Dimethyl Disulfide (DMDS): a New Soil Fumigant to Control Root-Knot Nematodes, *Meloidogyne* spp., in Protected Crops in Sicily, Italy

S. Leocata[a] and G. Pirruccio
ARA s.r.l.
S. Giovanni la Punta (CT)
Italy

E. Medico[c]
Certis Europe B.V.
Saronno (VA)
Italy

A. Myrta[b]
Certis Europe B.V.
Brussels
Belgium

N. Greco[d]
Istituto per la Protezione delle Piante - CNR
Bari
Italy

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Abstract

Dimethyl Disulfide (DMDS), as an EC formulation, was applied via drip-irrigation to control the root-knot nematode *Meloidogyne incognita* in two trials in protected crops. The trials were carried out in Sicily (Italy) during 2012 on melon and on tomato, according to a randomized block design with 4-5 replicates per treatment. The soil of the sites was sandy and naturally severely infested by the nematode. DMDS was applied at the rates of 300, 400, and 500 kg/ha (the last rate only in tomato). The soil was covered with virtually impermeable film (VIF) before fumigation and uncovered 2 weeks later. DMDS treatments were compared with 1,3-dichloropropene (140 L/ha for melon and 180 L/ha for tomato) and a non-treated control. Yield was recorded in all trials. The nematode attack on the roots was evaluated according to the 0-10 Zeck's scale modified (0 no galls and 10 root system completely deformed by large and numerous galls). In the melon trial, 60 days after transplanting (DAT), both DMDS doses (400 and 300 kg/ha) showed low root gall indices (1.2 and 1.5, respectively), compared to the non-treated control (5.5) and 1,3-dichloropropene (4.2). In the tomato trial 120 DAT, DMDS at 500, 400 and 300 kg/ha showed root gall indices of 2.9, 2.7, and 3.0, respectively, compared to 9.6 of the non-treated control and 3.6 of 1,3-dichloropropene. All nematicidal treatments significantly increased marketable yields of melon and tomato in comparison to non-treated controls. Therefore, DMDS, once registered, could be a new effective solution to control root-knot nematodes in protected crops and because of its favourable ecotoxicologial profile, it could also be considered for IPM programmes of protected crops.

INTRODUCTION

Dimethyl disulfide (DMDS) is one of the main biofumigant components of *Alliaceae* residues (garlic, onion or leeks) able to control soil-borne pathogens and it is the only disulfide found in soils following incorporation of *Brassicaceae* plant materials (Arnault et al., 2013). DMDS has shown very good nematicide and fungicide effect. Among nematode species, there are several experimental field works showing its very good control of several plant-parasitic species, such as *Meloidogyne* spp., *Heterodera* spp., *Globodera* spp., *Pratylenchus* spp., *Tylenchulus semipenetrans*, etc. (Coosemans, 2005; Fritsch, 2005; Charles et al., 2010; Curto et al., 2014; Zanon et al., 2014; Fritsch et al., 2014).

Having been a chemical intermediate in refineries and chemical industry since

[a] sleocata@aracatania.it
[b] myrta@certiseurope.com
[c] medico@certiseurope.com
[d] n.greco@ba.ipp.cnr.it

long time, the biological properties of DMDS are now being explored and the compound patented by Arkema for soil fumigation. The formulated product of DMDS (Paladin^{TM}) is now commercially available for use in the USA, Israel, Morocco, Turkey, Jordan, and Egypt, to control nematodes and other soil-borne pathogens of vegetable crops (Arnault et al., 2013; P. Charles, pers. commun.).

In 2012 the EU registration was initiated for Paladin 99.1% (w/w) DMDS pure active ingredient to be used by shank and Paladin EC 94.1% (w/w) to be applied via drip-irrigation. This paper reports on the effectiveness of DMDS EC (94.1%) against *Meloidogyne incognita*, in protected melons and tomatoes crops as the nematode causes severe yield losses under both field and protected conditions in Italy (Di Vito et al., 1985).

MATERIALS AND METHODS

Two field experimental trials were conducted in the Ragusa Province: at Acate for tomato cultivar 'Durinta' and at Vittoria for melon cultivar 'Polis'. Both trials were carried out in greenhouses naturally and severely infested with the root-knot nematodes and in which the previous tomato crops had 100% of the roots showing a galling index of from 5 to 8 in the greenhouse selected for the melon trials and from 3 to 6 in that for the tomato trial. The soil was very sandy in both trial 89-91%. The effects of soil treatments with the fumigants were compared with an untreated control. All treatments were set according to a randomized complete block design (RCB) with 4 (melon) and 5 (tomato) replicates. In each site, a net of interconnected drip irrigation PE tubes was placed on the soil (chemigation system). For melon, each plot was of 32 m^2 (4×8 m) and irrigated with 25 L water/m^2, through line of tubes spaced 80-120 cm apart and having 4 L/h drippers at 15 cm distance between them. For tomato these values were the same except that the plots were of 51 m^2 (6×8.5 m). Once the chemigation system was positioned, all treated plots were covered with VIF film (Agriplast - Eco-Brom Natural Bobina) and irrigated lightly before fumigation to favour an even distribution of the fumigants in the soil profile.

The fumigants were applied through the installed drip irrigation system using a dedicated equipment supplied by the SIS company. Water flow and fumigant injection into the system were electronically managed and measured and nitrogen-pressure injection was used for the release of the product.

DMDS (Paladin EC) was applied at the rates of 300 and 400 L/ha in both trials and also at 500 L/ha in the tomato trial, whereas the standard 1,3-dichloropropene (Condorsis EC) was used at the rates of 140 L/ha (for melon) and 180 L/ha (tomato). The concentration of DMDS EC during application never exceeded 0.25% and the time of application was 45-60 min for each treatment. After application of the fumigant only water was distributed through the chemigation system to clean the tubes.

Soil temperatures during the two weeks after fumigants application were 24-42°C in Acate and 30-46°C in Vittoria, while soil moisture content was at field capacity in both trials. The plastic films were kept until 14 days after applications and then they were removed and the soil left uncovered for aeration the following 7 days. Transplanting was made on 8 June 2012 for melon and 20 August 2012 for tomato. To assess the effects of the treatments on nematode infestation, from each plot, 4 plants at intermediate crop cycle and 16 at the end of the crop cycle were uprooted for melon and 12 and 32, respectively, for tomato. The roots of these plants were observed to determine the percentage of infected plants (incidence) and the severity of symptoms on the roots according to the Zeck's 0-10 scale. The damage index on roots (GSI - Gall Severity Index) was calculated by McKinney formula. The root gall assessments were made 28 days after transplant (DAT) and at harvest (60 DAT) for melon and 28 and 70 (DAT) and at harvest (120 DAT) for tomato.

Number of marketable fruits and weight of the fruits were evaluated on 20 plants from the central rows of each plot, at harvest for melon and during 6 pickings for tomato.

The data were checked for homogeneity of variance by Bartlett's test and analyzed using a two-way analysis of variance (ANOVA). The Student Newman-Keuls' multiple comparison test was used to separate any treatment difference that may be implied by ANOVA test. The Abbot equation was used to calculate the percentage of control compared with untreated plots.

RESULTS AND DISCUSSION

In the melon trial, 100% of the roots in the untreated plots were already infected by *Meloidogyne incognita* (Table 1) 28 DAT with a damage index of 1.63, compared to the standard product with 81.2% of the plants infested and a damage index of 1.19. In comparison, in the plots fumigated with DMDS only 18.7÷31.2% of the plants were infested with a damage index of 0.19÷0.31. At harvest, 60 DAT, the infested roots remained nearly at the same level in the non-treated plots (95.3%) but an increase was recorded of the damage index (5.52). At same observation, in the plots treated with the standard fumigant, the proportion of infested plants (90.6%) and the damage index (4.19) had increased to levels similar to those of the control plots. Instead, these values increased only slightly in the plots treated with DMDS in which the plants remained significantly less infected (32.8-53.1%) and damaged (damage index of 1.22-1.53). No significant difference was observed between the two rates of DMDS, but both rates performed significantly better than the standard fumigant although not statistically different. At harvest, no significant difference among treatments was observed in the number of small (non marketable) fruits and total fruits (Table 2), but more marketable fruits were counted in all fumigated plots, including the standard fumigant. The total fruits weight increased in all fumigated plots but only in those fumigated with 400 L/ha of DMDS the observed differences were significant compared with non treated plots.

For the tomato trial (Table 3), no symptom of attack by *Meloidogyne incognita* was observed in the treated plots 28 DAT, while the untreated check showed 100% of infested plants with a damage index of 5.85. Root infestation was already 100% 70 DAT in the control plots with a damage index of 8.17. Instead, these values were rather low and significantly less in all fumigated plots. The root infestation increased by the end of the crop cycle and was similar (100%) in the plots non treated or fumigated with the standard nematicide, while it was slightly but significantly less in the plots fumigated with the three different rates of DMDS. The damage index also increased in all plots but it remained significantly smaller in all the fumigated plots (2.88-3.62) compared to control plots (9.57). The number of fruits was similar in all plots during the first two harvests (80-91 DAT), then increased greatly in all fumigated plots until the last harvest and no fruit was harvested from the non treated plots 113 and 120 DAT. The weight of the fruits was already significantly higher than in the control plots since the first two harvests and continued to be so throughout the tomato crop cycle. However, there was no difference between the rates of DMDS nor between these and the standard fumigants.

No problem was encountered during the application of any of the products under test and no phytotoxic symptom was observed at any of the assessment timings.

Based on these results, DMDS appears as a good alternative to control nematodes as it has shown a performance similar or even better in comparison with the standard fumigant. In general DMDS has protected the roots better and longer from nematode attacks. The trials demonstrated the need of nematode control to achieve good yield performance of both melon and tomato in soil heavily infested with root-knot nematodes, *Meloidogyn*e spp. Although the results of incidence of the root galling show high levels of infection by the end of the crop cycle, nevertheless the reduced root infestation during most of the crop cycle appears sufficient to guarantee a satisfactory yield performance of the treated crop. However, further field trials are suggested to better define the most proper rate to use for nematodes control.

CONCLUSIONS

Certis Europe will continue the registration process of DMDS at European level. Trials carried out in different countries for key crops, as shown in this paper for melon and tomato in Italy, provide clear evidences that the product may represent a good technical solution for controlling nematodes, at the same or better level than other fumigant nematicides.

Considering how widespread root-knot nematodes are in protected crops and because of the severity of the damages they cause (Sikora et al., 2005), pre-planting soil fumigation appears necessary to obtain satisfactory crop yield. Other methods of controlling nematodes are also available but they may not be as effective as soil fumigation and others may not be suitable. For instance, the use of soil solarization is only suitable during hot months of the year and do not control nematodes in deep soil profile. Crop rotation is environmentally safe and reasonably cheap but it is difficult to use with nematodes having large host ranges as *Meloidogyne* spp. are. Resistant cultivars are effective but, unfortunately, they are not available for most crops and, besides, if they are used continuously, they may select virulent nematode population. Therefore, the use of DMDS, properly included in an IPM programs, appears very promising.

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Tables

Table 1. Effects of the treatments on the infestation of roots of melons by *Meloidogyne incognita.*

Treatments	28 DAT				60 DAT			
	Infested plants (%)	Abbot (%)	Damage index	Abbot (%)	Infested plants (%)	Abbot (%)	Damage index	Abbot (%)
Untreated check	100.00 a	-	1.63 a	-	95.31 a	-	5.52 a	-
DMDS 400	18.75 b	81.25	0.19 b	90.63	32.81 b	67.19	1.22 b	81.75
DMDS 300	31.25 b	68.75	0.31 b	84.72	53.13 b	46.51	1.53 b	76.84
1-3 D 140	81.25 b	18.75	1.19 b	32.43	90.63 a	5.77	4.19 a	21.37

Means followed by the same letter do not differ significantly for P=0.05 according to Student Newman-Keuls' multiple comparison test. DAT=Days after transplant.

Table 2. Yield of melon (20 plants/plot) as affected by different fumigant treatments.

Treatments	59 DAT			
	Small fruits (nr.)	Marketable fruits (n.)	Total fruits (nr.)	Total weight (kg)
Untreated check	9.50 a	6.00 b	15.50 a	22.95 b
DMDS 400	9.25 a	14.75 a	24.00 a	42.25 a
DMDS 300	8.00 a	12.75 a	20.75 a	35.20 ab
1,3-D 140	10.00 a	12.25 a	22.25 a	37.00 ab

Means followed by the same letter do not differ significantly for P=0.05 according to Student Newman-Keuls' multiple comparison test. DAT=Days after transplant.

Table 3. Effects of the treatments on the infection of roots of tomato by *Meloidogyne incognita*.

Treatments	70 DAT				120 DAT			
	Infested plants (%)	Abbot (%)	Damage index	Abbot (%)	Infested plants (%)	Abbot (%)	Damage index	Abbot (%)
Untreated check	100.00 a	-	8.17 a	-	100.00 a	-	9.57 a	-
DMDS 500	3.33 b	96.67	0.03 b	99.61	95.63 b	4.38	2.88 b	69.84
DMDS 400	3.33 b	96.67	0.03 b	99.59	90.63 b	9.38	2.65 b	72.43
DMDS 300	5.00 b	95.00	0.05 b	99.40	95.63 b	4.38	3.04 b	68.26
1,3-D 180	10.00 b	90.00	0.10 b	90.78	100.00 a	0.00	3.62 b	62.16

Means followed by the same letter do not differ significantly for P=0.05 according to Student Newman-Keuls' multiple comparison test. DAT=Days after transplant.

Table 4. Yield of tomato (20 plants/plot) as affected by different fumigant treatments.

Treatments	80 DAT+ 91 DAT		98 DAT+ 108 DAT		113 DAT+ 120 DAT		Total yield	
	Fruit (n.)	Weight (kg)	Fruit (n.)	Weight (kg)	Fruit (n.)	Weight (kg)	Fruit (n.)	Weight (kg)
Untreated check	225.8 a	12.50 b	195.0 b	8.62 b	0.00 b	0.00 b	420.80 b	21.12 b
DMDS 500	257.0 a	20.28 a	236.6 a	19.56 a	333.2 a	28.40 a	826.80 a	68.24 a
DMDS 400	258.2 a	21.04 a	242.0 a	20.10 a	341.4 a	27.94 a	841.60 a	69.08 a
DMDS 300	258.2 a	20.18 a	249.0 a	20.12 a	338.8 a	27.28 a	846.00 a	67.58 a
1,3-D 180	251.2 a	18.76 a	246.4 a	20.38 a	356.2 a	28.60 a	853.80a	68.74 a

Means followed by the same letter do not differ significantly for P=0.05 according to Student Newman-Keuls' multiple comparison test. DAT=Days after transplant.