

# The Control of *Verticillium dahliae* on Artichokes by Chemical and Non Chemical Soil Disinfestation Methods

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## Abstract

Since some years ago, the globe artichoke (*Cynara scolymus* L.) crops of Benicarló, at the north of Valencian Country (Alcachofa de Benicarló, appellation of origin) frequently suffer a tracheomicotic syndrome produced by *Verticillium dahliae*. The fungus remains in the soil, after it is infested, for long periods of time and lasts even in rotations of crops intercalated between two successive of artichoke. The crop use to remain in the field for two consecutive seasons or even more, if it is allowed by the plant health condition. In order to study the effect of soil disinfestation for disease control several systems of soil disinfestation are compared, based on chemical systems like the successive application of 1,3-dichloropropene (Telone II emulsifiable) at 18 g/m<sup>2</sup> followed by metam-Na at 72 g/m<sup>2</sup>, the mixture of 55.4% 1,3-dichloropropene and 32.7% chloropicrin (Agrocelhone emulsifiable) at 40 g/m<sup>2</sup>, all of them applied with the irrigation water, and physical methods like solarization with the addition of manure at 5Kg/m<sup>2</sup> or combined method like solarization with metam-Na at 72 g/m<sup>2</sup>. Methyl bromide at 30 g/m<sup>2</sup> under VIF sheet and a non disinfested check was used. as reference. The studied parameters include, the biocidal effect in the soil and efficacy over weeds, plant vigour, yield in quantity and quality as well as mortality due to *V. dahliae*. Excellent results were reached, on the first season, with all the compared systems except the check which had a high mortality rate and heavy yield losses. The effects for weed control and plant vigour follow the same pattern. On the second season the results are similar to the first one, but some problem of plant death affected all the treatments. The studied systems for soil disinfestation ensures the healthiness and quality of crop at least by the first season, and facing the next phase out of methyl bromide as a consequence of Montreal Protocol, some chemical and physical alternatives are offered.

## INTRODUCTION

*Verticillium dahliae* Kleb. is a wide range pathogen described in all Mediterranean basins. The first record of *V. dahliae* on artichoke was in Italy (Cirulli et al. 1994), since than it has been reported in France and other Mediterranean countries as well as in California (Bhat, et al., 1999). It is known in Spain on several crops such as cotton, olive, tomato, pepper, sunflower, peach tree (Andres et al, 1998) and eggplant.

The globe artichoke (*Cynara scolymus* L.) from the area of Benicarló A.O. (appellation of origin), at the north of Valencian country (Spain) is well known because of its earliness and special quality for fresh consumption. The interest of this crop is of such an importance that frequently is repeated for several successive crops on the same soil, or in the most favourable case there are other horticultural crops intercalated between two successive of artichokes.

The crops use to be installed in summer, along July, in order to begin the yield on November and ends on April or May depending on the market demand. The last yield use to be sold to the industry. If the plants have a good health condition at the end of the crop, can be pruned and allowed for a new consecutive season.

Since some years ago these crops frequently suffer a tracheomicotic syndrome such as that produced by the fungus *Verticillium dahliae* Kleb. starting by stunted growth of plants, followed by some basal chlorotic and wilted leaves; the cross section of stems show brown discoloration of vascular tissue and the longitudinal section shows the

necrotic tissue growing up to the apex of stem and leaves.

This fungus remains in the soil, after it is infested, for a long period of time and lasts even in rotation crops intercalated between two successive of artichoke. In addition one of the ways of infection is trough offshoots coming from diseased mother plants. Anyway this fungus is very cosmopolitan and can survive as a parasite of many other vegetal host species. Once installed in a soil it can survive many years by forming microsclerotia that remains into the soil.

Disease control can be achieved only by using healthy plant material grown in a healthy soil. But once the field infested, soil disinfestation is a good technique to reduce the soil inoculum to a level for a satisfactory crop (Tjamos & Paplomatas, 1988, Gamliel et al., 1997).

The most frequent disinfestations method in the region consists in the application of metam-Na with the irrigation water, generally without a further cover with a plastic sheet. This system offers a reasonable application fees to the grower, nevertheless it does not gives enough guarantee of success.

Other possible methods are the use of other fumigants such as methyl bromide, which is considered the most effective due to its biocidal wide activity and application facility, although because of its high hazardness, the applicators need a special training and permission and than the treatment costs are much higher.

The next phase out of methyl bromide on first January 2005, according to the Montreal Protocol, makes necessary to find out other alternatives in order to maintain similar conditions to the methyl bromide. Among the proposed alternatives are: the mixture of 1,3-dichloropropene and chloropicrin that combines the nematicidal action of the first with the fungicidal properties of the second. The well known fumigant metam-Na is recommended as well. A system that is growing in acceptance is soil solarization, mainly when its efficacy is improved by the addition of fumigants at low dosages or even some kind of organic matter (Cebolla, 1998; Gamliel and Stapleton, 1993).

This research has the aim to compare some soil disinfestation systems adapted to the special soil and climatic conditions of the crops in the area of Benicarló.

## MATERIALS AND METHODS

This research consisted in two experiments, the first one at laboratory scale to prove the pathogenicity of the isolate, and the second in a large scale field to compare some disinfestation systems.

Samples of stems and leaves were picked along the crop to isolate and identify the presence of the fungus in several part tissues.

Some colonies of *V. dahliae* grown on PDA petri dishes were transferred to sterile water, mixed and diluted up to a concentration of  $10^6$  conidia/ml to produce a conidial suspension. In the small scale laboratory experiment 20 seedling of artichoke were inoculated by root dipping in a conidial suspension of *V. dahliae* during 30 minutes. Other 20 seedlings were treated the same way, but without inoculum, and served as control. Plants were transplanted into small pots filled with sterile peat. And were allowed to incubate at room temperature and under natural light for 6 weeks.

In a field experiment to compare several disinfestations systems, two farms near Benicarló with a log history of repeated crops of artichoke were selected. Soil preparation, before disinfestations, was done by a deep plough followed by rototiller to break soil aggregates. Soil wetness was considered high enough to guarantee the most susceptible state for pathogens.

Treatments for soil disinfestations were as follows: soil solarization with the addition of metam-Na at a rate of  $72 \text{ g/m}^2$  covered with transparent polyethylene (PE) sheet along 30 days of July (SolMS); a mixture of 55.4 % of 1,3-dichloropropene and 32.7% chloropicrin emulsifiable (Agrocellhone NE) applied in the irrigation water at a rate of  $50 \text{ g/m}^2$  covered with PE along 10 days (ANE); two successive treatments with  $72 \text{ g/m}^2$  of metam-Na followed one week later by an irrigation with  $18 \text{ g/m}^2$  of 1,3-dichloropropene (Telone II) under the PE sheet. total duration of treatment was 2 weeks

(TelMS); the treatment consisted of the application of 5 kg/m<sup>2</sup> of a mixture 75% sheep manure and 25% chicken manure. Once incorporated to the soil with rototiller the plot was covered with PE sheet, and was irrigated under the sheet. The duration of treatment was 30 days (SolMan); all these treatments were compared with an application of methyl bromide at 30 g/m<sup>2</sup> under VIF (Virtually Impermeable Film) sheet, total duration of treatment was 5 days (Br30VIF); and a non disinfested control (Control).

Soil temperature during solarization was monitored with an automatic register system at a 10cm depth and compared to the non disinfested control.

After disinfestations the plots were planted with artichoke offshoots cv. Blanca de Tudela according to the A.O. Alcachofa de Benicarló, for a two seasons crop.

Plant vigour in each treatment was monitored as the average length of the longest leave of 10 plants. Plant mortality along the crop was also evaluated and the presence of *V. dahliae* was determined by isolating the fungus from apical, non discoloured pieces of leaves. Crop yield weight and number of heads harvested, were registered and classified in 1<sup>st</sup>, 2<sup>nd</sup> and debris qualities. Weed incidence was monitored by measuring the cost of weeding time and the most frequent weeds were identified.

Experimental design consisted in two blocs and two replicates per bloc. ANOVA and Duncan test at 95% were used to compare the individual treatments. Logarithmic transformation was used to compare yield and Arc sinus transformation was used to compare percentages.

## RESULTS AND DISCUSSION

Typical wilt symptoms and vascular discoloration were observed, in the laboratory experiment, only in the inoculated seedlings after 4 weeks of incubation and *V. dahliae* was isolated from that plants. 90% of plants were dead after 6 weeks of incubation and *V. dahliae* was isolated from that diseased plants.

In the field experiment *V. dahliae* was isolated repeatedly from control, non discoloured plant leaf tissues. The temperatures reached during soil solarization are shown in Fig. 1 where the cloudy days are distinguishable by the drop of maximal temperatures (35°C) nevertheless the temperatures reached are higher than 44°C most of the days.

Results of first season are shown in table 1. All treatments reduced the cost of weeding as compared to the control (Table 1). Treatments Br30VIF and ANE gave the best control. The main weeds present in the plots were *Chenopodium album* L., *Diploptaxis erucoides* (L.) DC., *Equinochloa crus-galli* (L.) Beauvais, *Portulaca oleracea* L., and *Stellaria media* (L.) Villars. The higher mortality occurred in the non disinfested control all the other treatments reduced significantly the number of dead plants. The same pattern occurred with regard to plant vigour (Table 1) where the control plants resulted in a more reduced size than the rest of treatments due to its lack of vigour. Treatments Br30VIF, ANE, SolMS and TelMS show the higher plant vigour. All treatments improved the amount of yield with respect to the control the differences are more distinguishable for the first quality yield (Fig. 2).

Data obtained the second year of crop show an increased mortality (table 1) in control plots with respect to all other treatments. In a similar way, size of plants, total and first quality yield of all disinfested treatments gave much better results than the control. Percent of debris was higher than the rest of treatments. As a summary the results of the second season confirm those of the first, although the yield is more reduced (46%) than the first one (Fig 3).

The choice between treatments must be considered taking into account the following features: The mixture 1,3-dichloropropene and chloropicrin must be operated by authorized contractors or applicators the same way as it occurs with methyl bromide. Other treatments such as solarization combined with metam-Na or with manure and the sequence metam-Na - 1,3-dichloropropene can be applied by the farmer itself. On the other hand the techniques related with solarization need more time for application (at least 10 days instead of 10 or 15 days for other fumigants) and than it can be difficult to fit the

schedule between the end of the crop and the start of the next one if we want to plant at the beginning of July, as is usually done in this area in order to get an early harvest.

## CONCLUSIONS

All these disinfection techniques reduce the disease incidence produced by *V. dahliae* and permit a successful yield as it can be achieved with methyl bromide, keeping good agronomical features at least for the first year of the crop. On the second year the yield drops drastically in all the studied treatments as compared to the first season.

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## Tables

Table 1. Plant mortality %, size of longest leaf (cm), weeding time (h/ha) and % of debris for 1<sup>st</sup> and 2<sup>nd</sup> season of artichoke crop cv. Blanca de Tudela.

Treatments	First season				Second season		
	Dead plants %	Leaf length	Weeding time	% debris	Dead plants %	Leaf length	% debris
Control	8.3 a	45.1 c	146.9 a	21.9 a	29.4 a	44.9 b	23.2 a
Br30VIF	0.0 b	65.7 a	18.1 b	15.05 b	6.1 bc	57.3 a	14.98 b
Tel&MS	6.0 ab	59.2 ab	62.2 b	14.66 b	14.9 b	57.0 a	12.50 b
ANE	2.5 ab	58.7 ab	15.0 b	13.81 b	8.1 bc	59.8 a	11.94 b
SolMS	4.6 ab	58.4 ab	34.7 b	14.46 b	6.5 bc	61.6 a	10.97 b
SolMan	4.7 ab	57.7 b	43.6 b	12.41 b	4.2 c	62.0 a	12.05 b

Figures with the same letter do not show significant differences ( $p < 0.05$ ).

**Figures**

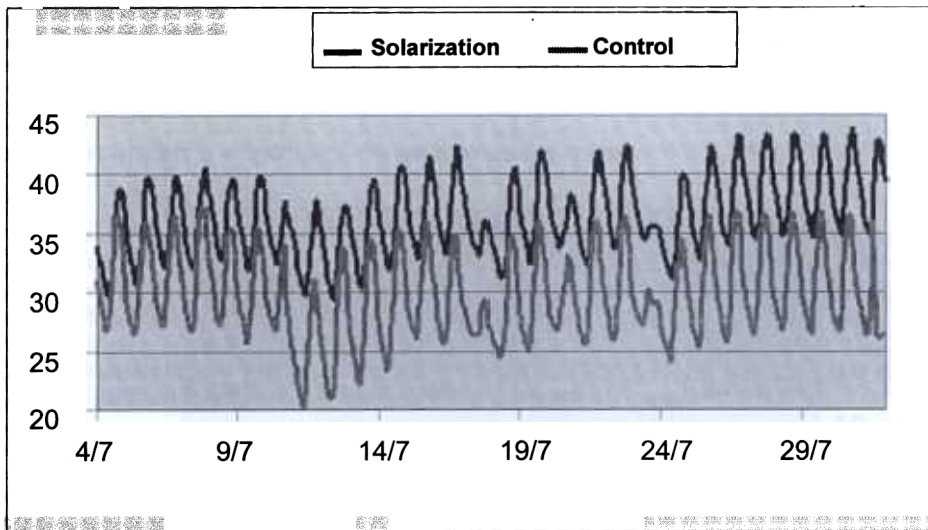
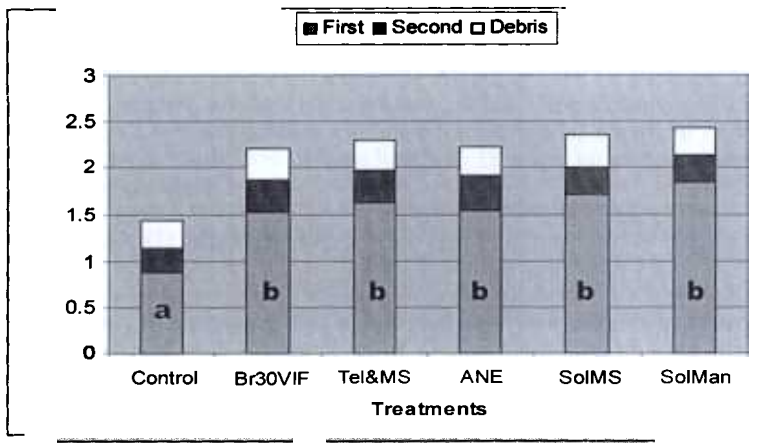


Fig 1. Soil temperatures (°C) at 10 cm depth for solarization and control treatments.



2. First season yield of artichokes classified as first, second and debris qualities.

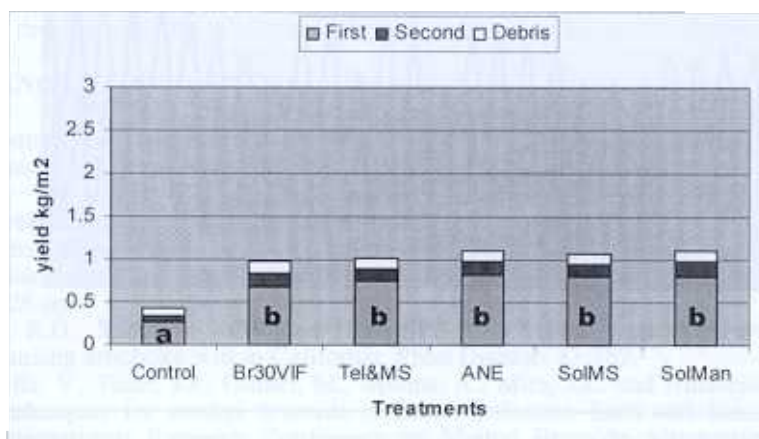


Fig. 3. Second season yield of artichokes classified as first, second and debris qualities. Treatments with the same letter do not show significant differences in first quality yield ( $p < 0.05$ ).