



Low Temperature Biodisinfection Effectiveness for *Phytophthora capsici* Control of Protected Sweet Pepper Crops in the Southeast of Spain

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Biodisinfection using fresh sheep manure in August is effective in controlling *Phytophthora* root rot in greenhouses in southeast Spain, but this is not the case after the beginning of September. The effectiveness of biodisinfection of new amendments has been evaluated beginning in October in sweet pepper greenhouses to control *Phytophthora capsici*. The amendments used were: T1: wheat husk + fresh sheep manure (FSM), 3.5 kg m⁻²; T2: sunflower pellets 3.5 kg m⁻²; T3: FSM: 3.5 kg m⁻²; T4: Control. Temperatures above 40°C were obtained in some amendments; anoxic conditions were found in all amendments, and also a reduction of the viability of *P. capsici* oospores with respect to the control, as well as a higher yield. The contribution of fresh sheep manure to the amendments did not lead to an improvement in their effectiveness. Autumn biodisinfection under low temperature conditions using fresh organic amendments that enhance soil anaerobic conditions may be a promising strategy for the control of *P. capsici* in pepper greenhouses in southeastern Spain.

Keywords: soil disinfestation, *Capsicum annuum*, organic amendment, *Phytophthora*, oospores, greenhouse

INTRODUCTION

Greenhouses of pepper (*Capsicum annuum* L.) in southeast Spain suffer from serious economic losses caused by the oomycetes *Phytophthora capsici* and *P. nicotianae*, which contaminate most of the region's soils (Lacasa et al., 2013). Both of these oomycetes limit the crop, leading to important losses in plants and harvests if preventive measures are not adopted before planting (Lacasa et al., 2010; Guerrero et al., 2013). Nowadays, *P. nicotianae* is the most prevalent (Lacasa et al., 2013; Blaya et al., 2014), favored by the climatic conditions in the greenhouses and farming practices. Chlamydospores and/or oospores may survive for up to 6 years (Erwin and Ribeiro, 1996). The main soil-borne pathogens of protected peppers are *Phytophthora* spp. and *Meloidogyne incognita* in addition to soil fatigue issues which are specific of this crop. The previous soil phytopathological problems are both caused by the repeated growing of pepper crops in the same soil for over 30 years (Guerrero et al., 2014).

Between 1982 and 2005, soil disinfestation with methyl bromide was the way to palliate the effects of *Phytophthora* root rot in more than 95% of the greenhouses (Lacasa et al., 2010). The mix

of chloropicrin and 1,3-dichloropropene has replaced methyl bromide in over 70% of the surface area. Biodisinfestation is used in 20% of the surface area, combining solarization with biofumigation (Lacasa et al., 2010; Guerrero et al., 2013).

The combination of solarization with biofumigation shows that the two methods have synergic effects (Katan, 2005). Sealing the soil with plastic produces the gas retention that occurs during organic matter decomposition (Bonanomi et al., 2007; Oka, 2010). If saturation levels are reached when the soil is wetted, then the effects of anaerobiosis are also added and phytoparasites and pathogens are affected (Blok et al., 2000).

If water saturation levels are not reached, but organic amendments containing a high carbon content are used anaerobiosis occurs under the plastic during biodecomposition (anaerobic soil disinfestation, ASD) which affects the pathogens (Shennan et al., 2014; Roszkopf et al., 2015). During the degradation process of the organic matter, changes occur in the composition of the microbiota that confer a suppressive character against some pathogens (Mazzola, 2011; Núñez-Zofío et al., 2011; Mazzola and Manici, 2012; Mazzola et al., 2012). The suppressive effects and the anaerobiosis depend on the soil characteristics, the organic amendment used, the soil temperature, the characteristics of the microbiota and the disinfectant effectiveness or the pathogens susceptibility (Butler et al., 2012, 2014). The choice of the organic matter used in the biodisinfestation is one of the determining elements for its efficacy. Bonanomi et al. (2007) suggested that the application of specific organic matters: crop residues and organic wastes, compost and peat could be useful to increase soil suppressiveness. Núñez-Zofío et al. (2013), found an effective biofumigant in beetroot stillage. Guerrero et al. (2013), checked the effect of *Brassica carinata* pellets on *Meloidogyne incognita* in pepper greenhouses in southeast Spain.

European Union restrictions on the use of chemical products, as well as the increase in the surface area dedicated to ecological agriculture, require the use of soil organic amendments for soilborne disease management. Biodisinfestation using fresh sheep manure in August gives good results in controlling *Phytophthora* in greenhouses in southeast Spain, although this is not the case when the process is performed after the start of September (Guerrero et al., 2010). The majority of the greenhouses soils in the region are clay loam (Haplocalcids), basic pH, with an organic matter content between 1.5 and 2.5%. The climate is Mediterranean with marine influence, hot dry summers, and mild winters. The greenhouse pepper crop is planted in December-January, and finishes in August-September.

In order to fit the start of the biodisinfestation to the end of the cropping season it is necessary to commence in October. Using the amendments that are typically used in August (fresh sheep manure), the biodisinfestation does not reduce the levels of pathogens, and are thus unviable (Guerrero et al., 2010, 2013; Lacasa et al., 2010), so it is necessary to improve the biodisinfestation effects by adding amendments that strengthen the anaerobic effect or increase soil temperature, both effects by the organic matter decomposition.

Moreover, the southeast of Spain is in a vulnerable zone due to nitrates (Directive 91/676/CEE, of 12 December, relating

to the protection of waters against contamination produced by agricultural origin nitrates) which means that there are restrictions on the contribution of nitrogen. This premise severely limits the use of organic matter in the zone.

The objective of this study was to obtain biodisinfestation amendments that are effective in controlling *Phytophthora* in pepper greenhouses with low temperatures whilst also reducing the amount of fresh organic amendments.

MATERIALS AND METHODS

Experimental Design and Treatments

The field experiment was carried out over two crop seasons in an experimental sweet pepper greenhouse of 1,000 m², at the experimental station of the IMIDA, located in the Campo de Cartagena, Region of Murcia (southeast Spain), where pepper has been grown periodically for over 20 years.

The clay loam soil was free from pathogens and was not previously disinfested.

The biodisinfestation was performed in October for both seasons, starting on 2 October 2019 and 9 October 2020, respectively. Treatments were arranged in a randomized complete block design with four replicates and were repeated in the same plots in each of the two seasons. Each experimental unit consisted of a 60 m² plot. The treatments evaluated were: T1: wheat husk (Alimer SCoop)+fresh sheep manure (WH+FSM) 3.5 (2+1.5) kg m⁻²; T2: sunflower pellets (Alimer SCoop) (S) 3.5 kg m⁻²; T3: (FSM): 3.5 kg m⁻²; T4: Control (non-amended and non-covered with plastic). Amendments and the non-amended control soil treatment were analyzed in the 2 years (Table 1).

The amendments were applied and plots were rototilled to a depth of 25–30 cm. The soil was irrigated using a drip irrigation system using 3 L h⁻¹ emitters spaced 0.40 × 0.60 m for 4 h on 2 consecutive days. Amended soil was covered with a 0.05 mm-thick transparent polyethylene film. The plastic was maintained for 6 weeks. The “Beniel” (Syngenta Seeds) pepper cultivar was planted in December at the habitual density of the zone: 1 m separation between rows and 0.4 m between plants in the same row (2.5 plants m⁻²).

Inoculum Production

The inoculum was obtained from a *P. capsici* isolate from pepper plants that presented symptoms and was conserved in the IMIDA mycology collection in PDA medium. When this isolate was inoculated into healthy pepper plants, it showed to be aggressive and maintained its pathogenicity. The isolate grew in controlled conditions at 25°C for 3 weeks until it reached the edge of a 9 mm diameter Petri dish. A micellar solution with a concentration of 1 × 10³ CFU mL⁻¹ was obtained by grinding the content of one dish in 100 mL of distilled sterile water and subsequent adjustment by dilution and direct count using a Neubauer counting chamber. A volume of 100 mL of soil, previously autoclaved at 120°C for 1 h on 2 consecutive days, was inoculated with 1 mL of micellar solution, and was placed inside muslin forming a bag. In three different points of each treatment

TABLE 1 | Composition of organic amendments and non-amended control soil treatment.

	Wheat husk	Sunflower pellets	Fresh sheep manures	Control
Total OM %	91.3 ± 22.1	93.8 ± 23.0	52.23 ± 3.92	2.82 ± 0.65
Total N g kg ⁻¹	22.8 ± 1.5	48.5 ± 2.3	16.5 ± 0.10	0.7 ± 0.39
C/N	20.4 ± 14.9	11.5 ± 14.1	17.9 ± 1.38	9.54 ± 0.81
P ₂ O ₅ g kg ⁻¹	25.2 ± 01.1	14.4 ± 3.2	0.95 ± 0.03	0,11 ± 0.04
K ₂ O g kg ⁻¹	20.1 ± 01.5	17.4 ± 1.2	3.98 ± 0.11	2.18 ± 0.13
pH	6.54 ± 0.5	6.47 ± 2.2	8.50 ± 0.05	7.71 ± 0.23
Electrical conductivity (25°C) dS m ⁻¹	2.59 ± 0.86	2.89 ± 0.41	8.62 ± 0.07	3.99 ± 0.54
Moisture%	8.12 ± 0.15	5.60 ± 0.12	51.53 ± 1.25	42,45 ± 1.07

Values are the mean of two replicates corresponding to the 2 years ± standard errors.

replicate plot, soil inoculated bags were buried at 15 and 30 cm soil depth.

Oospores of *P. capsici* were produced *in vitro* by pairing isolates of different mating types on soft pea agar following Núñez-Zofío (2011). Oospores embedded in nylon mesh were buried at 15 and 30 cm soil depth in three different points of each treatment replicate plot.

Variables Measured

- Ambient greenhouse temperature and soil temperatures in one replicate plot of each treatment at 15 and 30 cm depth were registered with 12-bit S-TMB-M017 temperature Hobo probes (accuracy < 0.2°C) connected to an H21-002 Hobo datalogger. Readings were taken every 30 min throughout the biodisinfestation treatment.
- Soil oxygen percentage content in one replicate plot of each treatment at 15 cm depth was registered with SO-200 galvanic cell type oxygen Apogee-Instruments probes (accuracy < 0.02% O₂) buried at a depth of 15 cm connected to an H22-001 Hobo datalogger. Readings were taken every 30 min throughout the biodisinfestation treatment.
- Viability of *P. capsici* oospores. First season. After 6 weeks of biodisinfestation treatment, *P. capsici* oospore viability was determined using the plasmolysis method (Jiang and Erwin, 1990), adapted by Núñez-Zofío et al. (2013).
- Infectivity of *P. capsici* inoculum soil buried. Second season. After biodisinfestation the bag with inoculated soil were removed, and the soil was placed in 150 mL pots into which “Lamuyo” cultivar (Clause Seeds) sensitive pepper plants were transplanted when they had four true leaves. The potted plants (three points for each treatment in each of the three replicate plots) were kept for 12 weeks in a chamber at 25°C and a relative humidity of 60–70% with 14:10 h light: darkness of photoperiod and were kept. Notes were taken on the presence of yellowing, wilting or death once per week and those that presented symptoms were analyzed in PDA medium. As plants died, damaged tissue was washed with tap water and fragments of roots and crown were placed on potato dextrose agar and on PARPH (Jeffers and Martin, 1986) medium and incubated at 25°C for 4–6 days. After that, the fungus was identified using microscopic observation. At the end of the bioassay, the plant roots were examined to detect symptoms of the disease. The results of infectivity were expressed as a

percentage of diseased plants (Coelho et al., 2000; Lacasa et al., 2015).

- Marketable yield. Each 15 days, from April through to August in each season, the production of the plants was harvested and weighed separately for each treatment replicate plot. This variable was expressed in kg m⁻². Each replicate plot consisted of two rows and 45 plants were harvested from each row.

Statistical Analysis

The effects of treatments were studied using two way analysis of variance (ANOVA) with the Software Statgraphics Centurion 16. In order to fulfill the assumptions of analysis of variance (homocedasticity and normality), the infectivity bioassay data were transformed using arcsine ($\sqrt{x/n}$), where x = total number of dead plants and n = total number of plants. Oospores viability data were transformed using arcsine ($\sqrt{x/n}$), where x = total number of oospores survival and n = total number of oospores. Data on crop yield were transformed using log transformation $\log(x+1)$, where x = total yield. Means for significant main effects (biodisinfestation treatment, soil depth) and all pairwise differences among least squares means within the biodisinfestation treatment × soil depth interaction were separated by Fisher's LSD test ($p < 0.05$).

RESULTS

Soil Temperatures

Temperatures exceeding 38°C were reached in the amendments consisting of wheat husk+fresh sheep manure, in the sunflower pellets, and fresh sheep manure, at 15 cm in the first season. In the second season, although the largest number of hours was accumulated in the order of 33°C, the soil was subjected to temperatures of up to 37°C for certain periods of time at both soil depths, 15 and 30 cm. In the second season, the lowest number of accumulated hours above 33°C was found in the non-treated control. There were no temperatures exceeding 42°C in any of the treatments (Table 2). The mean ambient greenhouse temperature ranged from 13.6 to 31.8°C in the first season, and from 15.9 to 44.3°C in the second.

There was a period of anoxia during the biodisinfestation process in both seasons in all the biodisinfestation treatments (Figure 1). Variations were found among the treatments and

TABLE 2 | Number of cumulative hours in each crop cycle at 15 and 30 cm soil depth within different temperature ranges.

Treatment	Depth (cm)	First season		Second season	
		34–37°C	38–41°C	34–37°C	38–41°C
Wheat husk + Fresh sheep manures	15	48	51	120	0
	30	0	0	39	0
Sunflower pellets	15	66	57	40	0
	30	0	0	18	0
Fresh sheep manures	15	60	52	223	0
	30	0	0	147	0
Control	15	0	0	30	0
	30	0	0	0	0

First and second season.

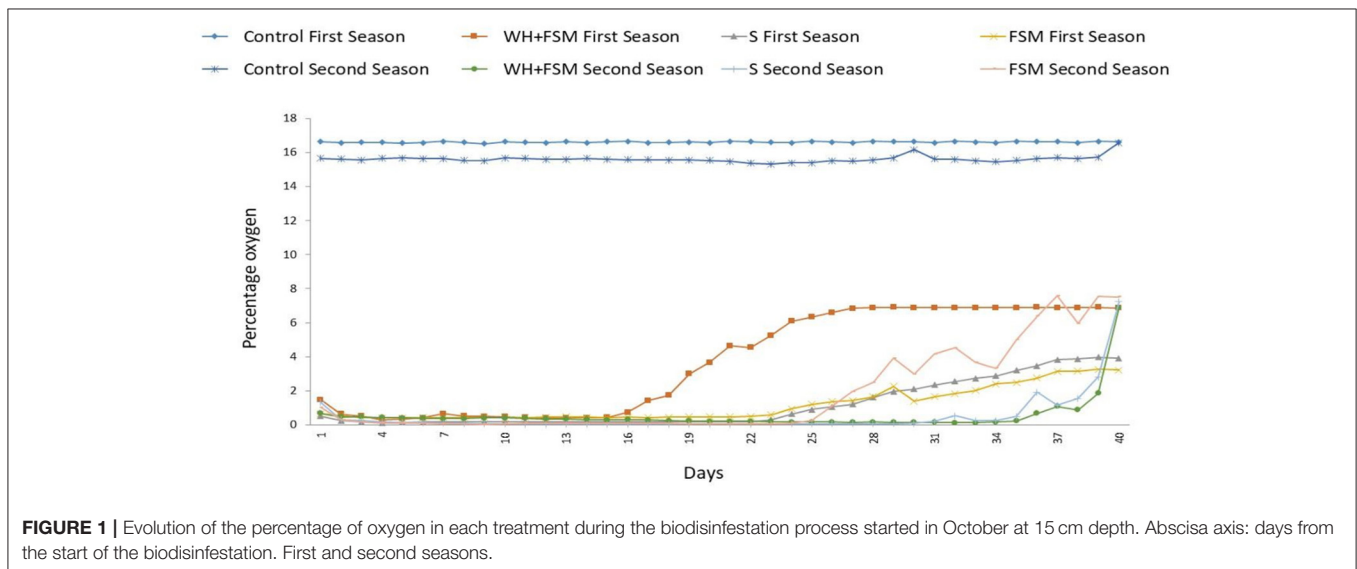


FIGURE 1 | Evolution of the percentage of oxygen in each treatment during the biodisinfestation process started in October at 15 cm depth. Abscisa axis: days from the start of the biodisinfestation. First and second seasons.

between years for the same treatment. In WH+FSM, at least 35.5% of the disinfestation time the O₂ level was below 2% in the first year, whilst in the second year this was the case 84.4% of the time. In FSM at least 64.4% of the time the oxygen levels were below 2%, whilst in the second year this occurred 57.7% of the time. In the first year the time that the oxygen level was below 2% in S and FSM was similar (at least 64.4%) whilst in WH+FSM the figure was, of at least 35.5%. In the second year, the oxygen level remained below 2% for 84.4% of the biodisinfestation time in both S and WH+FSM, whilst in FSM the proportion of biodisinfestation time with oxygen levels below 2% was at least 57.7%.

Effect of Treatments on *P. capsici* Oospore Viability

First Season

Table 3 shows the viability of the oospores in each treatment and at each depth. The depth had an influence on oospore viability. At 15 cm the oospore viability was reduced in all the treatments, except for the control, while at 30 cm the reduction in viability was greater in the WH+FSM and S treatments than in the FSM. Biodisinfestation reduced the viability of the oospores buried in the

amendments with FSM as well as in those that did not contain it [$F_{(3,11)} = 5.69$; $p = 0.0028$ at 15 cm; $F_{(3,11)} = 2.92$; $p = 0.004$ at 30 cm].

Effect of Treatments on Infectivity of Introduced Soil Inoculum of *P. capsici* Second Season

The results obtained in the bioassay were different at 15 cm and 30 cm soil depth (Table 4). There were differences between the percentage of dead pepper plants in each treatment and the control at 15 cm (Table 4). The percentage of dead plants in the bioassay at 30 cm differed between the FSM and the control, although this was not the case for the other two amendments [$F_{(3,11)} = 1.07$; $p = 0.040$ at 15 cm; $F_{(3,11)} = 3.30$; $p = 0.047$ at 30 cm].

Effect of Treatments on Marketable Yield

Yields were significantly higher in the biodisinfested plots than in the control. The effect of biodisinfestation treatments on marketable yield was significant in the first season [$F_{(3,15)} = 4.16$; $p = 0.009$], and in the second season [$F_{(3,15)} = 0.65$; $p =$

TABLE 3 | Effect of biodisinfestation treatments on *P. capsici* oospore viability percentage at two different soil depths (15 and 30 cm) after 6 weeks of exposition.

Biodisinfestation treatment	15 cm	30 cm
Wheat husk + Fresh sheep manure	2.89 ± 3.40bA	3.34 ± 2.23bA
Sunflower pellets	4.46 ± 4.93bA	4.66 ± 4.5bA
Fresh sheep manure	3.29 ± 2.91bB	8.88 ± 3.98aA
Control	22.8 ± 6.71aA	5.11 ± 2.85abB

x = mean percentage of viable oospores. Mean values ($n = 12$) ± standard deviation. Data were transformed using arcsine ($\sqrt{x+0.5}$) where x = mean percentage of viable oospores in each biodisinfestation treatment at each soil depth.

The significant differences among biodisinfestation treatments and each soil depth at 6 weeks were tested by two-way ANOVA.

Values in the same column followed by a different lower-case letter indicate significant differences between biodisinfestation treatments within a given soil depth and upper-case letters compare soil depths for a given biodisinfestation treatment based on the Fisher's LSD test ($p < 0.05$) within the interaction of biodisinfestation and soil depth treatments. When differences were non-significant, letters were omitted.

TABLE 4 | Infectivity of introduced soil inoculum of *Phytophthora capsici* at 15 and 30 cm depth in bioassays with sweet pepper variety "Lamuyo" plants expressed as percentage of dead plants during bioassays in each treatment.

Treatment	15 cm	30 cm
Wheat husk + Fresh sheep manures	77.7 ± 12 a	77.7 ± 12 ab
Sunflower pellets	77.7 ± 19 a	88.9 ± 19 ab
Fresh sheep manures	77.7 ± 18 a	100.0 ± 0 a
Control	44.4 ± 19 b	66.6 ± 3 b

Mean values ($n = 12$) ± standard deviation. Data were transformed using arcsine ($\sqrt{x+0.5}$) where x = mean percentage of lived plants at each soil depth.

Values followed by the same letter are not significantly different according to Fisher's LSD ($p < 0.05$).

TABLE 5 | Marketable yield (kg m⁻²) in each crop cycle.

Treatment	1st season	2nd season
Wheat husk + Fresh sheep manures	9.43 ± 1.06 a	6.86 ± 1.30 a
Sunflower pellets	9.72 ± 1.79 a	6.96 ± 0.80 a
Fresh sheep manures	9.21 ± 1.17 a	6.30 ± 1.20 a
Control	8.23 ± 0.44 b	5.00 ± 0.39 b

Mean values ($n = 4$) ± standard deviation. Data were transformed using log ($x+1$), where x = total yield. Values followed by the same letter are not significantly different according to Fisher's LSD ($p < 0.05$).

0.6028] (Table 5). The non-contribution of FSM had no negative influence on yield in the amendment composed of sunflower pellets. This fact increases the interest of the amendment, due to the situation of greenhouses in the zone subjected to restrictions in the N application with origin on livestock manures due to Nitrates Directive implementation.

DISCUSSION

In our trial, the temperatures obtained during the biodisinfestation carried out in October with the amendments of wheat husk+fresh sheep manure, sunflower pellets, and fresh sheep

manure exceeded 38°C in the first season, varying between 51 and 57 h, but no in the second. At both seasons soil temperatures accumulated in October during the biodisinfestation in the greenhouse were considerably lower than those obtained for the month of August in the same zone (Guerrero et al., 2019). Etxeberria et al. (2011) found that for 1,680 h at 15–35°C, the survival of the oospores ranged between 88 and 36%. The oospore viability in the two seasons was reduced by the presence of minimum temperatures above those reported by Etxeberria et al. (2011). The results also concur with those obtained by Lacasa et al. (2015) in which biodisinfestation in spring with *Sinapis alba* fresh green manure did reduce the infectivity of *Phytophthora nicotianae* chlamydospores introduced inoculum (Gandariasbeitia et al., 2019). The infectivity of the surviving inoculum corresponded to an insufficient reduction of the inoculum buried in the bags. Coelho et al. (2000) considered that inoculum levels in excess of 1 propagule per gram of soil are necessary in order to detect the surviving inoculum using plant indicators in bioassays.

The biodisinfestation with the assayed amendments induce anoxia in the soil for several weeks, which is one of the factors indicated for Gamliel et al. (2000) as cause of the decrease in the pathogen populations and of the efficacy of the disinfestation. In the first season, the level of oxygen in the soil was below 2% for more than 64% of the disinfestation time for S and FSM treatments, and over 35% of the time in WH + FSM. In the second season, the corresponding time was more than 57% in FSM and over 84% in S and for WH + FMS. The production of sporangia and the mycelial growth of several species of *Phytophthora* (including *P. capsici*) was considerably lower when the oxygen level was at 1% of oxygen in the crop solution. Below 2.5% of oxygen, the formation of sporangia is reduced and also the mycelial development of *P. cinnamomi*, affecting the viability of the inoculum (Davison and Tay, 1987).

The efficacy of the biodisinfestation when temperatures are low improves with the contribution of amendments that are rich in organic matter or by increasing the amount of carbon that is applied to the soil. The contribution of 4 mg C g⁻¹ of soil has been recommended to improve the efficacy of ASD at low temperatures (Butler et al., 2014; Roskopf et al., 2015). It is recommended that the carbon of the organic amendment should be labile, so that it can easily and rapidly be degraded by the soil microorganisms and create conditions for persistent anaerobiosis. Additionally, an adequate ratio of C/N must be considered so that, on the one hand, the N that is applied is enough so that N is not immobilized by the soil microorganisms in the degradation/mineralization of the organic amendments and, at the same time, there is not a potentially leachable excess of N. In our trials, the amendments presented a balanced composition, and ratios of C/N within the limits that are considered as adequate for biodisinfestation and the generation of hypoxia.

The efficacy or failure of soil biodisinfestation may be due to the soil characteristics, the degree of moistening, the electrical conductivity, the characteristics of the plastic, and the period of solarization (Chellemi, 2002). The temperate-humid climate has obstructed the application of biosolarization, very extended in

Mediterranean horticultural areas such as South-eastern Spain. However, previous experimentation with different amendments, doses and application times compatible with crop cycles showed the applicability of these practices in the humid temperate climate region of Northern Spain (Ojinaga et al., 2020).

In the north of Spain, the survival of oospores of *P. capsici* was reduced at 15 and 30 cm when the biodisinfestation was applied in August with both fresh and semi-composted manure (Núñez-Zofío et al., 2012). Using rice bran, rapeseed cake, or grape pomace as the carbon source, Serrano-Pérez et al. (2017) obtained significant reductions in chlamydozoospore viability and inoculum infectivity of pepper plants by *P. nicotianae*, when biodisinfestation was carried out in low temperature open air conditions of the spring season in south western Spain.

Despite the low disinfectant efficacy assessed by the reduction in the survival of *P. capsici* oospores in sub-optimal conditions for solarization (absence of thermal inactivation), improvements were observed in the suppressiveness and in the chemical, physical and biological soil properties when the organic amendments were applied in biodisinfestation, which caused a reduction of crop diseases and a yield increase (Núñez-Zofío et al., 2012; Roskopf et al., 2015; Gandariasbeitia et al., 2019). It was concluded that repeated biodisinfestation for the control of *Phytophthora* root and crown rot in protected pepper crops located in temperate climate regions can improve soil quality and suppressiveness, as well as enabling reduced doses of the organic amendment needed for biodisinfestation. However, this disinfectant efficacy worsened when the biodisinfestation was performed in March (Arriaga et al., 2011), and also when it was carried out in September (Núñez-Zofío et al., 2011).

One strategy employed when biodisinfestation fails due to the climate or crop cycles is to turn to anaerobic disinfection. This strategy has been applied in limiting solarization conditions in Japan (Shinmura, 2000; Momma et al., 2005, 2006, 2010, 2011; Katase et al., 2009) and also in the Netherlands (Blok et al., 2000; Agtmaal et al., 2015).

The effect of the application of organic matter produced a yield increase in both seasons. Although there was no naturally occurring presence of pathogens, the increased yield in the biodisinfested plots might be explained by the reduction of soil fatigue in the crop. The fatigue accumulated in greenhouses used for pepper monocropping is highly specific toward pepper (Guerrero et al., 2014). The greenhouse assayed in this study fits the conditions of pepper monocropping. Biodisinfestation with fresh sheep manure of greenhouses used to grow pepper crops, improved the physical and chemical characteristics of the soil (Fernández et al., 2005). The application of wood waste and sunflower husk biochar improved soil porosity and the structural stability of soil aggregates (Sokołowska et al., 2020). Improvement of soil water infiltration, soil drainage, and roots aeration have been associated with a better control of crop root rot caused by soilborne pathogenic species of *Phytophthora* (Ristaino, 1991).

The principal cause of this fatigue is considered to be biotic and related with the extent to which the soils are contaminated with *Fusarium* sp. (Martínez et al., 2009, 2011). It is additionally known that the fatigue accumulated in greenhouses used for pepper monocropping is highly specific toward pepper (Guerrero et al., 2014). In soils contaminated by *Fusarium* spp. and *Phytophthora* spp., Song et al. (2020a,b) obtained a significant reduction in the incidence of *Fusarium* and *Phytophthora* in strawberry crops in conditions of biodisinfestation with chicken manure or with maltose as carbon sources at 28°C and covering the soil with total impermeable film. They also observed a yield improvement related with the increase of soil available nutrients after the biodisinfestation treatment.

In those plots where FSM was not used in this study, the yields were similar to those in which it had been used. This fact is of great interest since the cropping zone of Southeast Spain is subject to organic matter restrictions. Moreover, it is known that the incorporation of organic correctives improves soil properties and fertility, as reported by Bonanomi et al. (2007).

The results obtained with the carbon-rich and balanced amendments and in time periods that are compatible with the pepper crop cycle in greenhouses in southeast Spain, showed a yield improvement. This increased yield may also be due to the effect highlighted by Núñez-Zofío et al. (2011) that repeated biodisinfestation treatments in temperate climate areas improve soil suppressiveness and quality. This would enable growers to extend the crop cycle and thereby the corresponding economic benefit.

In conclusion, using the specific organic amendments, significant reductions were obtained in the viability of the inoculum resting structures of *P. capsici* as well as increases in the marketable yield, when biodisinfestation is performed on dates that are compatible with the pepper growing cycle in the Campo de Cartagena. This way of soil disinfection is recommendable in the context of the strategies of sustainable integrated production.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MG designed the experiment and wrote the original draft. CL, VM, MM, and AM supervised the experiment and contributed to the data. SL revised the manuscript. All authors approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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