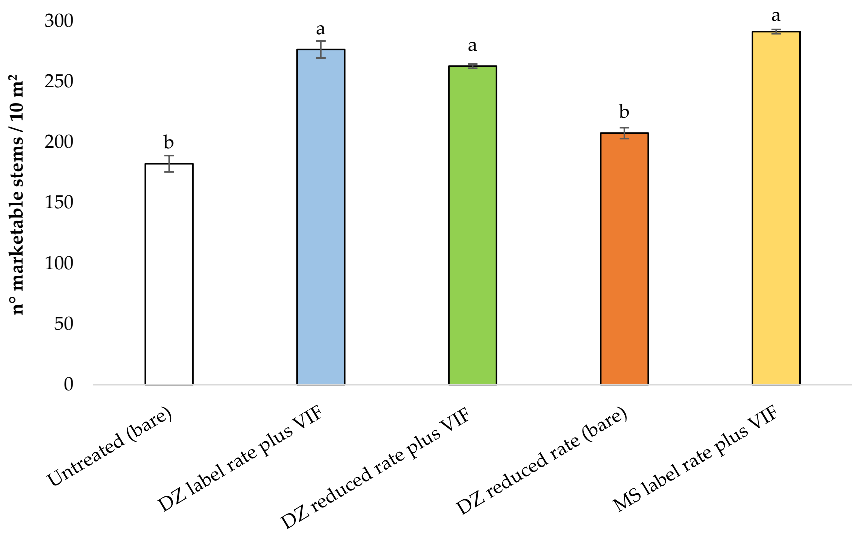

Figure 3. Performances of fumigation treatments in enhancing yield (commercial stalks of *Chrysanthemum* × *morifolium* cv Anastasia) detected in treated plots compared with control plot (white column). Data (from two combined trials) are means of four replicates (plots). Bars followed by the same letters are not significantly different among them according to Fisher's least significance difference test ($\alpha = 0.05$).

To analyze the survival of pathogen microsclerotia, all nylon mesh bags containing microsclerotia-colonized paper pieces were recovered after a 17-day treatment period from VIF mulched and bare plots, respectively. In both experiments, percentage recovery of viable inocula of isolates from infected paper debris was strictly dependent on the soil temperatures detected at 20-cm depths as reported above in the previous paragraph (Tables 1 and 2). However, the high performances of VIF in increasing soil temperatures achieved on mulched plots significantly reduced *V. dahliae* microsclerotia viability by almost 35% if compared to uncovered plots (data not shown).

4. Discussion

Verticillium wilt represents a serious threat for chrysanthemum crops especially in Italy and in many European producer countries (e.g., The Netherlands), where intensive cut flower production does not foresee any crop rotation. In addition, in some agroecosystems common practices can also include partial incorporation of cultural debris in the soil at the end of the crop season, thus resulting in an ever-increasing of *V. dahliae* potential inoculum over time [2,3,11]. Based on these aspects, management of Verticillium wilt should be focused on control measures involving reduction or inactivation of primary inoculum in soil, that are finalized to maintain yield losses below the economic damage threshold.

This paper provides basic information on DZ performance compared with standardized MS fumigation in reducing Verticillium wilt of chrysanthemum. Although the activity in semi-field and controlled conditions of DZ in reducing soilborne pathogens, pests and weeds is well-known [33,38–41], our findings represent the first confirmation of DZ efficacy in both reducing both natural infections of *V. dahliae* and yield losses over time in a productive scenario. Only Pecchia et al. [6] recently found the efficacy of another fumigant, dimethyl disulfide (DMDS) applied at label rate under VIF, in managing this fungal disease and avoiding yield losses comparable to those of standard MS fumigant. Similarly, DZ applied under VIF before transplanting herein provides the same performances against Verticillium wilt and provides high production of chrysanthemum cut-flowers to those achieved by MS fumigation performed under the same mulching.

Since DZ may be used in field and in greenhouse up to a maximum label rate of 693 kg ha^{-1} a.i. in Italy, it is noteworthy to underline as the reduction of up to almost one third of the label rate under low permeability film is able to effectively manage Verticillium infections and at the same time ensure a similar marketable cut flower production to that achieved with MS and DZ fumigation at label rate under VIF mulching. Our data are in accordance with a previous study demonstrating as a reduced DZ rate, under low permeability film, maintains good efficacy in reducing viable inoculum of another fungal pathogen [33]. Unlike this, our findings derive from two-year in field research and, therefore, a more sustainable use of this fumigant under low permeability film could be proposed on large scale to prevent Verticillium attacks on greenhouse chrysanthemum plantations.

Moreover, overall data let us to suppose that the high temperatures reached under VIF covered soils (up to more than maximum value of 39 °C) may have played an important role in enhancing the performances of fumigation treatment in accordance with data reported in literature [25,42]. According to our data and other recent findings, the application of low permeability materials (e.g., VIF) should be promoted to replace LDPE or HDPE materials for their high performances against *V. dahliae* herein reported and other targeted phytopathogens [33,43,44], because they are more environmentally friendly [31] and allow a significant reduction of fumigant rates [33]. Surprisingly, we noticed in our trials that VIF soil mulching alone is able to significantly reduce within a short period the inoculum of soilborne *V. dahliae.* Most likely, the disease reduction observed in our experiment could be related, to a significant extent, to high thermal regimes reached in the soil covered with this innovative material. However, additional studies should be performed to confirm these hypotheses. Although it well known that *V. dahliae* inoculum cannot definitively be eradicated from soil [45], VIF and other materials with low permeability and high thermal performances [29] may contribute to reduce worldwide the impacts of Verticillium wilt in production areas at high risk of disease epidemics on susceptible plant hosts. However, the research should be addressed in the future to compare performances of innovative, low permeability, classic PE and EVA, photo-selective, biodegradable and ultra-thin film in promoting adequate thermal regimes for soil disinfection from soilborne pathogens.

On the other hand, further studies should be focused on the ability of VIF application in enhancing performances of other natural and low impact fumigant such as botanical matrixes releasing the precursors of M-ITC compounds tested here and already proposed for nematode control [20] and in the past to suppress Verticillium inocula [46]. This will allow a decrease over time up to a definitive withdrawal of chemicals in the not too distant future.

5. Conclusions

This paper represents clear evidence that fumigant rate reduction is fully achievable and, for example, a DZ-VIF combination can represent a valid soil disinfection option for a conspicuous reduction of these chemical inputs in short to medium term, as nowadays required by GND policies.

Our findings could also open the way to reach a long-term soil disinfection solution aiming to integrate low permeability materials at high thermal performances with other eco-sustainable control measures.

Author Contributions: Conceptualization, I.C. and A.V.; methodology, I.C., A.D., A.B. and A.V.; software, A.V.; validation, I.C., A.D., A.B. and A.V.; formal analysis, I.C. and A.V.; investigation, I.C., A.D., A.B. and A.V.; resources, G.P.; data curation, I.C., A.D. and A.V.; writing—original draft preparation, I.C., A.D. and A.V.; writing—review and editing, A.V.; visualization, I.C., A.B. and A.V.; supervision, A.V.; project administration, I.C., G.P. and A.V.; funding acquisition, G.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded and supported by the following grants: Programma Ricerca di Ateneo MEDIT-ECO UNICT 2020–2022 Linea 2-University of Catania (Italy); Starting Grant 2020, University of Catania (Italy); Fondi di Ateneo 2020–2022, University of Catania (Italy), Linea Open Access. Research Project 201-62018, University of Catania 5A722192134.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: All authors are grateful to Certis Europe B.V. for their partial technical and funding support (Research agreement "Efficacy evaluation of dazomet fumigant at low rates in controlling natural infections of Verticillium wilt on cultivated chrysanthemum crops").

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

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