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ORIGINAL ARTICLE

Soil disinfestation with dimethyl disulfide for management of Fusarium wilt on lettuce in Italy

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Abstract The efficacy of dimethyl disulfide (DMDS) applied in pre-planting treatment by shank injection was investigated on the lettuce *Fusarium oxysporum* f.sp. *lactucae* (FOL) pathosystem in Northern Italy (Piedmont), in three experimental trials. DMDS was tested alone or in combination with metham sodium at 35.9 g/m^2, on lettuce cultivars showing different levels of susceptibility to the pathogen. DMDS, at 60 g/m^2, reduced FOL symptoms on the highly susceptible butterhead type of 70, 97 and 99%, and of 87, 97 and 100% on the moderately susceptible cultivar, respectively. DMDS at 30 and 40 g/m^2 showed a only partial efficacy on both lettuce types used in naturally infested soil, with a disease reduction from 30.3 to 64.5%, significantly comparable with dazomet. The results provided by DMDS at 40 g/m^2 plus metham sodium at 35.9 g/m^2 were statistically similar to those obtained with DMDS alone at 60 g/m^2, and better than those provided by dazomet alone. A positive effect on lettuce yield and weed control by DMDS, at the highest dosage tested and by DMDS plus metham sodium, was also observed.

Keywords *Lactuca sativa* · *Fusarium oxysporum* f. p. *lactucae* · Pre-plant treatments · Weeds · Soil-borne pathogens

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Introduction

Lettuce (*Lactuca sativa* L.) is a high-value crop in Italy, grown under greenhouse or in open field, for a total of 19,696 Ha (15,860 open field and 3836 under protection) [43]. During the past years, lettuce cultivation gained a significant economic importance, being subject to a continuous varietal and technological evolution, due to the conversion of many farms to the production and marketing of fresh cut and ready-to-eat salad mixes [10].

Lettuce is interested by severe losses caused by many pathogens [16, 7]; among them soil-borne pathogens play a major role [28, 31]. The most important soil-borne pathogen, affecting lettuce worldwide, is *Fusarium oxysporum* f. sp. *lactucae* (FOL), causal agent of Fusarium wilt [26, 29, 42, 50, 54, 56, 59, 68].

In general, the management of soil-borne pathogens is complicated by the limited number of registered chemicals and by the restrictions in the use of pre-plant fumigants, including metham sodium (MS) and dazomet (DZ) [14]. New fumigants are intensively investigated to provide solutions to the many practical problems associated with the loss or limitation of use of effective fumigants encountered by growers [13]. Good agricultural practices (GAPs) are needed to reduce the human health risks, the environmental impacts of fumigants and their effectiveness. Generally, GAPs are part of labels on registered fumigants. Moreover, because the commercially available fumigants have low vapor pressures and high boiling points compared to methyl bromide [2], their efficacy in the control of soil-borne pathogens and pests is more dependent on the method of delivery into the soil, soil type and condition, and meteorological conditions [2]. Several studies have been carried out to investigate the effect of fumigant application methods on their effectiveness, soil persistence and emission [12, 35, 40, 46, 60, 69, 70]. However, [18] have made the application of soil fumigants through drip water applications very difficult due to the requirement of large buffer zones to reduce human and animal exposure to the chemicals. Shank injection generally resulted in more uniform distribution of fumigants in the soil gas phase than the drip application. Moreover, generally the efficacy of fumigants applied by drip irrigation systems resulted affected by the amount of irrigation water more than the application rate or concentration [24, 35, 40, 41].

Dimethyl disulfide (DMDS) is a soil fumigant recently introduced in the USA [66], Europe and Mediterranean countries for soil disinfestation in shank and drip applications. DMDS is actually commercialized also in Asia (Korea) and Mexico. This fumigant affects the mitochondrial complex by inhibition of cytochrome oxidase function and has a zero Ozone Depletion with a favorable toxicological and eco-toxicological profile [17, 30]. DMDS active ingredient is naturally found in soils amended with fresh residues of *Allium* and *Brassica* crops [5, 8, 22].

DMDS is a pale yellow liquid with vapor pressure of 29 mm Hg at 25 °C and water solubility of 1–10 g/L at 20 °C [26]. It is used as pre-plant treatment to control nematodes [15, 23, 47], soil-borne plant pathogens [1, 3, 22, 32, 34, 36, 38, 48, 58, 60, 61] and weeds [1, 25, 27].

This study was carried out in order to test the efficacy of pre-planting treatments with DMDS against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce under field conditions, also by evaluating the effect of the fumigant on the survival of the pathogen. The effect of DMDS on weed management was also taken into consideration.

Materials and methods

Field experiments, plant material and pathogen

Three experimental trials were carried out in 2013 and 2014 under field conditions at Torino and Cuneo provinces, Northern Italy (Table 1) on lettuce cultivars Analena Sintia and Badina belonging to butterhead type highly susceptible to Fusarium wilt and cv. Novelsky belonging to batavia type, which is moderately susceptible to Fusarium wilt [33].

Lettuce plants, 21–30 days old, were transplanted 7 days after soil uncovering (Table 1) with 16 plants/m^2. Lettuce plants were drip irrigated and grown according to the cultural practices adopted by commercial growers in the region.

Two successive crops of lettuce were grown on the same experimental area. Before transplanting the second crop cycle, the soil was superficially tilled and farmed as the first crop (Table 1).

The temporal organization of the experimental trials, the timing of artificial inoculations and treatments in the different trials are summarized in Table 1.

A highly virulent strain of *F. oxysporum* f. sp. *lactucae*, isolated in 2002 from infected lettuce plants in northwestern Italy, coded Mya3040, belonging to race 1 [59], was used in trials 2 and 3.

To achieve a uniform soil infestation and high disease incidence, the fungal biomass (prepared by adding the Mya3040 strain cultured in PDA to wheat kernels previously sterilized for 30 min at 121 °C) was incorporated into the soil at 30 g/m^2 by rototilling at a depth of 1–20 cm. Seven days before the application of the fumigants, a second infestation with the pathogen was carried out by mixing into the soil the chlamydospores of Mya3040, prepared in talc according to Locke and Colhoun [49], to achieve a final concentration in soil of 1×10^4 chlamydospores/ml (Table 1).

Soil disinfestation treatments

Soil disinfestation treatments were carried out in open field by following the timing and the dosages of application, corresponding to the manufacturer's instructions (Tables 2, 3, 4, 5).

DMDS (Accolade/Paladin, 991 g/kg, Arkema) was applied by shank injection into the soil at 65–75% of water field capacity at 20–25 cm depth (Table 1) with an average soil temperature ranging from 17 to 22.5 °C. In shank injection fumigation, the product is injected below the surface of properly prepared soil and applied in a narrow band as the fumigation equipment moves across the field. The surface of the soil is subsequently compacted by pulling a ring roller behind the fumigation equipment. In the present study, the application was carried out by using the Forigo Roteritalia srl. (Mantova, Italy) and Società Italiana Sterilizzazioni (S.I.S, Ragusa, Italy) srl. equipments in accordance with Good Practices Fumigation guidelines, including product Stewardship application guidelines [11]. Soil mulching was carried out by using a virtually impermeable, transparent film (VIF) in one-pass immediately after product injection (Agricolplast, Cuneo, Italy), of 25 µm thick.

Virtually impermeable films were manually removed from soil 14 days after the soil disinfestation treatment, in accordance with the experimental protocol.

The commercial formulation of dazomet (Basamid, 965 g/kg, Kanesho Soil Treatment/Certis Europe) was mixed into the soil as commercial control. When metham sodium (Vapam, 470 g/kg, Taminco-Eastman) at 35.9 g/m^2 and DMDS were combined (Trial 3), DMDS treatment

Table 1 General information on the experimental layout and timing of the operations carried out thought the trials

	Trial 1	Trial 2	Trial 3
Site	Moretta (CN)	Carmagnola (TO)	Carmagnola (TO)
Crop system	Open field	Open field	Open field
Soil type	Sand/silt/loam 56:19:25	Sand/silt/loam 68.16:10.7:21.1	Sand/silt/loam 68.16:10.7:21.1
Soil infestation	Natural	Artificial, 30 g/m^2 + 1 × 10^4 chlamydospores/ml	Artificial, 30 g/m^2 + 1x10^4 chlamydospores/ml
Cv.	Badina, Novelsky	Badina, Sintia, Novelsky	Analena, Novelsky
Plants/m^2	16	16	16
Plot size	20 m × 4 m, 80 m^2	15 × 2, 30 m^2	15 × 2, 30 m^2
Replicates	5	4	4
Average soil T °C at 15 cm depth	17 °C	21 °C	22.5 °C
Soil moisture	65% of soil field capacity	65% of soil field capacity	75% of soil field capacity
Application method	Shank injection	Shank injection	Shank injection
Application date under VIF	4/06/2013	5/07/2013	7/08/2014
Plastic removal	19/06/2013	22/07/2013	21/08/2014
Planting date first and second crop cycle	26/06/2013	31/07/2013 and 11/09/2013	28/08/2014
End of the trial, first and second crop cycle	7/08/2013	4/09/2013 and 14/11/2013	7/10/2014

Table 2 Efficacy of soil disinfestation treatment with dimethyl disulfide (DMDS), dazomet (DZ) and DMDS+ metham sodium (MS) on the survival of *Fusarium oxysporum* (FuslLat 10RB) resistant to benomyl (RB) mixed into the soil profile at 10–12 cm depth expressed as Log_{10}

Treatment[1]	Dosage a.i.	*F. oxysporum* f. sp. *lactucae* RB		
		Trial 1	Trial 2	Trial 3
Untreated	–	4.0a[2]	4.1b	4.4c
DMDS	30 g/m^2	2.4a	–[3]	2.3b
DMDS	40 g/m^2	2.2a	1.8a	1.6ab
DMDS	60 g/m^2	1.1a	1.4a	1.0a
DZ[4]	49,5 g/m^2	2.1a	1.6a	1.8ab
DMDS+ MS[5]	40 g/m^2 + 35.9 g/m^2	–	–	1.0a

[1] Soil coverage of treated and untreated control under VIF, Virtually Impermeable Film, (Barrier film, 25 μm thick,) for 14 days with an average soil temperature from 17 to 22 °C

[2] Values in the same column followed by the same letter are not significantly different according to Tukey's HSD test ($p = 0.05$)

[3] Not tested

[4] Applied by mixing into the soil

[5] DMDS treatment was carried out immediately after the application of MS by using the Deeprino Forigo's equipment

was carried out immediately after the application of MS by using the same Deeprino Forigo's equipment. Control plots were untreated (Tables 2, 3, 4, 5).

Assessment of pathogen survival in soil (biological test) and statistical analysis

In order to evaluate the pathogen density after soil treatment, the strain *F. oxysporum* f. sp. *lactucae* (FusLat10 RB) marked with resistance to 10 mg/l of benomyl was used as talc formulation, as described by Ferrocino et al. [21]. The single-spore culture of each isolate was stored in glycerol at −80 °C.

Soil infestation with the selected Fusarium wilt resistant to benomyl was carried out 24 h before soil disinfestation treatment at 1–5 × 10^5 unit forming colony (UFC)/g of soil in 20 cm area placed in the center of the plots. Controls were represented by infested soil without treatments with the fumigants.

Table 3 Effect of soil disinfestation treatments with dimethyl disulfide (DMDS), dazomet (DZ) and DMDS+ metham sodium (MS) on Fusarium wilt control on cvs

Treatment[1]	Dosage a.i	Trial 1		Trial 2				Trial 3	
		Cycle I		Cycle I		Cycle II		Cycle I	
		'Badina'	'Novelsky'	'Badina'	'Novelsky'	'Sintia'	'Novelsky'	'Analena'	'Novelsky'
Untreated	–	78.0d[2]	50.8c	53.6c	43.6b	25.8c	13.0b	44.9c	21.9b
DMDS	30 g/m^2	54.4c	26.9b	–[3]	–	–	–	9.4b	2.0a
DMDS	40 g/m^2	33.0ab	18.1ab	4.2ab	3.3a	5.2ab	0.5a	5.5ab	5.5a
DMDS	60 g/m^2	23.3a	6.3a	1.6a	1.4a	2.2a	0.3a	0.4a	0.0a
DZ[4]	49.5 g/m^2	39.8b	16.9ab	11.4b	4.5a	12.0b	1.7a	3.9ab	0.4a
DMDS+ MS[5]	40 g/m^2 + 35.9 g/m^2	–	–	–	–	–	–	1.6a	0.4a

Badina, Novelsky and Analena. Data are expressed as disease index 0–100 at the end of the first and second crop cycle

[1] Soil coverage of treated and untreated control under VIF, Virtually Impermeable Film, (Barrier film, 25 µm thick,) for 14 days with an average soil temperature from 17 to 22 °C

[2] Values in the same column followed by the same letter are not significantly different according to Tukey HSD test ($p = 0.05$)

[3] Not tested

[4] Applied by mixing into the soil

[5] DMDS treatment was carried out immediately after the application of MS by using the Deeprino Forigo's equipment

Table 4 Effect of soil disinfestation treatments with dimethyl disulfide (DMDS), dazomet (DZ) and DMDS+ metham sodium (MS) on yield of cvs. Badina, Novelsky, and Analena

Treatment[1]	Dosage a.i.	Trial 1		Trial 2				Trial3	
		Cycle I		Cycle I		Cycle II		Cycle I	
		'Badina'	'Novelsky'	'Badina'	'Novelsky'	'Sintia'	'Novelsky'	'Analena'	'Novelsky'
Untreated	–	0.0b[2]	258.3d	0.0c	66.8b	738.9c	2058.8b	526.8b	1489.8a
DMDS	30 g/m^2	441.2b	2932.3c	–[3]	–	–	–	3451.0ab	4329.5a
DMDS	40 g/m^2	2029.8a	5141.9b	3238.3b	4289.6a	2913.5a	2823.9a	4600.8a	3580.0a
DMDS	60 g/m^2	3047.2a	7327.2a	5082.5a	6088.0a	2812.0ab	2611.3a	4786.3a	5113.8a
DZ[4]	49.5 g/m^2	2454.8a	6189.0ab	2529.4b	4568.5a	2241.9b	2508.5ab	4575.6a	5303.3a
DMDS+ MS[5]	40 g/m^2 + 35.9 g/m^2	–	–	–	–	–	–	5477.0a	5115.0a

Data are expressed as fresh weight of marketable lettuce (g/m^2) at the end of the first and second crop cycle in trial 2

[1] Soil coverage of treated and untreated control under VIF, Virtually Impermeable Film, (Barrier film, 25 µm thick,) for 14 days with an average soil temperature from 17 to 22 °C

[2] Values in the same column followed by the same letter are not significantly different according to Tukey's HSD test ($p = 0.05$)

[3] Not tested

[4] Applied by mixing into the soil

[5] DMDS treatment was carried out immediately after the application of MS by using the Deeprino Forigo's equipment

Fourteen days after fumigation, at soil uncovering, treated and untreated soil containing the FusLat10 benomyl-resistant strain was taken with a stainless cork borer and immediately transferred to the laboratory and processed. Three soil cores, collected randomly from each plot, were combined to make one soil sample of 50–100 g/treatment and replicate. A sub-sample of 5 g of soil was taken from each soil sample and used to evaluate the survival of soil population of the *Fusarium oxysporum* RB mutant by soil dilution plating on Fusarium selective medium [45] added with 10 mg/l of benomyl. The soil dilution plating method was used to quantify the resistant pathogen that had survived [21]. Three plates/dilution were used for each treatment and replicate. The survived populations of the Fusarium wilt agent were counted after 7 days at 22 °C. Data were expressed as unit forming

Table 5 Effect of different soil disinfestation treatments with dimethyl disulfide (DMDS), dazomet (DZ) and DMDS+ metham sodium (MS) on monocots and dicots control

Treatment[1]	Dosage a.i.	Trial 1					Trial 2					Trial 3			
		Number of weeds/m^2			Fresh weight (g/m^2)	% of soil coverage	Number of weeds /m^2			Fresh weight (g/m^2)	% of soil coverage	Number of weeds/m^2			Fresh weight (g/m^2)
		Monocot	dicot	Total weeds			Monocot	dicot	Total weeds			Monocot	dicot	Total weeds	
Untreated	–	0.7a[2]	40.1b	40.8b	4638.0c	96.0c	1.8b	74.0b	76.1b	1308.4a	59.4b	1.5a	32.5c	34.0c	186.8b
DMDS	30 g/m^2	0.1a	13.7ab	13.8ab	2755.0b	57.0b	–	–	–	–	–	0.0a	18.3bc	18.3bc	50.4a
DMDS	40 g/m^2	0.0	7.2a	7.2a	2449.0b	53.0b	0.4a	13.8a	14.1a	618.5a	21.3a	0.0a	14.5ab	14.5ab	29.3a
DMDS	60 g/m^2	0.2a	1.4a	1.6a	702.3a	10.0a	0.4a	9.6a	10.0a	815.0a	25.6a	0.5a	5.8ab	6.3ab	12.6a
DZ[4]	49.5 g/m^2	0.0a	0.6a	0.6a	188.7a	1.2a	0.0a	14.0a	14.0a	1041.5a	28.3ab	0.0a	0.0a	0.0a	0.0a
DMDS+ MS[5]	40 g/m^2+35.9 g/m^2	–	–	–	–	–	–	–	–	–	–	0.0a	3.8ab	3.8ab	5.2a

Data are expressed as average number of weeds per m^2 at 21–32 days after the soil uncovering and as % of soil coverage and total fresh weight of weeds (g/m^2) at the end of each trial

[1] Soil coverage of treated and untreated control under VIF, Virtually Impermeable Film, (Barrier film, 25 µm thick,) for 14 days with an average soil temperature from 17 to 22 °C

[2] Values in the same column followed by the same letter are not significantly different according to Tukey HSD test ($p = 0.05$)

[3] Not tested

[4] Applied by mixing into the soil

[5] DMDS treatment was carried out immediately after the application of MS by using the Deeprino Forigo's equipment

colony UFC/g of soil. Data, calculated as the mean Log count of the three plate replicates, were analyzed by univariate ANOVA in SPSS 22.0, and means were separated by multiple comparison Tukey's test ($p = 0.05$).

Efficacy trial

Trial 1 was carried out in a commercial field at Moretta (Cuneo, northern Italy) with a history of several lettuce cycles prior to the beginning of this study (Table 1), in a sandy loamy soil (sand/ silt/ loam 56: 19: 25 soil, pH 7.12 and 1.37% of organic material) naturally infested with the pathogen.

Trials 2 and 3 were carried out in an Experimental farm at Carmagnola (Torino, northern Italy) in a sandy loamy soil (sand/silt/loam 68.16:10.7:21.1 soil, pH 7.5 and 0.94% of organic material) in presence of artificial infestation with the pathogen (Table 1).

Plots were arranged in a complete randomized block design with 4 (trials 2 and 3) and 5 (trial 1) replicate per treatment.

Fusarium wilt and weeds assessment, yield and analysis

Plants were monitored weekly, and the data were recorded starting from 10 to 14 days after transplant of lettuce in the inoculated substrate, at the appearance of the first symptoms of yellow leaves and reduced growth. The number of infected plants showing wilting and stem necrosis was counted to assess disease incidence. Totally wilted (dead) plants were removed. The final disease rating took place four weeks after inoculation by dissecting each plant. The disease severity (DS) index used was: 0 = healthy plant, 25 = initial leaf chlorosis, 50 = severe leaf chlorosis and initial symptoms of wilting during the hottest hours of the day, 75 = severe wilting and severe symptoms of leaf chlorosis; 100 = plant totally wilted, leaves completely necrotic.

The efficacy of different treatments in controlling lettuce Fusarium wilt corresponding to the percentage of DS reduction was calculated as:

$$\%\,\text{efficacy} = 100 - (\text{DS}_t \times 100/\text{DS}\ i_{\text{control}})$$

where, i = inoculated and untreated control t = tested treatments.

Weeds (monocot and dicot) densities were determined by counting the number of seedlings within a given area in square meters within each treatment and replicate. At the last evaluation, the weeds biomass expressed as fresh weight and the percent of area covered by weeds were evaluated [19].

Lettuce yield was measured at the end of the trial on 16 plants per treatment, by weighing the fresh biomass of marketable and unmarketable plants (g/m^2).

All data were analyzed by univariate ANOVA in SPSS 22.0, and means were separated by multiple comparison Tukey's test ($p = 0.05$).

Results

Pathogen survival (biological test)

All treatments significantly reduced the survival of the benomyl-resistant strain of *F. oxysporum* f.sp. *lactucae* artificially introduced into the soil profile, in pre-treatment. DMDS at 40 g/m2 reduced FusLat10RB from 63.9 to 96.9% and provided significantly similar results of DA (from 59.9 to 99.6% of reduction). When DMDS was combined with MS, the efficacy in FusLat10RB suppression was 77.3%, significantly comparable to DMDS used at 60 g/m^2 (Table 2).

Fusarium wilt control

The first symptoms of Fusarium wilt were observed in all trials 14- 16 days after transplant (Table 3). At the end of trial 1 (cycle I) disease severity in the untreated control ranged from 50.8 (cv. Novelsky) to 78.0 (cv. Badina). The best control was provided by DMDS at 40 and 60 g/m^2 with a disease reduction of 57.6 and 70.1% (cv. Badina), and of 64.3 and 87.6% (cv. Novelsky), respectively. DZ reduced fusarium wilt symptoms from 40.5% (cv. Badina) to 74.3% (cv. Novelsky) (Table 3).

At the end of the trial 2 (cycle I), the untreated control showed a disease severity of 43.6 and 53.7 on cvs. Novelsky and Badina, respectively. DMDS at 40 g/m^2 provided results significantly similar to DZ, with a wilt reduction on cv. Badina of 92.2 and 78.8%, respectively. DMDS at 60 g/m^2 was significantly better than DZ on cv. Badina (disease reduction of 97%) (Table 3). All treatments significantly reduced Fusarium wilt on lettuce cv. Novelsky with a disease reduction ranging from 92.4 to 96.8% (Table 3). At the end of the trial 2 (cycle II), all the tested treatments significantly reduced Fusarium wilt symptoms on lettuce cv. Sintia; the best control was provided by DMDS at 60 g/m^2, (91.5% of reduction) followed by DMDS at 40 g/m^2 (79.8% of reduction) and of DZ (53.5% of reduction). All soil disinfestation treatments showed a significant control of Fusarium wilt on lettuce cv. Novelsky with a disease reduction from 86.9 to 97.7%.

Lettuce yield varied with the fumigation treatment (Table 4), with the lowest yield in the untreated plots. DMDS provided significantly similar results to DZ as fresh weight of cv. Badina, while, the marketable yield of cv. Novelsky was improved by DMDS at 60 g/m^2.

In Trial 2, all soil disinfestation treatments significantly improved the marketable yield of cvs. Badina and Novelsky compared with the untreated control. DMDS already at the lowest dosage gave the same results as DZ.

The marketable fresh weight of lettuce cv. Sintia grown in the second crop cycle in the same soil treated with DMDS at 60 g/m^2 and DZ was not significantly different, while the best results were provided by DMDS at 40 g/m^2 (Table 4). The same trend was observed on lettuce cv. Novelsky.

In trial 3, the fresh marketable weight of lettuce cv. Analena recorded from plants grown in the soil treated with DMDS at 60 and 40 g/m^2 was significantly similar to that obtained in plots treated with DZ (4.6 kg/m^2) and DMDS +MS (5.5 kg/m^2) (Table 4).

Effect of soil disinfestation treatments on weeds

Portulaca oleracea was the most represented weed (from 94 to 98%) in the experimental site in trials 1–3, followed by *Stellaria media*. *Amaranthus retroflexus*, *Chenopodium album.* Among the monocots the prevalent weeds were *Galinsoga* sp. and *Echinochloa crus-galli*.

In trial 1, DMDS at 60 g/m^2 provided significantly similar results to DZ in monocot and dicots control as average number of weeds/m^2, fresh weight and percentage of soil coverage, while a partial reduction was provided by DMDS applied at 30 and 40 g/m^2 (Table 5).

A similar trend was observed in trial 2 (Table 5). DMDS at 40 and 60 g/m^2 provided a weed reduction of 69.0 and 77.6% significantly similar to DZ. In trial 3, the best results in reducing weed density was provided by DMDS (81% of efficacy), and by the combination of DMDS with MS, which reduced of 89% the total number of weeds compared with the untreated control, with results statistically similar to DZ (Table 5).

Discussion

Lettuce Fusarium wilt is a severe disease that is responsible of heavy losses to growers worldwide [20, 52]. Its spread is increasing in new cultivation areas also because the possibility of its diffusion with infected seeds or seedlings has been proved [39, 55].

The tactics available to manage this disease include mainly the use of preventative measures, such as minimize dissemination in field, use of plant resistant cultivars whenever possible, the adoption of proper crop rotation, and removal and destruction of infested plant material [37, 44, 62].

Among the various measures, soil disinfestation remains a practice of economic significance in crop production, especially because a broad spectrum of activity against soil-borne pests (fungi, nematodes, soil insects and weeds) is generally required. However, at present, no chemical or nonchemical method used alone exhibit the same efficacy of some fumigants used in the past [44].

Among chemicals for soil disinfestation treatments, MITC generators such as metham sodium (sodium *N*-methyl dithio-carbamate) and dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) as granular formulations either, alone or in combination with other measures, are proving as effective in many situations [3, 4, 40]; however, their limitations in use as consequence of UE regulatory decisions must be considered [13]. DMDS is under EU process of evaluation for inclusion in Annex 1, an emergency use was granted in Italy (2015) for the use of Accolade 94 EC and the campaign was successfully conducted in protected fruiting vegetables. Following that, two new campaigns were granted to Certis Europe in 2016: one in Spain (Murcia region) as commercial large-scale experimental permit for use in protected tomato and another in Italy as emergency use in several protected crops (Solanaceous crops, Cucurbits, lettuce), which are ongoing until 31.12.2016.

EU registration for DMDS as active ingredient was initiated in late 2012 and is ongoing, to support the shank application with the product Paladin/Accolade 99.1% (w/w) and drip irrigation with Paladin/Accolade EC 94.1% (w/w). DMDS is now commercially available in USA [66], Mexico, Israel, Morocco, Turkey, Jordan, Lebanon, Egypt and Korea to control nematodes and other soil-borne pathogens of vegetable crops.

DMDS exhibits a good activity against root-knot nematodes [3, 15, 23, 36, 64], while often conflicting data are available concerning its fungicidal effect.

Fritsch [22] showed that DMDS significantly reduced the populations of *Sclerotium rolfsii*, *Verticillium dahliae* and *Rhizoctonia solani*. Moreover, DMDS provided significant reduction of Verticillium wilt on chrysanthemum comparable with CP and MS in naturally infected soils [60]. In contrast, only partial results in controlling *Pythium ultimum* and *Fusarium oxysporum* by DMDS have been previously reported by Gerik [32] and by Cabrera et al. [9].

In the present study, DMDS applied at 30, 40 and 60 g/m^2 alone or in mixture with MS has been tested in field under artificial infestation with FOL and in a naturally infested soil, in a commercial farm.

The occurrence of wilt was notably serious in the untreated plots, at the first cycle of cultivation in trials 1–3, with a DS ranging from 44.9 to 78.0 in the highly susceptible butterhead lettuce cultivars (cvs. Badina and

Analena) and from 21.9 to 50.8 in the moderately susceptible (cv. Novelsky).

DMDS, at the highest tested dosage under our field trials, reduced FOL symptoms on the highly susceptible butterhead type of 70, 97 and 99%, and of 87, 96.8 and 100% on the moderately susceptible cultivars. However, DMDS used at 30 and 40 g/m^2 showed a partial efficacy on both the lettuce type used under natural infested soil (DS reduction from 30.3 to 64.5%), significantly comparable with DZ.

DMDS at 40 g/m^2 reduced DS from 75 to 92.5% providing significantly comparable results to DMDS used at the highest dosage (60 g/m^2), and DZ at 50 g/m^2.

The fungicidal effect of DMDS was influenced by the type of lettuce cultivar. Indeed, different levels of susceptibility of lettuce to FOL are reported, and disease severity can be affected by other factors as soil temperature and age of the plants [30, 33, 53, 62, 63]. The lack, at present, of cultivars belonging to butterhead type resistant to FOL, makes interesting soil disinfestation with DMDS under practical conditions.

Our field trials revealed that the combination of DMDS with MS successfully suppressed FOL and reduced the colony-forming units (CFU), and was not significantly different from DZ at the dose of 50 g/m^2 but showed higher lettuce yields. The results of DMDS plus MS were statistically similar to those of DMDS alone (at the dose of 60 g/m^2), but better than those provided by DZ alone (at the dose of 50 g/m^2).

DMDS did not cause, under laboratory test, a complete mortality of FOL (from 77 to 99.9% at 60 g/m^2) and of 70% when it was combined with MS. A clear effects in reducing the inoculum density of *Fusarium oxysporum* (from 90.15 to 98.46%) and *Phytophthora* spp. (from 72.69 to 99.4%), were found in laboratory tests for soils treated with DMDS at 60 g/m^2 by Mao et al. [51] and by Papazlatani et al. [58]. On the other hand, the inoculum density of *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum f. sp. radices-cucumerinum* and *F. oxysporum f. sp. lycopersici* was significantly decreased by DMDS at 40 and 60 g/m^2, but the expression of the disease was not prevented [36].

The present study provides evidence of the efficacy against *F. oxysporum* f. sp. *lactucae* of DMDS applied as pre-plant treatment, with a positive effect on the lettuce yield and weeds. The positive effect on lettuce yield was shown in the second cropping cycle, too.

The synergistic activity of the combinations of DMDS with DZ has been previously reported [48, 67]. Our study shows the positive effect in controlling lettuce Fusarium wilt and weeds by combining DMDS and MS. Fumigant compatibility must be evaluated before combinations can be applied simultaneously [24]. The simultaneous application of fumigants, sometime may provide negative effects. In the case of the combination of methan-sodium and halogenated fumigants (e.g., Pic and 1,3-D), a reduced efficacy of metham sodium has been observed probably due to an accelerate degradation [71]. However, MS controls a broad spectrum of soil-borne pests, including weeds [65].

In the soil disinfestation sector the sustainability of fumigation practices can be reached by integrating the treatment with alternative techniques having low environmental impact, such as solarization, biological solutions, grafting, varietal resistance, biofumigation.

Our study provides additional information concerning the effect of DMDS, applied as shank irrigation in preplanting soil disinfestation treatments, on Fusarium wilt of lettuce under practical conditions. Moreover, DMDS was as effective as DZ in terms of increasing lettuce yield.

Moreover, DMDS at the highest dosage tested or in combination with MS is of particular interest in lettuce production systems showing efficacy against both Fusarium wilt of lettuce and weeds.

The unpleasant odor that may occurs near the application area under particular environmental conditions (i.e., temperature inversion, air stagnation and light winds) [57] can be a concern to the use DMDS in practice, particularly in populated areas, and requires to be properly managed with stewardship measures. Alongside implementation of Best Practice principles and improved shank injection equipment for DMDS, such as the use of totally impermeable films (TIF), should be recommended to ensure the management of odor for soil fumigation treatment.

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