agronomy

MDPI

Article

Influence of Season and Organic Amendment on the Effectiveness of Different Biosolarization Treatments against *Fusarium oxysporum* f. sp. *lactucae*

María del Mar Guerrero [1,*], Carmen Mª Lacasa [1], Victoriano Martínez [1], Antonio Monserrat [1], José Antonio López-Pérez [2], Raúl Ortega [3], José Carlos Nieto [3], Isabel Miralles [3] and Santiago Larregla [4]

[1] Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario y Medioambiental, Dpto. Protección de Cultivos, s/n, 30150 Murcia, Spain
[2] Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental (CIAPA), Lab Suelos, 19180 Marchamalo, Spain
[3] Department of Agronomy & Center for Intensive Mediterranean Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria, 04120 Almería, Spain
[4] Department of Plant Production and Protection, NEIKER, Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), 48160 Derio, Spain
* Correspondence: mariam.guerrero@carm.es

Abstract: One strategy presented as an alternative to avoid using chemical substances in soil disinfestation consists in the technique of biosolarization. The aim of the present study was to analyze the effect of seasonality on the effectiveness of biosolarization with different organic amendments for the control of *Fusarium oxysporum* f. sp. *lactucae* (FOLAC) on lettuce plants, and to compare the results obtained using a classical soil infectivity bioassay and a qPCR-based molecular technique. None of the plants subjected to biosolarization in the summer season (469–700 and 0–463 h with temperature > 42 °C at 15 and 30 cm soil depth, respectively) showed damage by the pathogen except the untreated control. Conversely, in autumn (3–5 and 0–0 h at temperature = 38–40 °C at 15 and 30 cm soil depth, respectively), only two biosolarization treatments (wheat + semi-composted manure, sunflower pellets) that reduced FOLAC inoculum in soil and plants did not show any disease at the lowest depth (15 cm) in the soil infectivity bioassay. This same result was only obtained at 30 cm soil depth in the biosolarization treatment with sunflower pellets. The number of FOLAC sequences per gram of soil determined with qPCR was null in the biosolarization treatments in summer at both soil depths and corresponded to the absence of disease in the soil infectivity bioassay. A threshold of 145 sequences per gram of soil determined by the qPCR-based molecular technique corresponded to the presence of 10% of diseased lettuce plants infected by FOLAC. Therefore, this molecular technique has been shown to be useful for establishing the soil inoculum thresholds required for crop infection by pathogens, while reducing the time and execution tasks necessary to perform soil infectivity bioassays.

Keywords: lettuce; sheep manure; wheat husk; sunflower pellets; soil pathogens

Citation: Guerrero, M.d.M.; Lacasa, C.M.; Martínez, V.; Monserrat, A.; López-Pérez, J.A.; Ortega, R.; Nieto, J.C.; Miralles, I.; Larregla, S. Influence of Season and Organic Amendment on the Effectiveness of Different Biosolarization Treatments against *Fusarium oxysporum* f. sp. *lactucae*. *Agronomy* **2023**, *13*, 1498. https://doi.org/10.3390/agronomy13061498

Academic Editor: Leire Molinero-Ruiz

Received: 24 March 2023
Revised: 12 May 2023
Accepted: 24 May 2023
Published: 30 May 2023

1. Introduction

Vascular wilt diseases of economically important crops throughout the world are caused by the *Fusarium oxysporum* species complex that accommodates a multitude of pathogenic wilt-inducing strains [1]. Host-specific strains of *F. oxysporum* are assigned to formae speciales (f. sp.). The formae speciales designation has no taxonomic standing and is considered as a biologic form or variant of a single species, rather than separate taxa [2].

One such phytopathogenic formae speciales is *Fusarium oxysporum* f. sp. *lactucae* (FOLAC), the causal agent of fusarium wilt of lettuce. This is an important soil-borne fungal disease occurring across different continents and which is responsible for significant

economic losses according to the European and Mediterranean Plant Protection Organization [1,3]. Affected plants show leaf yellowing and wilting. Crown tissues and upper roots usually show reddish-brown necrosis followed by decaying. Vascular necrosis can often be seen on leaf veins. Infected plants are stunted and often die, resulting in significant losses for the growers. This soil-borne fungal disease is considered as one of the limiting factors for commercial production of lettuce during the summer season, particularly when lettuce is grown as a monoculture, as is the case of Northern Italy, Northern Portugal, and Southeastern Spain [4,5]. Among the four races of *F. oxysporum* f. sp. *lactucae* that can be distinguished based on their different pathogenicity on specific lettuce cultivars, races 1 and 4 are the only races present in Europe and are closely related [6]. Race 1 was found in Southeastern Spain (Murcia province) in 2017 [5] and race 4 has been recently reported also in Southeastern Spain (Albacete province) in 2021 [7]. The fungus penetrates the plants via natural apertures or wounds on the roots, and its long-lasting resting spores called chlamydospores can remain viable in the soil or on plant debris for a considerable time. The pathogen is not spread by windblown spores. In addition, studies carried out in Italy have showed that this fusarium wilt was also transmitted through seeds [8,9]. The optimum growth of the fungus is favored by soil temperatures between 24–28 °C, although it can be active down to 10 °C. Infection may lead to the total destruction of the crop under favorable climate conditions of warm temperatures and/or in crop systems such as glasshouse crops or protected summer crops. However, in contrast to race 1, the recently reported race 4 may be more competitive at lower soil temperatures and could become a serious threat to Spain's lettuce production [7,10].

There is currently an ongoing peak in pathogenic organisms (viruses, bacteria, fungi, nematodes, and parasitic plants) in crops; one of the main factors triggering this is the use of monocropping agricultural models. Intensive monocropping, although highly productive in the short term, leads to a loss in diversity in the medium and long term, which facilitates infection by phytopathogens [11,12]. In the light of this increase in crop pathogens, the main strategy implemented involves the use of chemical products, such as fungicides, herbicides, or pesticides, amongst others. However, using that type of substances entails risks for both consumers and workers, as well as for the ecosystem where the crop is to be found. The serious drawbacks arising from the use of that type of substances have driven new research lines focused on the development of strategies that are safe, healthy, and which respect the environment and human health [13].

The control of FOLAC is difficult. Research has been carried out on the effects of soil chemical disinfestation with fumigants, crop rotation, crop management (e.g., date of planting), use of tolerant/resistant cultivars, and the use of bacteria or fungi as biological control agents [10]. Ecological agents such as *A. tubingensis*, *A. alabamensis*, and *A. oryzae* have been used commercially for the defense of pepper seedlings against *Fusarium* wilt [14]. The use of Maxifos Ca, Greencal, and *A. platensis* as alternate therapeutic nutrients of eco-destructive chemically synthesized fungicides appears to be an effective method for reducing the harmful effects of *Fusarium* wilt on pepper plants [15].

Several non-chemical soil disinfestation strategies could be used for the control of a soil-borne disease, such as *Fusarium* wilt of lettuce. One of them is solarization, which consists in the use of a thin plastic sheet to cover the soil to obtain high temperatures. It is effective against pathogens and weeds, but environmental conditions must be taken into account to achieve high enough temperatures for good efficacy. [16,17]. In this aspect, the climate conditions may vary depending on the season, so some periods may be inadequate for an effective solarization. Another strategy is biofumigation, in which fresh organic matter (manure, crop remains, industrial residues, etc.) is incorporated into the soil, where it decomposes, thereby liberating natural gases that control the phytopathogenic microorganisms and stimulate the microorganisms that are beneficial for the soil [18]. Lastly, the strategy that stands out for its excellent results in pathogen control is biosolarization [19–21]. This method is a combination of the above-mentioned strategies, and whose improved efficacy lies in the combined use of mechanisms such as the thermal

inactivation of pests, the release of mineral nutrients and volatile biocides, and changes in the microbiota. All these changes improve soil fertility and contribute to farming systems that are environmentally more friendly and economically more profitable [13,22]. The effectiveness of biosolarization is determined by several factors, such as the properties of the plastic used [23–25], the type and amount of organic amendment used [26,27], and, above all, by climatic conditions, since in colder seasons the temperatures [28–30] and anoxia conditions [31] may be insufficient to eradicate the pathogens.

As with other control methods, the use of organic amendments in biosolarization for pathogen control has certain limitations. One limitation to the use of organic amendments with a high nitrogen content is that the large amount needed in order to obtain satisfactory control can often cause phytotoxicity in the crop, or be economically unfeasible [31], in addition to possible environmental problems such as the emission of greenhouse gases or nitrates leaching. Due to the risks of water pollution by nitrates [32], animal manure amendment dosage has been progressively reduced so that, in nitrate-vulnerable areas such as Campo de Cartagena in Southeastern Spain, an amendment amount equivalent to 170 kg ha^{-1} of mineralized nitrogen is the limit established by the European Union 91/676/EEC Nitrates Directive. Due to the aforementioned problem, legislation only allows the application of a dose of semi-composted manure for biosolarization that does not exceed 15,000 kg ha^{-1}. Therefore, there is a need to find other amendments that might contribute to increase the efficiency of biosolarization whilst also reducing the risk of water contamination by nitrates. Some agri-food by-products can contribute to the achievement of this double objective, by increasing the soil anaerobic disinfectant mechanism.

Experiments to determine treatment effectiveness against certain soil-borne diseases have traditionally been based on measurements of infectivity on susceptible plants exposed to pathogen inoculum in soil. However, such experiments do not enable to quantify the presence of pathogens and, if the pathogens are present, whether they are below the thresholds which cause disease, but that would lead to disease if measures are not taken. In recent times, the development of qPCR-based molecular techniques has enabled the mentioned issues in infectivity analyses to be resolved.

Therefore, the aim of the present study was to analyze the effect of seasonality on the effectiveness of biosolarization with different organic amendments for the control of FOLAC on lettuce plants, and to compare the results obtained using a classical soil infectivity bioassay and a qPCR-based molecular technique.

2. Material and Methods

2.1. Location and Design of the Experiments

The field trials were carried out during the summer and autumn seasons in 2021 in a 1000 m^2 greenhouse, in the experimental station of the Institute of Agricultural and Environmental Research and Development of Murcia (IMIDA), located in the Campo de Cartagena, Region of Murcia (Southeast Spain).

The soil in the plot was of a clay loam type, with a total Organic matter (OM) of 2.07%, the nitrogen (N) content was 0.62 N g kg^{-1} (ratio C/N 8.29), and calcium carbonate content was 42%, which resulted in a basic pH of 7.8. The presence of pathogens was not detected prior to the trial.

The surface area of the trial was divided into two blocks in which the biosolarization treatments were applied on two different dates, one in the summer season which began on 26 July, and the other in the autumn season which began on 6 October. The design of the treatments in each zone was performed by a randomized complete block design with two replicates; each experimental plot covered 60 m^2. Three biosolarization treatments based on different origins and doses of the organic amendment were assessed. These treatments were: T1: Wheat husk + semi-composted sheep manure 3.5 (2 + 1.5) Kg m^{-2}, T2: sunflower pellets 3.5 Kg m^{-2}, T3: semi-composted sheep manure 3.5 Kg m^{-2}, T4: control (without amendment and no plastic cover). Composition of organic amendments: T1, T2, T3: total organic matter %: 93.59, 94.10, 51.24; total Nitrogen%: 2.44, 4.4, 0.8;

carbon/nitrogen: 20.6, 11.5, 9.5; P_2O_5 %: 2.46, 1.64, 0.11%; K_2O: 1.9, 1.74, 2.1; pH: 6.7, 7.7, 8.4; electrical conductivity (mS/cm): 2.79, 3.9, 7.9; moisture content %: 8.1, 5.6, 54.5.

Management of the treatments consisted initially in drip irrigation with 3 L h^{-1} emitters spaced 0.40 m × 0.60 m for 4 h on two consecutive days. Subsequently, the amendments were applied following the protocol in Guerrero et al. [21] and then rototilled at a depth of 30 cm. Finally, the plots were covered with transparent polyethylene plastic with a thickness of 0.05 mm for six weeks. These treatments were performed in summer and autumn in each of the zones designated for the seasonal treatments.

2.2. Production and Burial of the Inoculum with the Pathogen

The inoculum of FOLAC was obtained from strains of lettuce plants from the mycological collection of the Institute of Agricultural and Environmental Research and Development of Murcia (IMIDA). Koch's postulates were performed to confirm the causal agent, and an especially aggressive isolate (IMIDA code A1-20) was selected for inoculum production. The isolate was grown in controlled conditions in a Petri dish (9 mm) at 25 °C for three weeks, until it reached the dish edges. A spore suspension of FOLAC, mainly containing microconidia, was prepared at a concentration of 10^6 UFC mL^{-1}. For that, the content of the dish was milled in 100 mL of sterile distilled water, filtered, and adjusted to the desired final concentration by counting conidia in a Neubauer counting chamber and by dilution with sterile distilled water. Once the conidia suspension was prepared, a 100 mL volume sterilized soil sample was artificially infested with 1 mL at an inoculum density of 10^6 UFC mL^{-1} of FOLAC and placed inside a muslin bag. The soil sample was previously sterilized in autoclave at 120 °C for one hour on two consecutive days. The bags of inoculated soil were buried at 15 and 30 cm soil depth in three different points of each replicate plot per treatment.

2.3. Climate Variables Measured

The soil temperature in the greenhouse was measured in one replicate for each treatment at depths of 15 and 30 cm using Hobo S-TMB-M017 12-bit temperature probes (accuracy < 0.2 °C) connected to a Hobo H21-002 data logger. Readings were taken every 30 min throughout the whole biosolarization period. The number of hours accumulated at different temperature intervals (<38 °C, 38–40 °C, 40–42 °C, >42 °C) was calculated for each biosolarization treatment, as well as for the non-disinfested control, and for each soil depth (15 cm and 30 cm) in order to assess the effect of thermal inactivation on the fungal pathogen to be controlled in each season.

The percentage of oxygen in the soil was measured in one replicate per treatment at a depth of 15 cm, using Apogee Instruments galvanic cell-type oxygen probes SO-200 (accuracy < 0.02% Oxygen), buried at the same depth and connected to a Hobo H22-001 data logger. Readings were taken every 30 min throughout the whole biodisinfestation treatment.

2.4. Infectivity Trials in Lettuce

The infectivity of the pathogen was measured after six weeks of biosolarization, when the plastic was removed and the muslin bags containing the inoculated soil were recovered. The soil contained in each bag was placed in 150 mL plant pots, into which sensitive Metalia cultivar plants (Nunhems) with four true leaves were transplanted. The plants in the pots were kept in a chamber at 25 °C and a relative humidity of 60–70% with a 14:10 photoperiod (light:dark) for 12 weeks, with ten plants per treatment. The occurrence of yellowing, wilting, or plant death was recorded weekly. Plants presenting symptoms were analyzed in PDA medium, being incubated at 25 °C for 3–4 days. Microscope study was then used to identify the fungus and after six weeks all the plants were analyzed and the percentage of plant deaths by FOLAC was noted.

2.5. Microbiological Analysis in Soils

The presence of the pathogen was analyzed using molecular techniques for all the inoculated bags extracted after biosolarization. To do so, firstly DNA of the organisms present in the soil was extracted using a Qiagen DNeasy Powersoil Kit. Subsequently, the detection and quantification of the pathogen was undertaken, using real-time PCR (qPCR) and primers with a Microgaia Biotech SL PhytAlert SCP0131 species-specific probe kit.

2.6. Statistical Analyses

Variance analysis (ANOVA) was carried out to compare differences in the biosolarization treatments. When significant differences (p-value < 0.05) were detected, the LSD test with a 95% confidence interval was applied. The response variable of the percentage of dead plants in each experimental unit was analyzed using a binomial distribution and the generalized linear mixed models (GLMM) procedure (proc GLIMMIX) of SAS 9.4 software.

3. Results

3.1. Evolution of Temperature and Oxygen Concentration during Biosolarization

Clear temperature differences were found between the summer and autumn biosolarization seasons. Whilst in autumn, merely a couple of the treatments exceeded temperatures of 38 °C for a few hours, in summer, this value was widely exceeded in all the treatments, with temperatures in excess of 42 °C being reached for prolonged periods (Table 1). The control treatment T4 only showed 60 h over 42 °C and only in the first 15 cm. Regarding the biosolarization treatments, the time above 42 °C was between 7.5 and 11.5 times more in the first 15 cm (between 453 and 700 h) than in the samples at a depth of 30 cm.

Table 1. Soil temperature distribution during the biosolarization periods (6 weeks) in the different seasons trialed (summer and autumn).

Treatments	Depths	Season	<38 °C	>38–40 °C	40–42 °C	>42 °C
T1: Wheat husk + SCM	15 cm	Sumer	147	186	236	469
	30 cm		96.5	176.5	312	453
T2: Sunflower pellets	15 cm		68	71	199	700
	30 cm		712	99	150	77
T3: SCM	15 cm		130	197	215	496
	30 cm		290.5	424.5	323	0
T4: Control	15 cm		703	144	131	60
	30 cm		641	351	44	0
T1: Wheat husk+ SCM	15 cm	Autumn	1008	3	0	0
	30 cm		1011	0	0	0
T2: Sunflower pellets	15 cm		1006	5	0	0
	30 cm		1011	0	0	0
T3: SCM	15 cm		1011	0	0	0
	30 cm		1011	0	0	0
T4: Control	15 cm		1011	0	0	0
	30 cm		1011	0	0	0

SCM: Semi-composted manure.

In relation with the oxygen concentration, all the treatments except for the control treatment managed to reach anoxia in both seasons. In summer, treatments T1 and T2 were the treatments that reached oxygen levels below 2% most rapidly (2 days), whilst in treatment T3 it took one day more (Figure 1). In autumn, all the treatments produced anoxia conditions from the second day (Figure 2).

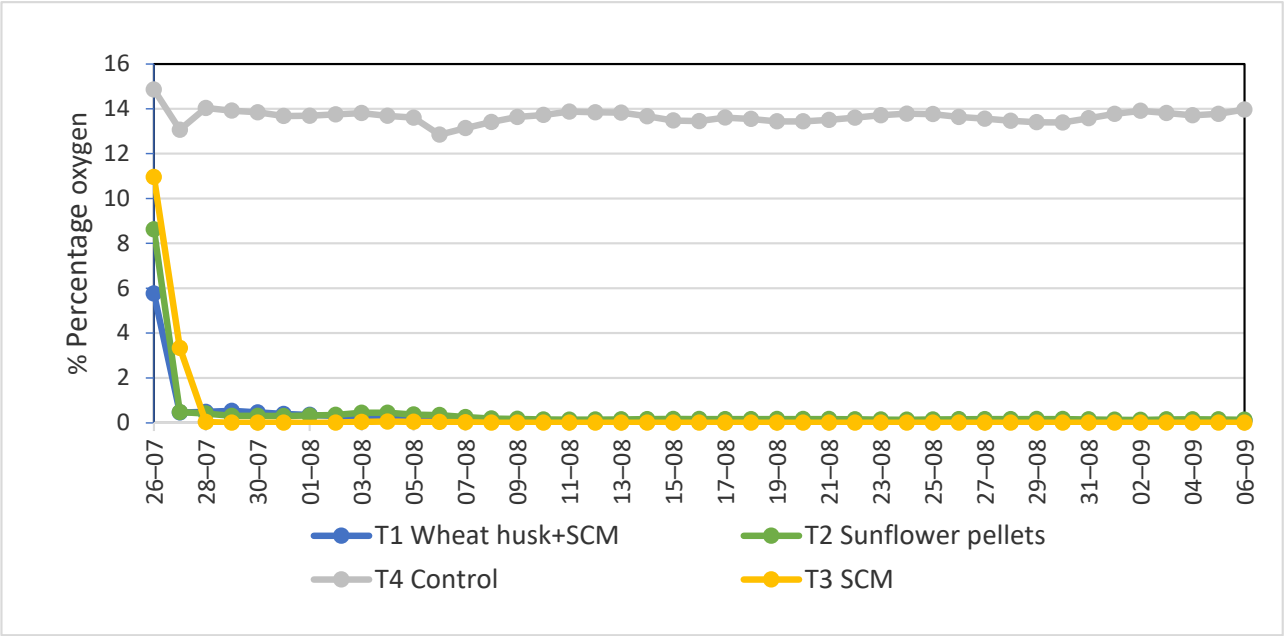

Figure 1. Soil oxygen percentages during the summer biosolarization season. SCM: Semi-composted manure.

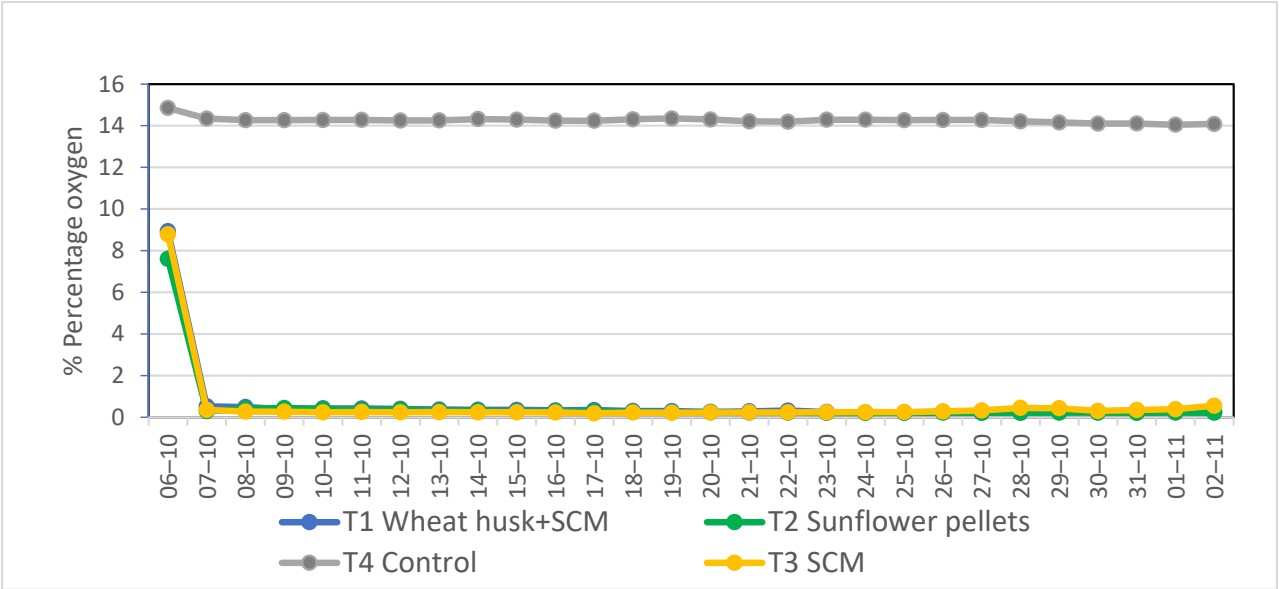

Figure 2. Soil oxygen percentages during the autumn biosolarization season. SCM: Semi-composted manure.

3.2. Infectivity Trials

In the summer season none of the plants established in the inoculated soils and subjected to biosolarization showed damage caused by the pathogen. Conversely, all the plants in the control treatment were affected by FOLAC (Table 2) ($F_{3,9} = 6.40$; $p = 0.016$).

For the autumn season, not all the biosolarization treatments managed to eliminate the presence of the pathogen, with infested lettuce plants being observed at both 15 cm ($F_{3,9} = 76$; $p \leq 0.001 < 0.000$), and at 30 cm ($F_{3,9} = 24$; $p \leq 0.001 < 0.000$).

Only treatments T1 and T2 showed no affected plants at the depth of 15 cm. With regard to the depth of 30 cm, only treatment T2 had no diseased plants, with the remaining

treatment having affected plants, in order of increasing incidence: T1 (10%), T3 (20%), and T4 (100%) (Table 2).

Table 2. Infectivity trial of FOLAC in Metalia (sensitive cultivar) lettuce plants on biosolarized soils.

Treatments	Percentage of Affected Plants			
Season	**Summer**		**Autumn**	
Depth	**0–15 cm**	**15–30 cm**	**0–15 cm**	**15–30 cm**
T1: Wheat husks + SCM	0 a	0 a	0 a	10 a
T2: Sunflower pellets	0 a	0 a	0 a	0 a
T3: SCM	0 a	0 a	10 a	20 a
T4: Control	40 b	40 b	100 b	100 b

Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$). SCM: Semi-composted manure.

3.3. Soil Pathogen Quantification by qPCR

The treatments were shown to be significantly different in the quantification of the pathogen in the soil for summer at 15 cm ($F_{3,5} = 3.91$; $p < 0.043$), summer at 30 cm ($F_{3,5} = 12.23$; $p \leq 0.001 < 0.000$), and autumn at 30 cm ($F_{3,5} = 3.33$; $p < 0.03$). No differences were found for autumn at 15 cm ($F_{3,5} = 1.59$; $p < 0.21$). The number of 21 sequences per gram of soil detected in the T1 treatment at 15 cm in autumn was insufficient to cause plant death (Table 3). The results of the qPCR-based molecular analyses showed that treatment T2 was the only one to be totally effective in eliminating FOLAC in both seasons (Table 3). Treatments T1 and T3 only proved effective in the summer, but they did not totally eliminate FOLAC in the autumn, although with differences. Treatment T1 presented a smaller amount of the pathogen at both soil depths, whilst T3 presented greater amounts of the pathogen at both depths (15 and 30 cm) in autumn.

Table 3. Presence of FOLAC pathogen in soil by season and treatment based on qPCR molecular analysis.

Treatments	N° Sequences/g Soil			
Season	**Summer**		**Autumn**	
Depth	**0–15 cm**	**15–30 cm**	**0–15 cm**	**15–30 cm**
T1: Wheat husk + SCM	0 a	0 a	22 ns	145 a
T2: Sunflower pellets	0 a	0 a	0	0 a
T3: SCM	0 a	0 a	1248	507 a
T4: Control	2349 b	1428 b	2255	4871 b

Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$). SCM: Semi-composted manure.

In any case, all the biosolarization treatments were, to a greater or lesser extent, effective at reducing the FOLAC levels. This was demonstrated when they were compared to the control treatment values (T4), in which the pathogen values increased in both seasons and in considerable amounts (Table 3).

4. Discussion

An especially relevant factor in the soil disinfestation process through biosolarization is to achieve soil anoxia conditions with the aim of achieving a reduction in pathogen populations [33–36]. From an environmental point of view, the biosolarization strategy with organic amendments is advantageous compared to soil disinfestation with chemically synthesized fumigants [37]. Davison and Tay [38] reflected that inoculum viability of *Phytophthora cinnamomi* is affected at oxygen levels below 2.5%, due to the diminished forming of sporangia and micellar development. In the present study, there were no noteworthy

differences in terms of oxygenation among biosolarization treatments, reaching favorable conditions (anoxia) for pathogen control during practically the whole biosolarization period. On the contrary, the control treatment exhibited soil oxygen levels close to 14% which could be related to the higher levels of FOLAC inoculum found in soil in both seasons.

On the contrary, the temperature differences measured in the soil during biosolarization would seem to have been the most important factor in the control of the FOLAC pathogen in the different seasons (summer and autumn). Thus, while in summer the number of hours above 42 °C was quite high, in autumn the temperatures barely exceeded 38 °C. Similar temperatures had been found during biosolarization treatments in summer in a previous study in the same area [21].

Lower temperatures, especially in autumn, limit the efficacy in the effectiveness of biosolarization. Butler et al. [39] and Rosskopf et al. [13,40] stated that the nature of the organic amendment is crucial for maximizing the disinfestation action of biosolarization. The application of amendments rich in labile organic carbon increases the biological activity of soil microorganisms since it can be readily degraded and at the same time promotes a slight additional increase in soil temperature through the exothermic process generated by microbial degradation [33]. Moreover, an adequate C/N ratio should be taken into account in order to ensure that the contributed nitrogen is not immobilized during amendment mineralization and in turn, avoid leaching of possible excess nitrates. The choice of amendments may therefore prove to be pivotal in effective biosolarization [41,42]. In our case, the amendment of sunflower pellets (T2) was clearly the most effective in increasing the temperature in summer (700 h above 42 °C between 0–15 cm) and was the only one that exceeded, albeit for scarcely five hours, 38 °C in autumn. On the other hand, at the depth of 15–30 cm, treatment T1 (Wheat husk + SCM) was the one that most notably obtained the highest temperature for a sustained period of time in summer (469 h above 42 °C). In autumn, no amendment exceeded 38 °C in the deepest part (15–30 cm).

This differential increment in the temperatures for the different treatments employed owing to the different natures of the organic matter used, had a key influence on the effectiveness of the biosolarization, an aspect corroborated by the data regarding infestation and pathogen determination by means of qPCR. No copies of FOLAC sequences were detected in the T2 treatment at any soil depths or any of the two seasons, which is consistent with the higher soil temperature data registered with this amendment. The high N content (4.8%) and the low C/N ratio (11.5) of the sunflower pellets (husked seed cake from oil extrusion), as occurs with other oilseed cakes, has been related to the biocidal effect of ammonia (basic pH soils) or nitrous acid (acidic pH soils) against different soilborne fungi [43,44] and phytopathogenic nematodes [31]. The sunflower pellets were also effective in reducing oospores viability of the phytopathogenic fungus *Phytophthora capsici* in biodisinfestation trials under sub-optimal temperature conditions in Southeastern Spain, and it was related with its ability to increase anoxia conditions during exposure to the biodisinfestation treatment [45]. In trials in pots and soil amended with compost made from olive oil mill waste, aubergine plants showed less prominent symptoms and slower disease development on account of *Verticillium dahliae* through biological control mechanisms [45,46]. Different studies, such as those by Etxeberria et al. [47] and Lacasa et al. [8] showed that above the threshold of 35 °C the presence of the pathogens *Phytophthora capsici* and *Phytophthora nicotianae* began to decrease considerably. Our study confirms that these temperatures also reduced the presence of FOLAC in soil, given the beneficial effects in all treatments compared to the untreated control T4. Despite not obtaining temperatures above 38 °C in autumn, it was not possible to detect inoculum in the biosolarization treatment with sunflower pellets at any soil depth, confirming previous results with other soil fungal pathogens during soil biosolarization [47,48].

Our results also highlight a higher resolution in the molecular technique and also enable us to determine an infectivity threshold. Thus, in treatment T1 (0–15 cm) in autumn, plants were not infected (Table 3), although values of 22 fungal sequences/g of soil were detected (Table 3). However, at values of 145 sequences/g of soil in the samples of this

same treatment and season, but at the depth of 15–30 cm, 10% of the plants became infected. Coelho et al. [14] had already pointed out the existence of thresholds to the resistance to infection by plants. The threshold observed to obtain lettuce plants affected by FOLAC in our infectivity bioassay was established at 145 fungal sequences per gram of soil. A number between 1428 and 4871 sequences per gram of soil generated a percentage of diseased plants between 40 and 100%. Thus, although there is presence of inoculum in the soil, a threshold is required to produce the death of plants.

Finally, it was observed that biosolarization efficacy decreased with depth, since a lower reduction in the presence of the pathogen was observed at 15–30 cm than at 0–15 cm. This result was explained by the effect of plastic that prevents evaporation of surface water, and retains gases generated during the biosolarization process [33,39].

5. Conclusions

Seasonality and adequate selection of the organic amendments used are essential to the effectiveness of biosolarization treatments as a technique for soil disinfestation. However, while in summer all the used treatments managed to eradicate the FOLAC soil inoculum, in autumn only the sunflower-based treatment managed to eliminate it. The success of this treatment was based in a greater soil temperature increase during the amendment's decomposition, as there were no differences with regard to the oxygen values (anoxia values were achieved in all the treatments throughout practically all the biosolarization period). Finally, the method of determining FOLAC soil inoculum using the qPCR-based molecular technique is useful to establish the relation between the number of sequences per gram of soil and diseased lettuce plants. Therefore, this molecular technique has been shown to be useful for establishing the soil inoculum thresholds required for crop infection by pathogens, while reducing the time and execution tasks necessary to perform soil infectivity bioassays. In view of the results obtained in this work, we recommend evaluating FOLAC soil inoculum density before lettuce crop planting to decide if it is necessary to carry out biosolarization treatments.

Author Contributions: Methodology, M.d.M.G. and J.A.L.-P.; Formal analysis, R.O., I.M. and S.L.; Investigation, M.d.M.G.; Resources, M.d.M.G., C.M.L. and V.M.; Data curation, M.d.M.G.; Writing—original draft, J.A.L.-P. and J.C.N.; Project administration, A.M. All authors have read and agreed to the published version of the manuscript.

Funding: FEDER1420-31: FEDER 2023–2027 Development of sustainable models of agricultural, livestock and aquaculture production project and MEDIBEES project (Grant number 2011) funded by PRIMA (Partnership for research and innovation in the Mediterranean area) programme supported by the European Union.

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

Abbreviations

FOLAC	*Fusarium oxysporum* f. sp. *lactucae*
SCM	Semi-composted manure

References

1. Gordon, T.R. *Fusarium oxysporum* and the *Fusarium* Wilt Syndrome. *Annu. Rev. Phytopathol.* **2017**, *55*, 23–39. [CrossRef]
2. Snyder, W.C.; Hansen, H.N. The species concept in *Fusarium*. *Am. J. Bot.* **1940**, *27*, 64–67. [CrossRef]
3. European and Mediterranean Plant Protection Organization. EPPO. 2023. Available online: https://gd.eppo.int/taxon/FUSALC (accessed on 1 July 2020).
4. Rana, A.; Sahgal, M.; Johri, B.N. Fusarium oxysporum: Genomics, Diversity and Plant–Host Interaction. In *Developments in Fungal Biology and Applied Mycology*; Satyanarayana, T., Deshmukh, S., Johri, B., Eds.; Springer: Singapore, 2017; pp. 159–199. [CrossRef]
5. Guerrero, M.M.; Martínez, M.C.; León, M.; Armengol, J.; Monserrat, A. First Report of Fusarium Wilt of Lettuce Caused by *Fusarium oxysporum* f. sp. *lactucae* Race 1 in Spain. *Plant Dis.* **2019**, *104*, 1858. [CrossRef]

6. Claerbout, J.; Van Poucke, K.; Mestdagh, H.; Delaere, I.; Vandevelde, I.; Venneman, S.; Decombel, A.; Bleyaert, P.; Neukermans, J.; Viaene, N.; et al. *Fusarium* isolates from Belgium causing wilt in lettuce show genetic and pathogenic diversity. *Plant Pathol.* **2023**, *72*, 593–609. [CrossRef]
7. Gálvez, L.; Brizuela, A.M.; Garcés, I.; Cainarca, J.S.; Palmero, D. First Report of *Fusarium oxysporum* f. sp. *lactucae* Race 4 Causing Lettuce Wilt in Spain. *Plant Dis.* 2023, *in press*. [CrossRef]
8. Garibaldi, A.; Gilardi, G.; Gullino, M.L. Seed transmission of *Fusarium oxysporum* f.sp. *lactucae*. *Phytoparasitica* **2004**, *32*, 61–65. [CrossRef]
9. Gilardi, G.; Vasileiadou, A.; Garibaldi, A.; Gullino, M.L. Low temperatures favour *Fusarium* wilt development by race 4 of *Fusarium oxysporum* f. sp. *lactucae*. *J. Plant. Pathol.* **2021**, *103*, 973–979. [CrossRef]
10. Gordon, T.R.; Koike, S.T. Management of *Fusarium* wilt of lettuce. *Crop Prot.* **2015**, *73*, 45–49. [CrossRef]
11. Al-Ani, L.K.T.; Furtado, E.L. The effect of incompatible plant pathogens on the host plant. In *Molecular Aspects of Plant Beneficial Microbes in Agriculture*; Sharma, V., Salwan, R., Al-Ani, L.K.T., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 47–57. [CrossRef]
12. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* **2019**, *3*, 430–439. [CrossRef]
13. Rosskopf, E.N.; Serrano-Pérez, P.; Hong, J.; Shrestha, U.; Rodríguez-Molina, M.D.C.; Martin, K.; Kokalis-Burelle, N.; Shennan, C.; Muramoto, J.; Butler, D. Anaerobic soil disinfestation and soilborne pest management. In *Organic Amendments and Soil Suppressiveness in Plant Disease Management*; Meghvansi, M., Varma, A., Eds.; Springer: Cham, Switzerland, 2015; pp. 277–305.
14. Attia, M.S.; Elsayed, S.M.; Abdelaziz, A.M.; Ali, M.M. Potential impacts of Ascophyllum nodosum, Arthrospira platensis extracts and calcium phosphite as therapeutic nutrients for enhancing immune response in pepper plant against Fusarium wilt disease. *Biomass Conv. Bioref.* **2023**. [CrossRef]
15. Attia, M.S.; Salem, M.S.; Abdelaziz, A.M. Endophytic fungi Aspergillus spp. reduce fusarial wilt disease severity, enhance growth, metabolism and stimulate the plant defense system in pepper plants. *Biomass Conv. Bioref.* **2022**. [CrossRef]
16. Katan, J. Solar heating (solarization) of soil for control of soilborne pests. *Ann. Rev. Phytopathol.* **1981**, *19*, 211–236. [CrossRef]
17. Stapleton, J.J. Soil solarization in various agricultural production systems. *Crop Prot.* **2000**, *19*, 837–841. [CrossRef]
18. Srivastava, J.N.; Ghatak, A. Biofumigation: A control method for the soil-borne diseases. *Int. J. Plant Prot. Phytopathol.* **2017**, *10*, 453–460. [CrossRef]
19. Coelho, L.; Mitchell, D.J.; Chellemi, D.O. Thermal inactivation of *Phytophthora nicotianae*. *Phytopathology* **2000**, *90*, 1089–1097. [CrossRef]
20. Domínguez, P.; Miranda, L.; Soria, C.; de los Santos, B.; Chamorro, M.; Romero, F.; Daugovish, O.; López-Aranda, J.M.; Medina, J.J. Soil biosolarization for sustainable strawberry production. *Agron. Sustain. Dev.* **2014**, *34*, 821–829. [CrossRef]
21. Guerrero, M.; Lacasa, C.M.; Martínez, V.; Martínez, M.C.; Larregla, S.; Lacasa, A. Soil biosolarization for *Verticillium dahliae* and *Rhizoctonia solani* control in artichoke crops in southeastern Spain. *Span. J. Agric.* **2019**, *17*, e1002. [CrossRef]
22. Martínez, M.A.; Martínez, M.C.; Bielza, P.; Tello, J.; Lacasa, A. Effect of biofumigation with manure amendments and repeated biosolarization on *Fusarium densities* in pepper crops. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 3–11. [CrossRef] [PubMed]
23. Cascone, G.; D'Emilio, A.; Polizzi, G.; Grillo, R. Effectiveness of greenhouse soil solarization with different plastic mulches in controlling corky root and rootknot on tomato plants. *Acta Hortic.* **2000**, *532*, 145–150. [CrossRef]
24. Chellemi, D.O.; Mirusso, J. Optimizing soil disinfestation procedures for fresh market tomato and pepper production. *Plant Dis.* **2006**, *90*, 668–674. [CrossRef]
25. Vitale, A.; Castello, I.; Cascone, G.; D'Emilio, A.; Mazzarella, R.; Polizzi, G. Reduction of corky root infections on greenhouse tomato crops by soil solarization in South Italy. *Plant Dis.* **2011**, *95*, 195–201. [CrossRef] [PubMed]
26. Klein, E.; Katan, J.; Gamliel, A. Soil suppressiveness to *Meloidogyne javanica* as induced by organic amendments and solarization in greenhouse crops. *Crop Prot.* **2012**, *39*, 26–32. [CrossRef]
27. Mauromicale, G.; Monaco, A.L.; Longo, A.M.G. Improved efficiency of soil solarization for growth and yield of greenhouse tomatoes. *Agron. Sustain. Dev.* **2010**, *30*, 753–761. [CrossRef]
28. Garibaldi, A.; Tamietti, G. Attempts to use soil solarization in closed glasshouses in northern Italy for controlling corky root of tomato. *Acta Hortic.* **1984**, *152*, 237–244. [CrossRef]
29. Oka, Y.; Shapira, N.; Fine, P. Control of root-knot nematodes in organic farming systems by organic amendments and soil solarization. *Crop Prot.* **2007**, *26*, 1556–1565. [CrossRef]
30. Ozores-Hampton, M.; McSorley, R.; Stansly, P.A. Effects of long-term organic amendments and soil sanitation on weed and nematode populations in pepper and watermelon crops in Florida. *Crop Prot.* **2012**, *41*, 106–112. [CrossRef]
31. Oka, Y. Mechanisms of nematode suppression by organic soil amendments—A review. *Appl. Soil Ecol.* **2010**, *44*, 101–115. [CrossRef]
32. OJ. Directive 91/676/EEC of the Council of the European Communities of December 12 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Off. J. Eur. Union L* **1991**, *375*, 1–8.
33. Gamliel, A.; Austerweil, M.; Kitzman, G. Non chemical approach to soilborne pest management with organic amendments. *Crop Prot.* **2000**, *19*, 847–853. [CrossRef]
34. Momma, N.; Kobara, Y.; Momma, M. Fe^{2+} and Mn^{2+}; potential agents to induce suppression of *Fusarium oxysporum* for biological soil disinfestation. *J. Gen. Plant Pathol.* **2011**, *77*, 331–335. [CrossRef]

35. Katase, M.; Kubo, C.; Ushio, S.; Ootsuka, E.; Takeuchi, T.; Mizukub, T. Nematicidal activity of volatile fatty acids generated from wheat bran in reductive soil disinfestation. *Jpn. J. Nematol.* **2009**, *39*, 53–62. [CrossRef]
36. Agtmaal, M.; Os, G.J.; Gera Hol, W.H.; Hundscheid, M.P.J.; Runia, W.T.; Hordijk, C.A.; De Boer, W. Legacy effects of anaerobic soil disinfestation on soil bacterial community composition and production of pathogen suppressing volatiles. *Front. Microbiol.* **2015**, *6*, 701. [CrossRef] [PubMed]
37. Blok, W.J.; Lamers, J.G.; Termorshuizen, J.; Bollen, G.J. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* **2000**, *90*, 253–259. [CrossRef] [PubMed]
38. Davison, E.M.; Tay, F.C.S. The effect of *cinnamomi* waterlogging on infection of *Eucalyptus marginata* seedlings by *Phytophthora*. *New Phytol.* **1987**, *105*, 585–594. [CrossRef]
39. Butler, D.M.; Kokalis-Burelle, N.; Albano, J.P.; McCollum, T.G.; Muramoto, J.; Shennan, C.; Rosskopf, E.N. Anaerobic soil disinfestation (ASD) combined with soil solarization as a methyl bromide alternative: Vegetable crop performance and soil nutrient dynamics. *Plant Soil* **2014**, *378*, 365–381. [CrossRef]
40. Rosskopf, E.; Di Gioia, F.; Hong, J.C.; Pisani, C.; Kokalis-Burelle, N. Organic amendments for pathogen and nematode control. *Annu. Rev. Phytopathol.* **2020**, *58*, 277–311. [CrossRef]
41. Gandariasbeitia, M.; Ojinaga, M.; Orbegozo, E.; Mendarte, S.; Larregla, S. Winter biodisinfestation with Brassica green manure is a promising management strategy for *Phytophthora capsici* control of protected pepper crops in humid temperate climate regions of northern Spain. *Span. J. Agric. Res. Arch.* **2019**, *17*, e1005. [CrossRef]
42. Ojinaga, M.; Gandariasbeitia, M.; Orbegozo, E.; Ortíz, A.; Guerrero, M.M.; Lacasa, C.M.; Arizmendi, J.; Otaño, A.; Camino, C.; Enbeita, G.; et al. Biodisinfestation for *Meloidogyne* and *Verticillium* control in commercial protected crops in the Basque Country Atlantic area (northern Spain). *Acta Hortic.* **2020**, *1270*, 327–336. [CrossRef]
43. Tsao, P.H.; Oster, J.J. Relation of Ammonia and Nitrous Acid to Suppression of *Phytophthora* in Soils Amended with Nitrogenous Organic Substances. *Phytopathology* **1981**, *171*, 53–59. [CrossRef]
44. Tenuta, M.; Lazarovits, G. Ammonia and Nitrous Acid from Nitrogenous Amendments Kill the Microsclerotia of *Verticillium dahliae*. *Phytopathology* **2002**, *92*, 255–264. [CrossRef]
45. Markakis, E.A.; Tjamos, S.E.; Chatzipavlidis, I.; Antoniou, P.P.; Paplomatas, E.J. Evaluation of compost amendments for control of vascular wilt diseases. *J. Phytopathol.* **2008**, *156*, 622–627. [CrossRef]
46. Guerrero, M.M.; Lacasa, C.M.; Martínez, V.; Martínez, M.C.; Monserrat, A.; Larregla, S. Low temperature biodisinfection effectiveness for *Phytophthora capsici* control of protected sweet pepper crops in the Southeast of Spain. *Front. Sustain. Food Syst.* **2021**, *5*, 659290. [CrossRef]
47. Etxeberria, A.; Mendarte, S.; Larregla, S. Thermal inactivation of *Phytophthora capsici* oospores. *Rev. Iberoam. Micol.* **2011**, *28*, 83–90. [CrossRef]
48. Lacasa, C.M.; Martínez, V.; Hernández, A.; Ros, C.; Lacasa, A.; Guerrero, M.M. Surival reduction of *Phytophthora capsici* oospores and *P. nicotianae* chlamydospores with Brassica green manures combined with solarization. *Sci. Hortic.* **2015**, *197*, 607–618. [CrossRef]