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Control Fusarium wilt with non-chemical methods

Effect of simulated soil solarization and organic amendments on Fusarium wilt of rocket and basil under controlled conditions

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Abstract

Four plot trials were carried out under controlled conditions in order to evaluate the effectiveness against Fusarium wilt of rocket (*Fusarium oxysporum* f. sp. *conglutinans*) and basil (*F. oxysporum* f. sp. *basilici*), of soil amendments based on a patented formulation of *Brassica carinata* defatted seed meal and compost, combined or not with a simulation of soil solarization. The soil solarization treatment was carried out in a growth chamber by heating the soil for 7 and 14 days at optimal (55 to 52°C for 6 h, 50 to 48°C for 8 h and 47 to45°C for 10 h/day) and sub-optimal (50 to 48°C for 6 h, 45 to 43°C for 8 h and 40 to 38°C for 10 h/day) temperatures similar to those observed in summer in solarized soil in greenhouses in Northern Italy. Two subsequent cycles of cultivation were carried out in the same soil. Even at sub-optimal temperature regimes, 7 days of thermal treatment provided very interesting results in terms of disease control on both rocket and basil. In general, the thermal treatment was more effective against *F. oxysporum* f. sp. *basilici* than against *F. oxysporum* f. sp. *conglutinans*. Control of Fusarium wilt of rocket is improved with 14 days of thermal treatment. The combination of organic amendments with a short period of soil solarization (7 or 14 days), although not providing any improvement to the level of disease management, permits a significant increase in the biomass, with a positive effect on yield.

Key words: Thermal treatment; biofumigation; compost; *Fusarium oxysporum* f. sp. *conglutinans*; *F. oxysporum* f. sp. *basilici*; integrated disease management

Introduction

Soil solarization is a non-chemical method of soil disinfestation leading to pathogen control either directly, through physico-thermal action, or indirectly by stimulating antagonists or by weakening the pathogen’s resting structures present in the soil, with a consequent exposure to the activity of the microorganisms (Gamliel and Katan 2012). Incorporation of organic amendments in to soil in order to control soilborne pathogens has been widely studied and exploited, since it represents a low cost and ecologically sound method (Gamliel and Stapleton 1997). Organic amendments, such as compost and crop residues, have shown great potential in controlling soil-borne pathogens (Bonanomi et al. 2007; 2010; Noble and Coventry 2005; Pane et al. 2011; Hadar and Papadopoulos 2012). Brassica crops incorporated as green manures have the ability to contain multiple soil-borne problems (Mazzola et al. 2007; Larkin and Griffin 2007; Lazzeri et al. 2009; Motisi et al. 2009) either alone or when combined with other disinfestation methods such as soil solarization (Gamliel and Stapleton 1997). Disease suppression by organic amendments could be related to specific microorganisms involved in predation, parasitism, and competition (Waller et al. 2002), or correlated with the metabolic activity of some groups of microorganisms (Hoitink and

Boehm 1999), as well as to the microbial population of the soil that can be affected differently (Termorshuizen and Jeger 2008).

Indeed, due to the fact that soil solarization is a climate-dependant process, under favorable environmental conditions in warm climates it must last at least 4 weeks, while under less favorable weather conditions and during years with predominantly overcast summers, the duration should be increased to 6 weeks (Katan and De Vay 1991; Gamliel and Katan 2012). In the Mediterranean area the application of soil solarization requires a soil covering period of 4 to 6 weeks, which is sometimes not very compatible with intensive agricultural systems (Granados et al. 2010; Garibaldi and Gullino 1991; Gullino and Garibaldi 2012). To make soil solarization a more widely adopted method, it is imperative to reduce its length by effective combination with other control measures, including the use of beneficial microbial agents (Gamliel and Kapulnik 2012) or reduced dosages of pesticides (Gamliel 2012).

The loss of effective fumigants as well as the need to use more environmentally friendly methods makes the combination of soil solarization with organic amendments particularly interesting and investigated (Gamliel and Stapleton 2012).

The incorporation of soil amendments can improve the efficiency of solarization, by extending the spectrum of pathogens controlled, reducing the solarization duration, and preserving soil microbial communities from the negative effects of heating (Stapleton 1984; 2000; Klein et al. 2011 b; Tjamos et al.2000). Previous studies carried out under field conditions showed improved control of Fusarium wilt and dry root rot of clusterbean by combining 14 days of soil solarization with urea and farmyard manure application (Lodha 1995). Similar results were obtained under greenhouse conditions by combining 31 days of soil solarization with biofumigation using cabbage residues, against *Pythium aphanidermatum* of cucumber (Deadman et al. 2006).

Methods to study and validate the different possible variations are needed in order to determine the best combination for soil-borne pathogens control. A controlled laboratory system for simulating soil solarization with or without organic amendments, using 2 L soil containers exposed to controlled and constant aeration, and to temperature fluctuation similar to those occurring naturally during soil solarization, has been developed by Klein et al. (2007) and tested against *F. oxysporum* f. sp. *radicis-lycopersici* on tomato.

This study was carried out by simulating the effect of soil solarization under favorable and less favorable temperature conditions, with or without organic soil amendments (*Brassica carinata* defatted seed meals and compost), in order to screen different possible combinations for the management of the two causal agents of Fusarium wilt of rocket and basil.

Rocket (*Eruca sativa*) and basil (*Ocimum basilicum*) are high value crops affected by emerging soil-borne pathogens (Gullino and Garibaldi 2010; Gullino et al. 2012). *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* were recently observed in Italy on crucifer crops such as cultivated (*E. sativa*) and wild (*Diplotaxis tenuifolia*) rocket (Garibaldi et al. 2006), while Fusarium wilt, caused by *F. oxysporum* f. sp. *basilici* has long been known in Italy (Grasso 1975).

Material and methods

Layout of trials, thermal soil treatment and plant material.

Four experimental trials (two on basil and two on rocket) were carried out at Agroinnova facilities (Grugliasco, Italy), during March to November 2012. All trials started with the thermal treatment under growth chamber conditions, using plastic containers (50 x 40 x 20 cm corresponding to 20-L of soil capacity) filled with a mixture (70:30 v/v) of sandy loam soil (sand, 71.8% ± 5 ; silt, 5.4% ± 5; clay, 22.7% ± 5; pH, 7.3; organic matter content, 2.2%; cation exchange capacity, 2.7 meq100 g^{-1} soil) and peat substrate (Tecno 2, 70% white peat and 30% clay, pH 5.5-6, N 110-190 mg L^{-1}, P_2O_5 140-230 mg L^{-1}, K_2O 170-280 mg L^{-1},Turco Silvestro terricci, Bastia d'Albenga, SV, Italy). The characteristics of the final substrate obtained were: sand, 68.8% ± 5 ; silt, 6.8% ± 5; clay, 26% ± 5; pH, 7.1; organic matter content, 2.4%; cation exchange capacity, 5.7 meq100 g^{-1} soil).

A thermal treatment for simulating soil solarization was carried out under growth chamber conditions by heating the soil mix described above to optimal (55 to 52°C for 6 h, 50 to 48°C for 8 h and 47 to 45°C for 10 h/day) or sub optimal temperature conditions (50 to 48°C for 6 h, 45 to 43°C for 8 h and 40 to 38°C for 10 h/day) for 7 and 14 days. The two temperature regimes were selected according to the temperatures reached in the soil under greenhouse conditions in northern Italy (Gullino et al. 1998; Tamietti and Garibaldi, 1987). Immediately before starting the trial, soil was irrigated with water at 4.5 L/pot corresponding to soil moisture capacity. Treated soil was covered with polyethylene (PE) sheets (50 μm thick) immediately after the application of soil amendments, and moved in to growth chambers in order to start heating. Soil temperature was monitored at the depth of 10 cm in the middle of the solarized plot by using a Digital Data Logger EM50 (Decagon Devices, USA) at 60 min intervals. The untreated control soil was kept between 25 and 28 °C under greenhouse conditions.

In all four trials, at the end of each thermal treatment carried out under growth chamber conditions, treated and untreated soil was transferred into 10 plastic pots of 2 L capacity and kept in a greenhouse with temperatures ranging from 25 to 28 °C and 70-80 UR% (Table 2).

Plants of cultivated rocket (*Eruca sativa*, cv. Coltivata, Bertolino) and basil (*Ocimum basilicum*, cv. Fine verde, Furia Sementi), both highly susceptible to Fusarium wilt, were used. Rocket seeds were sown in plug trays (160 plugs/tray) and 15-20 day old seedlings were used for transplanting in to the 2-L pots, (5 plants/pot), while 50 to 70 basil seeds were sown in each treated and untreated 2-L pots. Two subsequent cycles of cultivation were carried out in the same soil into treated and untreated soil. Each crop cycle lasted 40 to 44 days after transplanting rocket or sowing basil (Table 1). Plants were irrigated daily.

Artificial inoculation and soil treatments

To achieve a high disease pressure, talc formulations of *Fusarium oxysporum* f. sp. *basilici* strain Fob009RB (resistant to 10 mg L^{-1} of benomyl), and *Fusarium oxysporum* f. sp. *conglutinans*

ATCC16600RB (resistant to 10 mg L^{-1} of benomyl) (Lu et al., 2010), prepared according to Locke and Colhoun, (1974), were incorporated into the soil at $5x10^4$ FCU ml^{-1} (Table 1 and 2).

Brassica carinata, as defatted seed meal (Biofence, N organic 3%, P 2.2%, K 2%, organic C 52%, Triumph, Italy), was mixed into the soil at 2.5 g L^{-1}. A municipal compost (Acea Pinerolese, Pinerolo, Italy), prepared from the organic fraction of municipal solid and biodegradable waste, was used at 4 g L^{-1} of soil. The soil was amended with *B. carinata* seed meal and/or compost before starting the thermal treatment (T0), or at the end of thermal treatment (T 14) at soil uncovering, at the dosages reported above, (Table 1).

Disease and growth parameters evaluation

The effectiveness of different treatments on the severity of *F. oxysporum* f. sp. *conglutinans* on rocket and *F. o oxysporum* f. sp. *basilici* on basil was evaluated weekly during the trials. Throughout the experiments wilted plants were counted and removed. The final disease rating was carried out 3 to 4 weeks after transplanting rocket and sowing basil by evaluating the vascular discoloration. At the end of rocket trials disease incidence (DI) was assessed on a 0 to 100 scale: 0: corresponded to healthy plants; 12.5: plants growing regularly with slight vascular discoloration; 25: slight leaf chlorosis and reduced growth, vascular discoloration; 50: chlorosis, growth reduction, vascular discoloration, initial symptoms of wilting; 75: extended vascular discoloration, strong leaf chlorosis, severe growth reduction and wilting symptoms; 100: whole leaves yellow, plants totally wilted and dead. The effect of the different treatments on basil was evaluated by counting the number of healthy and diseased plants/replicate. Disease incidence was expressed as percentage of diseased plants at the end of the trials,

At the end of each trial, after completing disease rating, the total fresh plant biomass was weighed by using a technical balance (Orma SNC, Italy) in order to evaluate the effect of each treatment on plant growth.

Experimental design and data analysis

Trials were carried out by adopting a completely randomized block design with five replications for each treatment. Disease incidence data were analyzed to check the normal distribution with Shapiro-Wilk Test and arcsine transformation was made when necessary. The data was subjected to the analysis of variance (ANOVA). All data were statistically analyzed according to Tukey test (P=0.05).

Results

The methodology used for soil infestation led to a good disease incidence in the control plots in all trials for both pathogens on the two crops (Tables 2 to 5). The disease index for control plants ranged from 55 to 97.5 in the case of *F. oxysporum* f. sp. *conglutinans* on rocket (Tables 2 and 3) and from 40.9 to 80.4 in the case of *F. oxysporum* f. sp. *basilici* on basil (Tables 4 and 5).

In the case of Fusarium wilt of rocket, in the presence of a disease incidence of 55 in the inoculated and untreated plots, one week of soil solarization carried out at both sub-optimal and optimal temperature regimes, lead to complete control (first crop) and almost complete control(second crop) of the disease in the first trial (Table 2). The use of compost alone, applied at 4 g L^{-1} of soil at the time of artificial infestation, resulted in quite effective disease control at the first crop cycle, but not as effective at the second crop cycle. *Brassica carinata*, added at 2.5 g L^{-1} of soil, was only very partially effective at the first cycle, providing less than 20% disease reduction. On the second crop, *B. carinata* applied alone at T0 caused no significant effect in disease incidence reduction (Table 2). One and two weeks of thermal treatment, both at optimal and sub-optimal temperature regimes were quite effective in reducing Fusarium wilt. The combination of thermal treatment and soil amendment completely controlled Fusarium wilt of rocket on the first crop and significantly reduced wilt incidence on the second crop. However, none of the combinations could improve the effect of the thermal treatment by itself (Table 2). The soil amendments applied, although not effective in terms of disease control, provided, in general, a positive effect on plant biomass produced. The best results, in terms of fresh weight, were provided by the mixture of *B. carinata* and compost, with and without the thermal treatment. The positive effect on biomass provided by such a mixture was more evident on the first crop cycle and was observed when the amendments were applied at T0 or T14 (Table 2).

In the second trial carried out against Fusarium wilt of rocket, in the presence of a very high disease incidence, one week of thermal treatment alone was very effective when the optimal temperature regime was adopted (Table 3). When sub-optimal temperatures were used, two weeks of thermal treatment were needed to obtain a satisfactory reduction of Fusarium wilt. The same trend was observed on the first and second cycle of the crop (Table 3). In the presence of such a high disease incidence (DI), the soil treatment with *Brassica carinata* seed meal and compost, alone or combined, was not effective at reducing DI on the first crop cycle. The same treatments were more effective on the second cycle (Table 3). The use of soil amendments, alone or combined, lead to a significant disease reduction when combined with 7 days of thermal treatment at sub-optimal temperature regimes. One week of soil solarization combined with soil amendments of *B. carinata* with or without compost at T 14 lead to the highest plant biomass. As already observed in the first trial, the positive effect of soil amendments on plant biomass is more evident on the first crop cycle (Table 3).

One and two weeks of thermal treatment, at both optimal and sub-optimal temperatures, were very effective against *F. oxysporum* f. sp. *basilici*, on both the first and second crop cycles of basil (Table 4). *B. carinata* seed meal alone and combined with compost only partially reduce disease incidence at the first cycle (37%, 13% and 45% of reduction compared with the untreated control), while at the second cycle no significant differences with the untreated control were observed (Table 4). All combinations tested were very effective at reducing DI on the first and second cycles, however they were not able to improve the level of control offered by one and two weeks of thermal treatment alone (Table 4). The highest biomass was obtained with the combination of thermal treatment and soil amendments with *B. carinata* seed meal and compost; the increase in biomass in comparison with the thermal treatment alone was observed on both crop cycles (Table 4).

Similar results, in the presence of a lower disease incidence of 52.4 and 64.5 in control plants, were observed in the second trial carried out against Fusarium wilt of basil. One and two weeks of thermal treatment, with optimal and sub-optimal temperature regimes, were very effective in reducing disease incidence on the first and second crop cycles of basil (Table 5). *B. carinata* and compost, applied alone, did not reduce Fusarium wilt on both cycles, while their combination did partially provided a significant disease incidence reduction of 58% and 57% on first and second cycles, respectively (Table 5). All combinations of thermal treatments and soil amendments were very effective but not able to improve the efficacy provided by the thermal treatment alone (Table 5). However, the combination of thermal treatment and compost did affect plant biomass; the highest fresh weight was observed in the plots solarized and amended with compost (Table 5).

Discussion

Soil solarization has been largely exploited all over the world wherever the climatic conditions allow it. For instance, in northern Italy, its practical application is still primarily limited to greenhouse production of high value crops (Gullino and Garibaldi 1991 and 2012). Besides climate dependency, the length of soil solarization treatment represents a major drawback to its broader implementation (Katan and Gamliel 2012). Many approaches have been developed to reduce the length of soil solarization in order to encourage more growers adopt this method . Also, environmentally controlled methods that simulate the soil solarization process have been developed and validated (Klein et al. 2007) with the aim of better evaluating the different parameters involved in soil solarization as well as the effects of organic amendments against soil-borne pathogens. The system adopted in this study permits a better evaluation of several combinations of thermal treatment of the soil and use of organic amendments, in order to determine the best combination for different crops.

The two temperature regimes adopted (optimal and sub-optimal) for the simulation of soil solarization correspond to the temperatures reached in greenhouses under natural conditions in northern Italy, where average increases in soil temperature of 9.1 and 4.6 °C were observed at 12 and 25 cm depths, respectively, in several experimental trials (Tamietti and Garibaldi 1987; Garibaldi and Gullino 1991).

The results obtained show that, even when a sub-optimal temperature regime is tested, 7 days of thermal treatment provides very interesting results, in terms of Fusarium wilt control. Disease control is improved with 14 days of treatment at sub-optimal temperatures and with 7 or 14 days of treatment at optimal temperatures.

In general, the thermal treatment was always more effective against *F. oxysporum* f. sp. *basilici* than *F. oxysporum* f. sp. *conglutinans.* Differences in response to the thermal effect of soil solarization among different pathogens, and even among *formae speciales* of the same species, are known and well documented (Katan and Gamliel 2012). For this reason, the selection of the treatment length should consider the variability in thermal susceptibility existing among the different pathogens (Bollen 1969). In the mean time, short solarization treatments could fit in well between short cycle crops, particularly under conditions of consistent insolation and when high temperatures are reached. This situation is typical of southern European countries.

Although soil heating is a major factor in soil solarization, it is not the only one. It has been documented that soil heating also enhances a number of beneficial microbial processes, thus improving disease control and also increasing plant growth and yield (Katan and Gamliel 2012). The incorporation of soil amendments into the soil did not provide, under our experimental conditions, satisfactory disease control. This was probably due to the relatively short duration of the trials. It is well known that organic amendments need long periods in order to be effective since their activity is due to decomposition and release of volatiles (Bonamomi et al. 2010).

However, the combination of organic amendments with a short period of soil solarization (7 or 14 days), while not providing any improvement to the level of disease management, significantly increase the biomass of plants. The positive effect on plant biomass was evident on rocket especially on the first crop cycle, while it was more long lasting on basil. The plant biomass increase is of particular interest in the case of leafy vegetable crops, because it leads to a yield increase, with significant economic advantages.

When a shorter period of soil solarization is adopted due to practical constrains, this treatment can be combined with the use of other methods (biocontrol agents, soil amendments, reduced dosages of fumigants, ...), to exploit all possible additive or synergistic effects(Minuto et al. 2000; 2006). This kind of approach, which is very compatible with an IPM approach, will achieve the best results under practical conditions.

The results obtained in this study, in the presence of a very high disease pressure, show the potential to develop different options for shorter solarization periods in combination with the use of organic amendments, for a positive effect both on disease management and yield.

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Table 1. Main information on the trials carried out.

Cropping cycle	Timing (days after the artificial inoculation)	Operation carried out	Rocket		Basil	
			1st trial	2nd trial	1st trial	2nd trial
1°	T0	Artificial inoculation	5 March 2012	25 June 2012	29 March 2012	31 July 2012
	T0	Organic amendments application	5 March 2012	25 June 2012	3 March 2012	31 July 2012
	T0	Thermal treatment with simulation of soil solarization	5 March 2012	25 June 2012	3 March 2012	31 July 2012
	T7	Post solarization treatment	12 March 2012	2 July 2012	6 April 2012	7 August 2012
	T14	End of thermal treatment with simulation of soil solarization for 14 days	19 March 2012	8 July 2012	12 April 2012	14 August 2012
	T14	Post solarization treatment	19 March 2012	8 July 2012	12 April 2012	14 August 2012
	T15	Transplanting or sowing (cycle I)	20 March 2012	9 July 2012	13 April 2012	14 August 2012
	T44	End of the cycle I -Disease and biomass evaluation	18 April 2012	29 August 2012	22 May 2012	27 September 2012
2°	T64	Transplanting or sowing (cycle II)	7 May 2012	19 September 2012	25May 2012	3 October 2012
	T106	End of the cycle II -Disease and biomass evaluation	18June 2012	7 November 2012	16 July 2012	23November 2012

Table 2. Effect of organic amendments alone, and combined with simulated optimal and sub-optimal thermal treatments, on disease severity caused by *F. oxysporum* f. sp. *conglutinans* (ATCC16600RB*)* on rocket (Cycles I and 2, Trial 1).

Thermal treatment (days)	Soil amendment g L^{-1}	Application of soil amendments [a]	Disease incidence 0-100								Fresh biomass weight g							
			Sub-optimal [b] thermal treatment				Optimal thermal treatment				Sub-optimal thermal treatment				Optimal thermal treatment			
			1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle	
Inoculated control	-	-	55.0	d[c]	57.5	c	55.0	d	57.5	b	12.4	f	2.7	i	12.4	h	2.7	g
7	-	-	0.0	a	6.0	a	1.0	a	4.0	a	26.3	e	9.7	g-i	27.4	g	7.2	e-g
14	-	-	0.0	a	8.5	a	1.0	a	13.5	a	25.7	e	12.0	f-i	30.0	g	9.0	e-g
-	*B. carinata* 2.5	T0	45.0	c	76.0	c	45.0	c	76.0	b	24.2	ef	5.5	hi	24.2	gh	5.5	fg
-	Compost 4		33.5	b	69.0	c	33.5	b	69.0	b	56.8	cd	4.7	hi	56.8	f	4.7	fg
-	*B. carinata* + compost 2.5+4	T0	26.5	b	56.0	bc	26.5	b	56.0	b	85.0	a	12.3	e-i	85.0	abc	12.3	d-g
7	*B. carinata* 2.5	T0	0.0	a	10.5	a	0.0	a	8.0	a	54.3	cd	25.5	b-e	71.6	de	17.8	c-e
7	Compost 4		0.0	a	21.5	a	0.0	a	13.5	a	49.3	d	19.4	c-g	72.8	b-e	13.5	d-f
7	*B. carinata*+ compost 2.5+4	T0	0.0	a	23.0	a	0.0	a	7.0	a	55.9	cd	28.7	b-d	85.6	a-c	30.6	ab
14	*B. carinata* 2.5	T0	0.0	a	11.5	a	0.0	a	6.0	a	47.1	d	24.8	b-f	68.0	d-f	20.6	b-d
14	Compost 4		0.0	a	16.5	a	0.0	a	4.5	a	46.6	d	17.4	d-h	80.5	a-d	14.8	c-f
14	*B. carinata* + compost 2.5+4	T0	0.0	a	28.5	ab	0.0	a	14.0	a	56.8	cd	31.5	a-c	85.8	ab	33.5	a
7	*B. carinata* 2.5	T7	0.0	a	18.5	a	0.0	a	4.0	a	51.2	d	21.2	c-g	61.2	ef	14.1	d-f
7	Compost 4	T7	0.0	a	14.0	a	0.0	a	14.0	a	56.7	cd	16.9	d-h	72.4	c-e	10.2	d-g
7	*B. carinata* + compost 2.5+ 4	T7	0.0	a	10.5	a	0.0	a	11.0	a	69.5	b	36.6	ab	89.7	a	25.2	a-c
14	*B. carinata* 2.5	T14	0.0	a	17.0	a	0.0	a	10.5	a	56.8	cd	26.7	b-d	62.0	ef	16.2	c-e
14	Compost 4	T14	0.0	a	17.5	a	0.0	a	10.5	a	56.3	cd	27.0	b-d	77.4	a-d	11.1	d-g
14	*B. carinata* + compost 2.5+4	T14	0.0	a	4.0	a	0.0	a	2.5	a	64.8	ab	42.4	a	89.8	a	29.8	ab
Not treated not inoculated control	-	-	0.0	a	0.0	a	0.0	a	0.0	a	26.6	e	10.6	ghi	26.6	g	10.6	d-g

[a]T0 immediately before starting thermal treatment at soil mulching; T14 immediately after thermal treatment at soil unmulching.
[b] Maximum temperature at 10 cm soil depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.
[c] Means of the same column, followed by the same letter, do not significantly differ following Tukey's test ($P<0.05$).

Table 3. Effect of organic amendments alone, and combined with simulated optimal and sub-optimal thermal treatments, on disease severity caused by *F. oxysporum* f. sp. *conglutinans* (ATCC16600RB) on rocket (Cycles I and II, Trial 2).

Thermal treatment (days)	Soil amendments (g L^{-1})	Application of soil amendments [a]	Disease incidence 0-100								Fresh biomass weight g							
			Sub-optimal [b] thermal treatment				Optimal thermal treatment				Sub-optimal thermal treatment				Optimal thermal treatment			
			1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle	
Inoculated and not treated control	-	-	97.5	g [c]	91.0	d	97.5	b	91.0	c	1.9	h	2.3	f	1.9	e	2.3	e
7	-	-	38.1	d-f	60.5	b-d	13.1	a	4.0	a	32.9	f-h	4.5	f	56.4	c-e	6.5	e
14	-	-	26.9	a-e	20.0	ab	8.8	a	6.0	ab	36.1	f-h	8.0	ef	61.5	b-e	11.8	e
-	*B. carinata* 2.5	T0	85.0	g	85.5	cd	85.0	b	85.5	c	20.6	gh	6.2	f	20.6	de	6.2	e
-	Compost 4		86.3	g	89.0	d	86.3	b	89.0	c	23.8	gh	4.8	f	23.8	de	4.8	e
-	*B. carinata* + compost 2.5+4	T0	65.0	fg	86.0	cd	65.0	b	86.0	c	61.0	e-g	8.2	ef	61.0	b-e	8.2	e
7	*B. carinata* 2.5	T0	41.3	ef	17.5	ab	23.1	a	9.5	ab	68.3	d-g	16.7	d-f	83.7	b-d	13.5	e
7	Compost 4		14.4	a-e	48.0	a-d	12.5	a	32.0	ab	98.4	c-e	11.4	ef	93.6	a-c	7.4	e
7	*B. carinata*+ compost 2.5+4	T0	0.0	a	29.5	ab	14.4	a	14.0	ab	128.1	bc	31.1	c-f	115.0	a-c	15.4	e
14	*B. carinata* 2.5	T0	23.1	a-e	28.5	ab	17.5	a	15.5	ab	53.8	e-g	24.3	d-f	63.1	b-e	28.9	c-e
14	Compost 4		11.9	a-e	28.0	ab	11.9	a	43.5	b	86.6	c-e	10.6	ef	97.5	a-c	8.8	e
14	*B. carinata* + compost 2.5+4	T0	33.1	b-f	16.0	ab	18.8	a	18.0	ab	78.8	c-f	46.6	b-d	77.3	b-d	27.3	c-e
7	*B. carinata* 2.5	T7	9.4	a-e	10.5	ab	22.5	a	4.0	a	153.5	ab	40.5	b-e	124.9	ab	48.5	b-d
7	Compost 4	T7	0.6	ab	37.0	a-c	7.5	a	30.0	ab	116.0	b-d	20.4	d-f	115.8	a-c	10.4	e
7	*B. carinata* + compost 2.5+ 4	T7	5.6	a-d	10.5	ab	8.8	a	8.0	ab	181.5	a	96.7	a	158.5	a	53.9	bc
14	*B. carinata* 2.5	T14	36.3	c-f	4.0	a	28.1	a	17.0	ab	53.1	e-g	57.2	bc	113.4	a-c	61.7	ab
14	Compost 4	T14	5.0	a-c	17.0	ab	3.1	a	19.0	ab	96.1	c-e	16.6	d-f	108.5	a-c	30.4	c-e
14	*B. carinata* + compost 2.5+4	T14	15.6	a-e	20.5	ab	20.6	a	12.0	ab	125.7	bc	65.3	ab	111.9	a-c	86.6	a
Not treated not inoculated control	-	-	0.0	a	0.0	a	0.0	a	0.0	a	116.8	b-d	19.7	d-f	116.8	a-c	19.7	de

[a]T0 immediately before starting thermal treatment at soil mulching; T14 immediately after thermal treatment at soil unmulching.
[b] Maximum temperature at 10 cm soil depth in sub-optimal (48°C for 6 h, 43°C for 8 h and 38°C for 10 h) and optimal (52°C for 6 h, 48°C for 8 h and 45°C for 10 h) conditions.
[c] Means of the same column, followed by the same letter, do not significantly differ following Tukey's test (P<0.05).

Table 4. Effect of organic amendments alone, and combined with simulated optimal and sub-optimal thermal treatments, on disease incidence caused by *F. oxysporum* f. sp *.basilici* (FOB009 RB) on basil (Cycles I and II, Trial 1).

Thermal treatment (days)	Soil amendments (g L^{-1})	Application of soil amendments [a]	Disease incidence 0-100								Fresh biomass weight g							
			Sub-optimal [b] thermal treatment				Optimal thermal treatment				Sub-optimal thermal treatment				Optimal thermal treatment			
			1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle	
Inoculated and not treated control	-	-	80.4	d [c]	40.9	cd	80.4	d	40.9	c	9.2	g	9.8	g	9.2	h	9.8	f
7	-	-	1.1	a	3.2	ab	0.0	a	1.6	a	28.9	c-f	21.0	e-g	34.0	d-f	17.2	ef
14	-	-	0.5	a	7.4	ab	2.4	a	4.4	a	27.2	d-g	26.0	d-g	36.5	d-f	14.5	ef
-	*B.carinata* 2.5	T0	50.9	b	44.2	d	50.9	b	44.2	c	15.2	fg	29.4	c-g	15.2	gh	29.4	c-f
-	Compost 4	T0	70.6	c	41.0	cd	70.6	c	41.0	c	19.1	fg	24.5	d-g	19.1	f-h	24.5	d-f
-	*B. carinata* + compost 2.5+4	T0	43.6	b	32.4	b-d	43.6	b	32.4	bc	19.2	fg	33.1	b-f	19.2	f-h	33.1	b-e
7	*B. carinata* 2.5	T0	0.2	a	17.0	a-d	0.8	a	2.8	a	31.7	b-f	44.2	a-d	41.0	c-e	54.1	a
7	Compost 4		0.6	a	10.8	a-c	0.3	a	7.1	ab	45.1	a-c	33.3	b-f	50.5	b-d	29.0	c-f
7	*B. carinata*+ compost 2.5+4	T0	0.7	a	9.6	a-c	0.3	a	5.0	a	55.2	a	55.7	a	48.9	cd	60.7	a
14	*B. carinata* 2.5	T0	2.0	a	6.1	ab	0.2	a	2.6	a	29.1	c-f	48.8	a-c	44.9	c-e	50.4	ab
14	Compost 4		1.2	a	13.5	a-d	0.0	a	5.9	a	42.8	a-e	32.8	b-f	71.6	a	26.6	c-f
14	*B. carinata* + compost 2.5+4	T0	0.2	a	6.0	ab	0.5	a	14.2	ab	52.1	a	40.6	a-e	56.1	a-c	57.7	a
7	*B. carinata* 2.5	T7	0.3	a	3.8	ab	0.0	a	1.3	a	27.8	c-f	47.9	a-c	30.3	e-g	44.9	a-c
7	Compost 4	T7	0.6	a	9.6	a-c	0.2	a	6.8	ab	44.0	a-d	30.9	b-g	49.8	b-d	27.5	c-f
7	*B. carinata* + compost 2.5+ 4	T7	0.8	a	9.2	a-c	0.0	a	3.8	a	47.7	ab	59.9	a	57.2	a-c	56.4	a
14	*B. carinata* 2.5	T14	3.2	a	12.1	a-d	0.3	a	4.2	a	25.3	e-g	52.2	ab	43.7	c-e	44.0	a-d
14	Compost 4	T14	1.8	a	14.0	a-d	0.2	a	9.5	ab	51.0	a	32.3	b-f	67.2	ab	23.8	ef
14	*B. carinata* + compost 2.5+4	T14	0.4	a	8.5	ab	0.3	a	12.4	ab	50.9	a	60.0	a	56.7	a-c	56.0	a
Not treated not inoculated control	-	-	0.0	a	0.0	a	0.0	a	0.0	a	28.1	c-f	18.1	fg	28.1	e-g	18.1	ef

[a] T0 immediately before starting thermal treatment at soil mulching; T14 immediately after thermal treatment at soil unmulching.
[b] Maximum temperature at 10 cm soil depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.
[c] Means of the same column, followed by the same letter, do not significantly differ following Tukey's test ($P<0.05$).

Table 5. Effect of organic amendments alone, and combined with simulated optimal and sub-optimal thermal treatments, on disease severity caused by *F. oxysporum* f. sp .*basilici* (FOB009RB) on basil (Cycles I and II, Trial 2).

Thermal treatment (days)	Soil amendments (g L^{-1})	Application of soil amendments [a]	Disease incidence 0-100								Fresh biomass weight g							
			Sub-optimal [b] thermal treatment				Optimal thermal treatment				Sub-optimal thermal treatment				Optimal thermal treatment			
			1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle		1st cycle		2nd cycle	
Inoculated and not treated control	-	-	52.9	cd [c]	65.4	c	52.9	cd	65.4	c	10.5	f	14.9	h	10.5	d	14.9	h
7	-	-	4.7	a	12.3	ab	6.3	a	10.4	a	25.7	d-f	20.4	gh	44.9	a-c	26.2	gh
14	-	-	5.6	a	4.2	ab	7.0	a	0.9	a	26.5	c-f	27.4	f-h	41.0	a-d	40.3	f-h
-	*B. carinata* 2.5	T0	40.4	c	55.0	c	40.4	c	55.0	c	30.0	b-f	48.7	c-h	30.0	b-d	48.7	e-h
-	Compost 4		60.5	d	54.0	c	60.5	d	54.0	c	15.5	f	29.5	e-h	15.5	cd	29.5	gh
-	*B. carinata* + compost 2.5+4	T0	22.1	b	27.8	b	22.1	b	27.8	b	38.5	a-f	55.0	b-h	38.5	a-d	55.0	c-h
7	*B. carinata* 2.5	T0	7.8	a	15.9	ab	1.5	a	2.2	a	21.3	ef	47.3	d-h	41.3	a-c	99.2	a-c
7	Compost 4		1.1	a	7.8	ab	2.6	a	0.4	a	53.7	a-d	43.7	d-h	53.9	ab	70.3	b-g
7	*B. carinata*+ compost 2.5+4	T0	6.3	a	21.0	ab	4.1	a	3.4	a	57.2	ab	61.5	a-g	60.4	ab	116.8	a
14	*B. carinata* 2.5	T0	6.6	a	5.4	ab	2.2	a	0.6	a	33.3	a-f	69.6	a-f	33.2	a-d	86.1	a-f
14	Compost 4		0.3	a	1.5	a	3.1	a	1.2	a	61.2	a	46.2	d-h	62.8	a	50.5	d-h
14	*B. carinata* + compost 2.5+4	T0	4.6	a	0.3	a	5.4	a	2.1	a	24.5	ef	75.4	a-d	41.3	a-c	90.2	a-e
7	*B. carinata* 2.5	T7	5.0	a	12.7	ab	2.9	a	1.1	a	37.2	a-f	92.6	ab	53.8	ab	102.9	ab
7	Compost 4	T7	2.0	a	17.4	ab	6.4	a	9.8	a	54.4	a-d	53.1	b-h	38.6	a-d	60.5	b-h
7	*B. carinata* + compost 2.5+ 4	T7	6.1	a	19.8	ab	2.2	a	2.3	a	54.3	a-d	72.5	a-e	53.2	ab	120.9	a
14	*B. carinata* 2.5	T14	7.5	a	3.6	ab	4.5	a	0.5	a	15.8	f	92.5	a-c	34.6	a-d	119.2	a
14	Compost 4	T14	1.9	a	0.9	a	5.1	a	0.8	a	55.5	a-c	77.7	a-d	43.2	a-c	95.8	a-d
14	*B. carinata* + compost 2.5+4	T14	0.4	a	3.3	ab	2.5	a	0.0	a	49.8	a-e	101.7	a	42.3	a-c	104.6	ab
Not treated not inoculated control	-	-	0.0	a	0.0	a	0.0	a	0.0	a	34.4	a-f	34.0	d-h	34.4	a-d	34.0	gh

[a] T0 immediately before starting thermal treatment at soil mulching; T14 immediately after thermal treatment at soil unmulching.
[b] Maximum temperature at 10 cm soil depth in sub-optimal (48°C for 6 h, 43°C for 8 h and 38°C for 10 h) and optimal (52°C for 6 h, 48°C for 8 h and 45°C for 10 h) conditions.
[c] Means of the same column, followed by the same letter, do not significantly differ following Tukey's test ($P<0.05$).