Spanish Journal of Agricultural Research
17 (1), e1002, 11 pages (2019)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2019171-13666
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Soil biosolarization for *Verticillium dahliae* and *Rhizoctonia solani* control in artichoke crops in southeastern Spain

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Abstract

The efficacy of soil biosolarization for the control of *Verticillium dahliae* and *Rhizoctonia solani* fungal pathogens was evaluated over two consecutive artichoke crop cycles in southeastern Spain. Soil biosolarization was applied in mid-June for 42 days. The evaluated soil treatments were: fresh sheep manure (FSM); beer bagasse (BB) plus FSM; broccoli crop residues plus FSM; and a control of non-disinfestated and non-amended soil. Different variables were analyzed: i) soil temperature during biosolarization; ii) soil inoculum density of *Verticillium* before and after biosolarization; iii) infectivity of *V. dahliae* and *R. solani* introduced inoculum after biosolarization treatments at 15 and 30 cm soil depth through bioassays; iv) crop disease incidence; and v) marketable yield. Treatments were randomized in a complete block design with four replicates. Biosolarization treatments reduced levels of both fungal pathogens in both years and had significant lower percentages of affected plants at the end of the crop. All biosolarization treatments significantly improved marketable yield 22-29% to 38-59% compared to the non-disinfestated control in 2015-2016 and 2016-2017 crop cycles respectively. Biosolarization with different organic amendments can be recommended as an effective management strategy for the control of soil-borne fungal diseases in artichoke crops in southeastern Spain, especially in repeated monocultures which are cultivated intensively.

Additional keywords: broccoli crop residues; beer bagasse; sheep manure; *Cynara cardunculus* var. *scolymus*.

Abbreviations used: BB (beer bagasse); BR (broccoli residues); BS (biosolarization); CFU (colony forming units); FSM (fresh sheep manure); OM (organic matter).

Authors' contributions: Conceived and designed the research: MMG and AL. Collected the data: CML, VM, MCML. Analysed the data: MMG. Drafted the manuscript: MMG, SL, AL. Revised the manuscript: SL.

Citation: Guerrero, M. M.; Lacasa, C. M.; Martínez, V.; Martínez-Lluch, M. C.; Larregla, S.; Lacasa, A. (2019). Soil biosolarization for *Verticillium dahliae* and *Rhizoctonia solani* control in artichoke crops in southeastern Spain. Spanish Journal of Agricultural Research, Volume 17, Issue 1, e1002. https://doi.org/10.5424/sjar/2019171-13666

Received: 04 Jul 2018. **Accepted:** 04 Mar 2019.

Funding: FEDER (Project 14-20-02); INIA-FEDER RTA (Project 2015-00060-C04-04).

Competing interests: The authors have declared that no competing interest exist.

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Introduction

Spain is one of the major producing countries of artichoke (*Cynara cardunculus* var. *scolymus*). In 2016, 16,001 ha were cultivated, with a total yield of 225,619 t. The country is also one of the largest exporters of flowers for fresh consumption or for use in conserves. Some 58.7% of the crops are in the Southeast of the country (provinces of Alicante and Murcia with 2,135 ha and 7,259 ha, respectively), with a production of 135.214 t (MAPAMA, 2017). In southeast Spain, the cropping is biannual or annual, depending on the incidence of viruses such as Tomato spotted wilt virus (TSWV) and crop yield (Lacasa *et al.*, 1996). It is planted in late August and early September; the harvest finishes between late April and mid-May. Most of the surface area is planted with vernalized stumps from other production zones (Lacasa *et al.*, 2016). There has been an increase in the surface area dedicated to growing seed plants (principally, hybrid cultivars) in recent years. The soils are clay loam, with a low nitrogen and organic matter (OM) content, average electrical conductivity and a pH of 7 to 8. The climate in the southeast of Spain is Mediterranean, with a maritime influence on the coast and a continental influence inland. The summers are hot, while the winters are mild; precipitations are scarce and are concentrated in autumn and winter. Artichoke crop is rotated with other horticultural crops such as broccoli, melon, lettuce or potato, with artichoke being grown in the soil again every two or three years. The crop intensity or crop rotation with others crops which are susceptible to the same soil pathogens (Ortega & Pérez, 2007), causes that such pathogens become a limiting factor of crop yield and emergence of phenomena of soil fatigue caused by the soil microbiological component. These phenomena reduce the soil fertility and crop yields in the same way that occurs in protected pepper crops grown in southeastern Spain (Martínez *et al.*, 2009; Guerrero *et al.*, 2014).

In Levante and the southeast of Spain, *Verticillium dahliae* and *Rhizoctonia solani* are the principal soil-borne pathogens, causing important production losses (Cebolla *et al.*, 2004; Armengol *et al.*, 2005). In the absence of a host plant, the most important pathogen in artichoke, *V. dahliae,* may persist as melanized microsclerotia in the soil for more than 10 years (Cirulli *et al.*, 1994; Pegg & Brady, 2002). Both of these pathogens are frequent and abundant in soils in Alicante and Murcia where artichoke is grown (Ortega & Pérez, 2007), and also in other crops such as melon or potato. Prospections carried out in the Region of Murcia between 2014 and 2017 found differences in the crops prevalence of both pathogens, depending on the production zones (Guerrero *et al.*, 2017). This prevalence appears to be related with the farming practices used, in particular with the type of irrigation, and with the environmental conditions.

The strategies to control both diseases are based on planting in uncontaminated soils, the use of stumps which are pathogen-free or the use of seed plants, in crop rotation programs with crops that are not susceptible to the two pathogens (Cirulli *et al.*, 1994). Soil chemical disinfestation in pre-planting using general disinfestants such as methyl isothiocyanate generators (metam sodium, metam potassium or dazomet) was satisfactorily indicated in Italy (Cicarese *et al.*, 1985), although it showed deficiencies in Levante, Spain (Cebolla *et al.*, 2003). The combination of metam sodium and broccoli residues did not improve *V. dahliae* control when the broccoli residues were used alone (Berbegal *et al.*, 2007a) nor with ovine manure (Cebolla *et al.*, 2003). The limitations in the use of methyl isothiocyanate general disinfectants (one application each three years in the same soil, Directive 2011/53/EU and Regulation (EU) nº 359/2012) condition the control strategy of both pathogens today. Pre-planting disinfestation with chloropicrin or with the mixture of chloropicrin and 1,3-dichloropropene is not authorised in Spain, although it has been shown to be effective (Cebolla *et al.*, 2003).

The approach of combining solarization and OM application is defined as biological solarization or biosolarization (Katan, 2005; Ros M *et al.*, 2008; Domínguez *et al.*, 2014). The effects of biosolarization on different soil-borne pathogens are associated with the action of several mechanism such as i) the solarization temperature (Katan *et al.*, 1976; Katan, 1981); ii) the effect of released gases from organic amendment biodecomposition (Kirkegaard *et al.*, 1993; Kirkegaard, 2014; Butler *et al.*, 2011, 2012); iii) the effect of anaerobiosis due to oxygen deficit in the tarped soil, mechanism that, according to the different countries, is denominated by several terms such as, biological soil disinfestation (Blok *et al.*, 2000; Goud *et al.*, 2004; Momma *et al.*, 2006; Messiha *et al.*, 2007), anaerobic soil disinfestation (Butler *et al.*, 2012; Rosskopf *et al.*, 2014; Serrano *et al.*, 2017), soil reductive sterilization (Yossen *et al.*, 2008) or reductive soil disinfestation (Katase *et al.*, 2009); and (iv) the amendment suppressive effect favouring microorganisms development which exert antagonistic effects on the pathogens (Gamliel & Stapleton, 1993; Mazzola *et al.*, 2001, 2007; Bonanomi *et al.*, 2007). The MBTOC (2007) denominated all these ways of disinfestation as biodisinfestation, since several actions concur when applying OM in any of these soil disinfestation techniques.

In the countries of the Mediterranean basin, solarization is presented as an effective disease management strategy for the control of *V. dahliae* and other soil-borne pathogens in numerous horticultural crops (Katan *et al.*, 1976, Katan, 1981; Cenis & Fusch, 1988; González *et al.*, 1993; Tamietti & Valentino, 2001), in orchard crops (López-Escudero *et al.*, 2003; Yolageldi *et al.*, 2012) and cotton (Melero *et al.*, 1995). Solarization has only been proven as a successful control strategy against *Sclerotinia* sp. and *R. solani* in Sicily and Tuscany (Triolo *et al.*, 1985, 1989; Cartia, 1987; Tamietti & Garibaldi, 1989). Solarization was effective in controlling *Verticillium* wilt of artichokes for three successive cropping seasons in Greece (Tjamos & Paplomatas, 1988). The incorporation of OM into the soil prior to the solarization (biosolarization/biodisinfestation) improves the efficacy of pathogen control in horticultural crops in greenhouse or in open field (Gamliel & Stapleton, 1993; Bonanomi *et al.*, 2007; Guerrero *et al.*, 2010; Núñez- Zofio *et al.*, 2011; Domínguez *et al.*, 2014). In Levante, Spain, solarization of cauliflower residues mixed with ovine manure was shown to be effective for *Verticillium* control in artichoke crops (Cebolla *et al.*, 2003, 2004; Berbegal *et al.*, 2007a, 2008) through soil inoculum reduction and significantly diminished the percentage of plants displaying symptoms and which were infested, and increased crop yield.

In protected pepper crops located in southeastern Spain, biosolarization improves the efficiency of solarization in the control of soil-borne pathogens such as *Meloidogyne incognita* (Ros C *et al.*, 2008) and reduces the effect of soil fatigue in the absence of the main pathogens (*Phytophothora capsici, P. parasitica* and *M. incognita*) which resulted in a crop yield increase (Martínez *et al.*, 2009; Guerrero *et al.*, 2014).

Given the difficulties in using chemical disinfestants to reduce the inoculum of *Verticillium* and *Rhizoctonia* in soil dedicated to growing artichoke and the possibility of using new local organic amendments, there is a need to evaluate the efficacy of new organic amendments for the control of *R. solani* and *V. dahliae*, the main soil-borne pathogens of artichoke crops in southeastern Spain. The aim of this work was to assess the effects of biosolarization using organic residues from local agri-food industries as organic amendments for the control of *V. dahliae* and *R. solani* in artichoke crops.

Material and methods

Plot establishment

Biosolarization trials were carried out during two crops seasons, 2015-2016 and 2016-2017, in two field plots naturally infested with *V. dahliae* and *R. solani* located in the coastal strip of the south of Alicante province where artichoke is repeatedly grown. The soil is clay loam, OM (%) 1.3 to 1.8, organic carbon (%) 0.8 to 1.08, total N (%) 0.08 to 0.1, C/N 7.8 to 10.6; phosphorous (ppm) 7.4 to 7.8, pH 7.4 to 7.8 and EC (dS m^{-1}) 3.4 to 6.3.

The treatments evaluated were: 1) biosolarization (BS) with 4 kg m^{-2} fresh sheep manure (FSM); 2) BS with 2 kg m^{-2} FSM plus 2 kg m^{-2} beer bagasse (BB); 3) BS with 2 kg m^{-2} FSM plus 2 kg m^{-2} broccoli residues (BR); and 4) non-solarized and non-amended soil (untreated control treatment). The FSM and FSM+BB amendments for the two years came from the company 'Abonos Orgánicos Pedrín' located in Abarán (Murcia). In order to know the uniformity of the amendments, the two years were analyzed (Table 1). BR came from broccoli crops grown in neighboring field plots.

The onset of biosolarization was in mid-June both years. Treatments were arranged in a randomized complete block design with four replicates and were repeated in the same plots in each of the two years of the trial. Each experimental unit consisted of a plot of 850 m^2. Organic amendments were added and incorporated into the soil at 25-30 cm depth using a rototiller. The amended plots were subsequently irrigated by a drip irrigation system using 3 L h^{-1} emitters spaced 0.40 × 0.60 m for 4 h the first day and 4 h the second day. The soil was covered with a transparent polyethylene 0.05 mm thick plastic film for a period of six weeks. Artichoke stumps of 'Blanca de Tudela' cultivar were planted in the third week of August, spaced 1.5 × 0.80 m. The crop season ended in May.

Variables measured

The following variables were measured:

— Soil temperature at 15 and 30 cm depth was monitored in a plot of each treatment with thermistor probes attached to a H8-4 32K Hobo datalogger.

— Soil inoculum density of *Verticillium* before and after biosolarization. Prior to the BS treatment, five soil samples at 0-25 cm depth were taken uniformly distributed along the experiment field on 13th June 2015 and on 7th June 2016. After the BS treatment, soil samples from five points evenly distributed were taken and mixed to constitute a composite sample per each treatment and replicate plot at 0-25 cm depth on 18th August 2015 and on 22nd August 2016. All the samples were processed using the wet sieving method described by López-Escudero *et al.* (2003), with the soil passing through a 0.08 mm sieve. Sub-samples of 25 grams were suspended in 100 ml of sterile distilled water. The suspension was shaken for one hour at 270 rpm, and it was then passed through 150 and 36 µm sieves placed in tandem. The residue that remained in the 36 µm sieve was collected in 100 mL of distilled water. Aliquots of 1 mL from this solution were placed on plates with Sorensen NP10 culture medium, semi-selective for *Verticillium* (Sorensen *et al.*, 1991) Ten sub-samples were taken from each sample. The plates were incubated for 25-30 days at 22 ºC in dark conditions. The plates were then washed with sterile distilled water to

Table 1. Organic amendments composition. Values are the mean of two replicates corresponding to the two years.

	FSM	FSM+ BB
Total OM, %	54.82 ± 4.62	69.87 ± 2.14
Total N, %	1.71 ± 0.12	0.89 ± 0.11
C/N	18.64 ± 1.49	13.23 ± 1.10
P_2O_5, %	0.90 ± 0.05	0.99 ± 0.08
K_2O, %	4.28 ± 0.13	2.26 ± 0.21
Na, %	0.26 ± 0.15	0.16 ± 0.04
pH	8.31 ± 0.07	7.17 ± 0.26
Conductivity (25ºC), dS m^{-1}	8.70 ± 0.47	8.56 ± 0.88
Humidity, %	47.26 ± 1.03	48.12 ± 2.18

OM=organic matter. FSM=fresh sheep manure. BB=beer bagasse.

eliminate soil particles and the number of *Verticillium* colonies were counted. The *Rhizoctonia* inoculum density was not assessed since no sufficiently reliable soil analysis method was available.

— Infectivity of *V. dahliae* and *R. solani* soil buried inoculum after biosolarization period.

● Inoculum production: The cultures of *V. dahliae* and *R. solani* used in this study were originally isolated from artichoke stumps presenting symptoms in commercial crops in one of the experimental stations in February 2015. Both isolates were kept in PDA culture medium in the collection of IMIDA (Murcia, Spain), being considered as aggressive in previous bioassays performed in controlled conditions, in both artichoke plants as well as in melon and aubergine. The *Verticillium* inoculum was obtained following the indications given by Tenuta & Lazarovits (2002). The isolate was grown in PDA medium for three weeks in the dark at 24ºC. The culture was crushed in sterile distilled water (one plate of 9 mm-diameter per 100 mL) and filtered through a 75 µm sieve to obtain microsclerotia of a smaller size. The microsclerotia were stored at 24°C in the dark prior to use. The *Rhizoctonia* isolate was grown in solid PDA medium until it covered the 9 mm-diameter Petri dish. The contents of one dish were crushed in 100 mL of sterile distilled water. A myceliar suspension containing 13,000 CFU mL^{-1} was obtained.

● Assay procedures: *Rhizoctonia* (1 mL containing 13,000 CFU mL^{-1}) and *Verticillium* (1 mL with 350 microsclerotia mL^{-1}) inocula were added to 100 mL of moist autoclaved soil from every replicate plot per treatment and then wrapped in muslin to form a small bag for each pathogen, which were buried at 15 and 30 cm soil depth in three points in each of the three replicate plots per treatment. Previous to the pathogen inoculation, the soil was autoclaved at 120ºC for one hour on two consecutive days. At the end of biosolarization treatment, bags of inoculated soil were removed and placed into pots (150 mL), where one artichoke plant cv. 'Lorca', susceptible to both fungal pathogens, was transplanted with four true leaves. Potted plants (3 points per each replicate plot and 3 replicate plots per treatment in each depth) were grown at 25ºC, relative humidity 60-70% with 14:10 h light:dark photoperiod in a growth chambers for 12 weeks. Once per week, symptoms (yellowing, wilting and/or death) were registered for every single plant; those plants which presented symptoms were analyzed in PDA. The roots and stem were washed with water; the tissue was cut into 1 cm sections with a sterile scalpel and placed on Petri dishes with PDA and incubated at 25ºC. After 4-6 days of incubation, the isolated fungi were identified by microscopic observation of their morphological characteristics.

— Crop disease incidence. The disease incidence was registered during the growing season every 15 days in each replicate plot (50 plants per replicate plot and four replicate plots per treatment) and those that presented symptoms of either of the two fungi were analyzed in PDA medium. When plants were dead, root tissues were thoroughly washed with tap water and then placed on Petri dishes with PDA medium and incubated at 25ºC. After 4-6 days the isolated fungi were identified by microscopic observation of their morphological characteristics.

— Marketable yield. Artichoke flowers were harvested throughout the crop season in 50 plants of each replicate treatment plot, three times a month from November to April. The flowers were classified according to the official commercial criteria of the cooperatives in the area. Calibers from 140-160 g flower^{-1} to more than 250 g flower^{-1} are common in the internal market. The yield was weighed at each time of harvest in each replicate plot and expressed as kg m^{-2}.

Data analysis

The effect of treatments was studied using analysis of variance (ANOVA). Means of significant treatments were separated by Fisher´s LSD test (p<0.05). In order to fulfill the assumptions of analysis of variance (homocedasticity and normality), data were transformed using the following transformations:

Data on *Verticillium* inoculum (CFU g^{-1} dried soil) in soil before and after biosolarization were transformed using arcsine ($\sqrt{x + 0.5}$), where x = number of CFU per gram of dried soil of *Verticillium* inoculum. Data on infectivity bioassay and disease incidence were transformed using arcsine ($\sqrt{x/n}$), where x = number of dead plants and n = total number of plants. Data on crop yield were transformed using log transformation log (x+1), where x=total yield.

Results

Soil temperatures

Major differences were found between years and also among treatments within the same year, and between depths and the number of cumulative hours at temperatures over 38ºC, 40ºC and 42ºC (Table 2). The temperature did not exceed 45ºC in any of the treatments.

In 2015-2016, the number of cumulative hours at temperatures above 38ºC, 40ºC and 42ºC was similar

Table 2. Number of cumulative hours in each crop cycle at 15 and 30 cm soil depth within different temperature ranges. Duration of biosolarization was 6 weeks. Temperatures were recorded hourly by a datalogger located in each treatment.

Treatment[1]	Crop cycle	Depth (cm)	>38ºC	>40ºC	>42ºC
FSM	2015-2016	15	633.0	416.5	206.0
		30	-*	-*	-*
	2016-2017	15	70.5	57.0	15.0
		30	36.0	29.0	20.5
FSM+BB	2015-2016	15	610.5	406.0	212.0
		30	449.5	134.5	0.0
	2016-2017	15	70.5	0.0	0.0
		30	0.0	0.0	0.0
FSM+BR	2015-2016	15	309.0	104.5	9.5
		30	161.5	0.0	0.0
	2016-2017	15	0.0	0.0	0.0
		30	0.0	0.0	0.0
Control	2015-2016	15	0.0	0.0	0.0
		30	0.0	0.0	0.0
	2016-2017	15	0.0	0.0	0.0
		30	0.0	0.0	0.0

[1]FSM=fresh sheep manure. BB=beer bagasse. BR=broccoli residues. *FSM 30 cm 2015-2016: no data.

in FSM and in FSM+BB at 15cm and almost double the number in FSM+BR. At 30 cm, neither 42ºC nor 40ºC were exceeded in FSM+BR. The temperature in the non-solarized and non-amended control did not exceed 38ºC at 15cm nor at 30 cm. In 2016-2017, only FSM exceeded 40ºC at both 15 cm and 30 cm. In FSM+BB the temperature of 38ºC was only exceeded at 15 cm. In FSM+BR and in the non-solarized and non-amended control, 38ºC was not exceeded at any depth.

Verticillium dahliae inoculum survival in natural field soils before and after biosolarization

The biosolarization with the three amendments significantly reduced the soil inoculum density when compared to the non-disinfestated control in both years (Table 3) ($F_{3,119}$=3.86; p=0.0172 in 2015-2016; $F_{3,119}$=1.0; p=0.004 in 2016-2017). No differences were found among the amendments in any of the two years; with a total soil inoculum reduction after biosolarization.

The inoculum in the non-disinfestated control decreased during the biosolarization period in the first year, but not in the second year. The variation in the amount of inoculum in samples from the same field was high, due to a non-homogeneous distribution of the natural inoculum. In the second year, the level of inoculum in both field was lower than in the first year.

Infectivity of introduced soil inoculum of *Verticillium* and *Rhizoctonia*

Inoculum of both pathogens was only introduced into the soil prior to the disinfestation in 2016-2017. The biosolarization with the three amendments significantly reduced the survival and infective capacity of *Verticillium* inoculum buried at both soil depths (p<0.0001 at 15 cm; p<0.0001 at 30 cm), in comparison with the non- disinfestated control (Table 4).

The three amendments significantly reduced the survival and infective capacity of *Rhizoctonia* inoculum buried at both soil depths ($F_{3,11}$=5.53; p=0.0036 at 15 cm; $F_{3,11}$=8.59; p=0.0032 at 30 cm), in comparison with the non- disinfestated control. No differences were found among amendments in the inoculum survival of the two pathogens at both soil depths. Total disinfectant efficacy was found for the *Verticillium* inoculum at both soil depths and for *Rhizoctonia* it was only total in FSM also at both soil depths. No differences were found between depths in inoculum survival for both pathogens in the non- disinfestated control.

Table 3. *Verticillium* inoculum (CFU g^{-1} dried soil) in soil before and after the biosolarization treatment.

Treatment[1]	2015-2016		2016-2017	
	Before BS[2]	After BS[3]	Before BS[2]	After BS[3]
FSM		0.0±0b		0.0±0b
FSM+BB		0.0±0b		0.0±0b
FSM+BR		0.0±0b		0.0±0b
Control	2.0±0.79	1.2±0.6a	0.13±0.45	0.13±0.05a

[1]BS=biosolarization. FSM=fresh sheep manure. BB=beer bagasse. BR=broccoli residues. [2]Before BS. Mean values (n=50) ± standard deviation. [3]After BS. Mean values (n=40) ± standard deviation. Data were transformed using arcsine $(\sqrt{x+0.5})$, where *x*=number of CFU g^{-1} dried soil of *Verticillium* inoculum before and after biosolarization. Treatments were arranged in randomized complete block design with four replicates per treatment. Values with the same letter in each column are not significantly different based on Fisher´s LSD test (p<0.05).

The disinfectant efficacy on the artificial inoculum buried in the soil prior to disinfestation is related with that found for the natural *Verticillium* inoculum (Table 3).

Crop disease incidence

The three biosolarization treatments significantly ($F_{3,15}$=4.85; p=0.0049 in 2015-2016; $F_{3,15}$=1.98; p=0.0017 in 2016-2017) reduced crop incidence of *Verticillium* in the two years when compared to the non- disinfestated control (Table 5), with no differences found among biosolarization amendments. Plants presenting symptoms (reduced development, yellowing and wilting on exterior leaves, darkened vessels) and infestation with *Verticillium* were only found in FSM+BB in 2016-2017. The *Verticillium* incidence in the non- disinfestated control was similar in both crop cycles: *Verticillium* wilt affected more than one third of the plants.

Bisolarization with FSM+BR significantly ($F_{3, 15}$=1.67; p=0.0042) reduced *Rhizoctonia* incidence in 2015-2016 when compared to the non- disinfestated control but no differences were found among the amendments (Table 5). In 2016-2017, the biosolarization did not reduce the *Rhizoctonia* incidence ($F_{3,15}$=0.69; p=0.5679 in 2016-2017). In 2015-2016, the *Rhizoctonia* incidence in the non- disinfestated control was double than that in the 2016-2017 crop cycle.

Marketable yield

Marketable crop yield in biosolarized soils was significantly higher than in the non- disinfestated control treatment in both crop cycles ($F_{3,15}$=7.99; p=0.0034 in 2015-2016; $F_{3,15}$=8.13; p=0.0032 in 2016-2017). No differences were found among amendments (Table 6). In the 2015-2016 crop cycle, the increase in the marketable yield as compared to the non- disinfestated control varied from 121% for FSM+BB to 129% for FSM+BR. In 2016-2017 the yield increases with respect to the non- disinfestated control were greater, varying from 138% for FSM to 159% for FSM+BB. The harvest in the non- disinfestated control fell by 31% in 2016-2017 with respect to that of 2015-2016. The incidence of the soil-borne pathogens would explain part of the increased yield in the biosolarization treatments, and soil fatigue would account for the reduction in yield in 2016-2017 with respect to 2015-2016.

Discussion

The inoculum density and the incidence of *Verticillium* wilt in the artichoke crop were significantly reduced by biosolarization and, an increase in marketable yield was achieved for two consecutive years. Solarization alone or with organic amendments (biosolarization) is considered as an effective way of controlling soil-borne pathogens in Mediterranean climates, both for greenhouse crops (González *et al.*, 1993; Guerrero *et al.*, 2010, 2014), as well as in open field (Tjamos & Paplomatas, 1988). Thermal increases in the soil during the disinfestation process have been shown to be particularly effective in controlling *Verticillium*, by drastically and sustainably reducing the inoculum (Pullman *et al.*, 1981a; Melero *et al.*, 1995; Pikerton *et al.*, 2000; Tamietti & Valentino, 2001; Goud *et al.*, 2004; Berbegal *et al.*, 2007a; Yolageldi *et al.*, 2012), directly or by the effects of temperature on the OM biodecomposition or the antagonist microbiota (Katan *et al.*, 1976; Tjamos & Paplomatas, 1988).

Table 4. Infectivity of introduced soil inoculum of *V. dahliae* and *R. solani* at 15 and 30 cm depth in bioassays with artichoke plants expressed as percentage of dead plants during biosolarization treatments in 2016-2017.

Treatments[1]	*V. dahliae*		*R. solani*	
	15 cm	30 cm	15 cm	30 cm
FSM	0.0±0b	0.0b	0.0±0b	0.0±0b
FSM+BB	0.0±0b	0.0b	22.2±0.10b	11.1±0.30b
FSM+BR	0.0±0b	0.0b	11.1±0.30b	33.3±0.24b
Control	100±0a	100±0a	55.5±0.28a	77.7±0.25a

[1]FSM=fresh sheep manure. BB=beer bagasse. BR=broccoli residues. Mean values (n=3) ± standard deviation. Values with the same letter in each column are not significantly different based on Fisher´s LSD test (p<0.05). Data were transformed using arcsine $(\sqrt{x/n})$, where *x*=number of diseased plants and *n*=total number of plants.

Table 5. Final percentage of plants infected by *V. dahliae* and by *R. solani* in each crop cycle.

Treatments[1]	2015-2016			2016-2017		
	Nº analyzed plants	*V. dahliae* plants %	*R. solani* plants %	Nº analyzed plants	*V. dahliae* plants %	*R. solani* plants %
FSM	11	0.0±0.0b	29.0±17.13ab	22	0.0±0.00b	20.3±11.23ns
FSM+BB	13	0.0±0.0b	29.1±14.23ab	17	4.15±4.16b	12.5±12.50
FSM+BR	18	0.0±0.0b	10.0±5.77 b	17	0.0±0.0b	29.15±17.16
Control	11	35.5±9.69a	54.3±20.40a	15	34.72±20.83a	24.15±19.02

[1]FSM=fresh sheep manure. BB=beer bagasse. BR=broccoli residues. Mean values (n=4) ± standard deviation. Values with the same letter in each column are not significantly different based on Fisher´s LSD test (p<0.05). ns: values are not significantly different. % Data were transformed using arcsine ($\sqrt{x/n}$), where x=number of dead plants and n=total number of plants.

The critical threshold to eliminate 90% of *V. dahliae* microsclerotia inoculum on artificial media at constant temperatures of 38, 40, 42, 45ºC required exposures times of 324, 275, 97 and 21 cumulative hours respectively (Tamietti & Valentino, 2001). In the first year of our study, although exposure time (310 h) in the FSM+BR biosolarization treatment was slightly below the mentioned critical threshold (324 h) at 38ºC, the treatment efficacy in inoculum inactivation could be related to the fact that *V. dahliae* microsclerotia on artificial media are more resistant to heat than those in natural solarized field soil (Pullman *et al.*, 1981a). According to Pullman *et al.* (1981a), thermal inactivation of *V. dahliae* microsclerotia required 14 days (336 h) at 37ºC of constant temperature in moist soil and 28 days at 35-37ºC of fluctuating temperature in solarized field soils in California. The thermal regime in the biosolarized soils of our first-year trial (2015-2016) exceeded that described as effective to inactivate *V. dahliae* and *R. solani* in solarized field soils in California. Although the number of cumulative hours above the thermal level that is considered to be lethal for the *Verticillium* inoculum was only reached at 15 cm in FSM and FSM+BB and at 30 cm in FSM+BB in the first cycle (2015-2016) of our experiment, Pikerton *et al.* (2000) indicated that the sublethal action of temperatures lower than the threshold considered to be lethal, or the fumigant effects of the amendments, could explain the drastic soil inoculum reduction after biosolarization in year 2015.

Table 6. Marketable yield (kg m^{-2}) in each crop cycle.

Treatments	2015-2016	2016-2017
FSM	2.45±0.14a	1.86±0.10a
FSM+BB	2.39±0.07a	2.15±0.06a
FSM+BR	2.52±0.07a	2.06±0.09a
Control	1.96±0.06b	1.35±0.29b

FSM=fresh sheep manure. BB=beer bagasse. BR=broccoli residues. Mean values (n=4). Values with the same letter in each column are not significantly different based on Fisher´s LSD test (p<0.05). Data were transformed using log (x+1) where x=total yield.

In the second crop cycle (2016-17), conversely, biosolarization treatments showed a much lower number of cumulative hours and were below the reference threshold values for an effective control of *V. dahliae* microsclerotia. Temperatures exceeding 38°C were not achieved in the non- disinfestated control in either of the two years of biosolarization (2015 and 2016).

The effectiveness of biosolarization treatments in which thermal inactivation thresholds were not reached (FSM+BR in 2015-16; FSM, FSM+BB, FSM+BR in 2016-17), could be explained by the synergistic effect of other factors such as the increase and retention of volatile toxic compounds in the amended tarped soil, the anaerobic soil conditions and the increase in biological activity (Gamliel, 2000; Stapleton, 2000; Blok *et al.*, 2000) and the effect of sub-lethal temperatures (Davis & Sorensen, 1986; Tjamos & Paplomatas, 1988).

Previous works have shown that both *Verticillium* and *Rhizoctonia* differ in their sensitivity to heat. Inoculum population density of *R. solani* declined faster than *V. dahliae* in solarized soils (Pullman *et al.*, 1981b). In controlled laboratory conditions, *Rhizoctonia* was the first fungus to lose viability at constant temperatures above 39ºC. At 42ºC of constant temperature, the exposure time required to kill mycelia in agar medium was 33 h for *R. solani* and 44 h for *V. dahliae*, although *R. solani* showed a shorter recovery time than *V. dahliae* for restarting mycelial growth after exposure at high temperatures of 37-50ºC.

Biosolarization treatments significantly reduced soil inoculum density of *V. dahliae* in soil and inoculum infectivity of both fungal pathogens compared to non-disinfestated soil in both years and presented significantly lower percentages of affected plants at the end of the crop in the case of *V. dahliae*. On the contrary, final crop percentage of *R. solani* infected plants in biosolarization treatments were not significantly different from the non-disinfestated control in both years, except the FSM+BR biosolarization treatment in the first year. The *Rhizoctonia* incidence in 2016-2017 (Table 5) did not appear to be directly related to the survival of the inoculum buried in the soil prior to disinfestation (Table 4); the reduction in the inoculum's survival with respect to the non-disinfestated control varied between 55.5% and 33.3% at the depth of 15 cm, and between 77.7% and 44.4% at 30 cm. Despite the reduction of soil inoculum infectivity of *R. solani* and *V. dahliae* after biosolarization, as estimated by the infectivity bioassays (Table 4), final crop incidence by *R. solani* was considerably higher than incidence by *V. dahliae* (Table 5). A possible explanation of the high final incidence of *R. solani* in the crop could be its greater saprophytic ability (Bonanomi *et al.*, 2007), which would favour its growth with the use of easily assimilable nutrients generated after amendment decomposition in the biosolarized soil treatments. The estimated inoculum density in the non-disinfestated soil for the two years of the present study (1.2 to 0.13 microsclerotia g^{-1} of soil) was low in comparison with the level found in artichoke crops in Levante, Spain (5 to 9 microsclerotia g^{-1} of soil) by Berbegal *et al.* (2007b); the incidence of *Verticillium* wilt (35.5% in 2015-2016 and 34.7% in 2016-2017) was also lower than in the trials carried out in Levante (almost 91%). However, the reduction in yield in the control with respect to the mean of biosolarization treatments was similar (30.1% in the Levante trial; 20.0% in 2015-2016 and 32.5% in 2016-2017 in our trials).

Although plants infection by *Rhizoctonia* was lower in the second season than in the first one (with the exception of treatment FSM + BR), the crop marketable yield was lower in the second season than in the first one in all the treatments, with a more pronounced decrease in the non-disinfestated control than in the biosolarization treatments. This fact could be explained by soil fatigue caused by repeated monoculture as described in other pathosystems (Zydlik & Pacholak, 2008; Guerrero *et al.*, 2014). All biosolarization treatments significantly improved the marketable yield by 22-29% to 38-59% compared to the non-disinfestated control in the 2015-2016 and 2016-2017 crop cycles, respectively. The reduction of marketable yield in the second crop cycle compared to the first crop cycle was 18% for the average of biosolarization treatments and 31% for the non-disinfestated control treatment, although the *Verticillium* incidence was similar in both years (Table 5) and that for *Rhizoctonia* did not increase in the second year. We consider such differences to be related to the effect of soil fatigue due to reiterated monocropping, as occurs in other intensive crops (Katán, 2005; Martínez *et al.*, 2011; Guerrero *et al.*, 2014).

In one solarization trial using crop cauliflower residues and these with the addition of metam sodium, Berbegal *et al.* (2007a) obtained similar yields per plant when comparing cauliflower residues, solarization alone and solarization combined with cauliflower residues. In contrast, crop disease incidence by *Verticillium* was 80% in the cauliflower residues treatment, whilst in the treatments of solarization alone or solarization combined with the cauliflower residues, disease incidences were 30% and 38%, respectively.

In addition to the effect of biosolarization on the pathogens it would also be necessary to assess the effect that the organic matter and its decomposition at high temperatures has on the soil's physical and chemical characteristics. Our trials have shown that biosolarization is an effective mechanism, continued over time, to control artichoke soil-borne pathogens. In southeastern Spain, a zone where vegetables like artichoke are cultivated intensively, especially in repeated monocultures, biosolarization with different organic amendments can be recommended as an effective management strategy for the control of soil-borne fungal diseases and the improvement of soil fertility and crop yield.

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